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SELECTIVE PHAGOCYTOSIS I

MICROSCOPIC OBSERVATIONS CONCERNING THE REGULATION OF THE BLOOD FLOW THROUGH THE LIVER AND OTHER ORGANS AND THE MECHANISM AND RATE OF PHAGOCYTIC REMOVAL OF PARTICLES FROM THE BLOOD

BY

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INTRODUCTION

The purpose of this paper is to present an integrated overall view of the selective phagocytic removal of coated foreign particles from the blood stream of frogs. It is necessary to understand selective phagocytosis from the blood stream in order to understand several aspects of certain pathologic conditions and diseases, particularly those in which the blood of the animal or patient is precipitated and agglutinated into a circulating sludge. Intravascular agglutination of erythrocytes has been seen or demonstrated in living animals and in human patients, and some of its results observed in vitro or in histological preparations by many investigators. Among these are: COCCIUS, 1852; DONDERS, 1864; HUETER, 1876; 1879 a, 1879 b; FLEXNER, 1902; LUEDDE, 1913; AREY, 1918; PLOMAN, 1920; WELCH, 1920; ELSCHNIG, 1921; FÅHRAEUS, 1921, 1928, 1929; FREEDLANDER and LENHART, 1922; RUEDEMANN, 1933, 1937; SALSBURY and MELVIN, 1936; SWINDLE, 1937; MÜLLER, 1937; KNISELY, STRATMAN-THOMAS and ELIOT, 1941; KNISELY and BLOCH, 1942, 1945; YOUNGNER and NUNGESTER, 1944; OLIVER-GONZALES, 1944; MILLS and DOCHEZ, 1944; KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH, 1945; KNISELY, ELIOT and BLOCH, 1945; KNISELY, BLOCH, ELIOT and WARNER, 1947. However, the factors which cause intravascular agglutination have not been thoroughly studied, nor have the roles of intravascular agglutination in the mechanisms of pathological physiology and in the defense reactions been intensively studied.

To introduce the subject "selective phagocytosis from the blood stream" it is necessary to describe first of all the structure and mechanical functioning of the liver lobule of frogs, and then to show how the phagocytic von Kupffer cells which line the sinusoids of the lobule selectively remove coated foreign particles from the blood stream.

Controls and Background.

Our chief aim in this, as in previous studies, has been to keep living tissues and organs under as nearly normal conditions as possible, while their structure, functions and reactions are observed with microscopes. The findings in the liver were made possible by a long intensive preliminary and concurrent study of the structure and reactions of the peripheral vascular beds of other organs which for the most part present simpler anatomical and physiological problems than the liver (cf. KNISELY

1940). Living internal organs have been transilluminated for microscopic study by means of fused quartz rods (KNISELY 1934 a, 1936 a, 1937, and 1938). HOERR, 1944 a, has described some recent technical advances in this method.

One of the chief advantages of studying living organs with the microscope is that structures, functions and reactions can be studied simultaneously. One of the difficulties of such studies is that of determining which reactions of the blood, vessels and tissues are responses to anesthetics, hemorrhages and trauma, and which are responses to the integrative activities of the animal's own internal regulatory mechanisms.

Beginning in 1931 a variety of anesthetics and operative techniques have been rather carefully tested. To do this the circulating blood and the reactions of vessels in the surface areas of several species of animal have been compared with the blood and reactions of vessels in internal organs. One set of such tests was made using Amblystoma larvae. The blood and vessel walls of an area of the broad transparent tail fin of a specimen were studied before the animal was anesthetized. In this tail fin, vessels which are deep enough in the tissue to be beyond the reach of small local surface injuries can be studied. Then that animal was given an anesthetic to be tested and the same area studied again. Then that animal's body cavity was opened with an operative technique to be tested, and the same area of tail fin studied again. And then that animal's internal organs were studied. A variety of modifications of such tests, some of which were described by KNISELY, 1936 a, have made it possible to select anesthetics and to learn to do careful enough operations so that the vessel walls of internal organs and the circulating blood of operated animals show none of the reactions to injury described by E. R. and E. L. CLARK, 1935 and KNISELY, ELIOT and BLOCH, 1945. More than 1100 Amblystoma were used in these studies. (The drouth of 1934 dried up the ponds around Chicago and made it a simple matter to catch large numbers of Amblystoma larvae in the quarter, half and nearly full grown stages.)

In frogs, observations of the structural patterns of, circulatory conditions in, and some reactions of the fine vessel systems of skin, tongue, dorsal surface of the brain, peripheral nerves, smooth muscle of the gastro-intestinal tract, stomach mucosa, mesenteries, bladder, various striated muscles, lung, suprarenal gland, kidney and liver, have been made under a variety of nearly normal and experimental conditions. About 3500 frogs have been used in these studies. Motion pictures of the circulation in several frog organs, including kidneys (glomeruli), striated muscles and liver, have been taken through the microscope, thereby recording some of the observations made. In mice, rats, guinea pigs and kittens, circulatory conditions in small vessels of pia-arachnoid, mesenteries, smooth muscles of stomach and intestinal walls and of uterus (KNISELY 1934 b) and spleens (KNISELY, 1936 b and c), and livers have been studied. More than 500 such small mammals have been used in these studies. Some simple generalizations about the structure and activities of peripheral vascular beds, based on summaries of these observations, have been published (KNISELY, 1940). These

studies were necessary in order to test the tissue transillumination techniques and to learn how to study living internal organs with microscopes.

The fused quartz rod living-tissue transillumination technique and the operations which permit microscopic observations of several internal organs have now been developed far enough so that we can handle living tissues and organs delicately enough to keep them reasonably near to normal for many hours at a time. The whole animal and the tissues under observation can be kept near enough to normal so that

(1) the blood retains its normal fluidity (cf. KNISELY, ELIOT and BLOCH 1945, and KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH 1945).

(2) the linings of vessels exhibit none of the well-known reactions to injury (cf. E. R. and E. L. CLARK 1935), and

(3) many microscopic-sized parts of the vascular beds of organs contract or dilate, or perform other acts, in apparently well-coordinated integrated behavior patterns, and thereby carry out, or participate in carrying out, previously well-known functions of the organs (cf. KNISELY 1934 b and 1936 b and c).

The first two of these three points need further elaboration. Much of the rest of this paper may be taken as one demonstration of the third point.

Operations can now be performed with a minimum of blood loss and trauma to the animal. With a minimal blood loss the animal's vascular system does not exhibit (1) the unduly prolonged spasms of somatic arterioles, nor (2) the hepatic autotransfusion reaction, to be described later, nor (3) the wide separation of all the moving red cells due to blood dilution, which are early reactions to hemorrhage and preliminary stages of hemorrhagic shock. With a minimum of mechanical trauma to the animal (1) precipitates are not formed in the plasma of blood flowing past the sites of trauma and then carried around in flowing blood in sufficient amounts (a) to be seen with the microscope in circulating blood, nor (b) to slow the rate of flow of blood through tissues, and (2) precipitated coatings are not formed on the red blood cells flowing past the sites of trauma. These two reactions begin immediately as a result of trauma and are the first microscopically visible, initiating stages of traumatic shock (KNISELY, ELIOT and BLOCH 1945, KNISELY and BLOCH 1945).

In normal unanesthetized animals and men, the red cells have no precipitated coatings and do not tend to stick to each other and agglutinate. In Memphis, during the summer of 1941, BLOCH studied the blood circulating in the small blood vessels of the bulbar conjunctiva of fifty unanesthetized normal medical students and student nurses, using a Shahan opthalmoscopic lamp and a stereoscopic 48 and 96 × microscope. In none of them did the red cells show any tendency to agglutinate as coated red cells do. In uninfected, unoperated animals and men and in the most carefully prepared uninfected, operated animals even rouleaux formation does not take place (for early concepts of rouleaux formation see ROBIN 1858; DOGIEL 1879; WEBER and SUCHARD 1880; JOLLY 1909; and FÅHRAEUS 1929); the red cells exhibit no tendency to stick to each other in normal animals and men; most of them are separated from each other; all are freely suspended in the fluid plasma. This agrees with AREY 1918.

In perfectly normal animals and men the red cells seem to repel each other slightly, as they might if they all carried small similar electrostatic charges. (See ABRAMSON, MOYER and GORIN 1942, p. 307—318.)

Following slight mechanical trauma to a mammal, the red cells begin to form rouleaux. Upon more severe trauma, but still less than that sufficient to cause traumatic shock, the red cells begin to agglutinate mildly, giving the so-called "granular flow" (see KROGH 1929, p. 13). When frogs or Amblystoma are mechanically traumatized the blood plasma begins to increase in viscosity, becoming sirupy, and the red cells may or may not exhibit a true agglutination (KNISELY, ELIOT and BLOCH 1945).

Thus in completely normal animals and men, and in animals opened with a minimum of trauma, the blood is fluid and the red cells exhibit no tendency to stick to each other. Consequently, the consistency and behavior of the blood flowing past the microscope is a continuous indicator showing whether the original operative trauma of the experiment was sufficient, or whether continuation of the experiment has introduced trauma which has become cumulatively sufficient, to cause changes in the mechanical consistency of the circulating blood.

Living internal organs can now be transilluminated for many hours without damaging the tissues enough to cause the linings of the small blood vessels to begin to exhibit their well-known reactions to injury. E. R. and E. L. CLARK have had wide experience studying transparent portions of unoperated amphibians and tissues in transparent chambers inserted into various parts of mammals. (A list of most of the Clarks' early papers can be obtained from the bibliography of CLARK and CLARK, 1935, and CLARK, CLARK and REX, 1936.) Many of their observations have been carried out at very high magnifications. Some of those made in rabbit ear chambers have been carried on day after day for weeks or months in one area of one animal. In 1935 they published a precise, well illustrated account of the visible reactions of white blood cells to normal and injured endothelium. Contrary to many previously published accounts, the Clarks stated that white blood cells do not stick at all to normal endothelium. In their preparations when white cells touched normal endothelium they slid right along its inner surface exhibiting no tendency to adhere at any point. When a segment of endothelium was mildly injured the white blood cells coming along in the blood adhered temporarily to the injured area. They often adhered slightly and were rolled along while they adhered; or if the injury was more severe they stuck tightly in one place. Thus white cells accumulated on the injured endothelium. As the endothelium recovered from the mild injury the leucocytes let go and were swept down stream. If the endothelium was more severely injured the leucocytes stuck much more tightly and for longer times; consequently, the area became almost completely covered with leucocytes closely adjacent to each other. (See the CLARKS' 1935 figures 7, 8 and 10.)

Our observations on these points agree precisely with those of the Clarks. White cells exhibit no tendency to stick to normal endothelium. (See KNISELY, ELIOT and BLOCH, and KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH, and the motion picture

"Knowlesi Malaria in Monkeys"[1].) When endothelium is mildly injured intentionally, the temporary sticking of leucocytes does occur and the degree of sticking is proportional to the degree of injury. Thus the temporary sticking of leucocytes is the first visible evidence of the mildest degrees of injury to endothelium. The endothelium can easily recover from those degrees of injury which do not cause more severe response than the sticking of leucocytes. Thus when small blood vessels are being studied, the behavior of leucocytes toward the linings of those vessels which are under observation is a continuous precise indicator which tells whether the endothelium is normal or has begun to be mildly irritated. It is, of course, possible to injure tissues so severely that the endothelium of the small vessels becomes permeable to plasma proteins, or that the vessel walls sacculate, or that red cells begin to diapedese out through the walls, or so severely that hemorrhage occurs or that the walls disintegrate and multiple hemorrhages occur. (See the CLARKS, 1935, fig. 10). Any one of these increasing degrees of response to increasing degrees of injury may occur so rapidly after a severe injury that the advanced response to injury develops before there is time for the blood to bring in enough leucocytes to coat the inner surface of the vessel wall. Further, in those animals and men in which the blood has been changed to a thick precipitated and agglutinated sludge (KNISELY, STRATMAN-THOMAS and ELIOT 1941, The same and BLOCH 1945; and KNISELY and BLOCH 1942, 1945) the moving agglutinated masses of coated red cells may, but do not always, push white cells off from the walls of vessels to which they are somewhat adherent. (For a specific example see KNISELY, ELIOT and BLOCH 1945.) Hence, it is possible to have damaged vessel walls without having leucocytes present on the inner surface of the damaged endothelium. But when the vessel linings exhibit none of the well-known visible evidences of severe injury, and the blood is in its normally fluid state, the behavior of leucocytes toward the linings of those vessels which are under observation is a continuous precise indicator which tells whether the endothelium is normal or has begun to be mildly irritated.

At this point one might suspect that some one or more features of the quartz rod technique altered the tissues in such a way as to prevent the vessels from exhibiting their normal responses to injury. But this is not necessarily so, for whenever vessels under observation are accidentally or intentionally injured they go through the standard reactions to injury, described by the Clarks 1935, the degree of the response being roughly proportional to the severity of the injury.

In summation, the operative and transillumination techniques have now been developed far enough to make it possible to keep a number of internal organs near

[1] At several points in this paper there are references to scenes in the motion picture "Knowlesi Malaria in Monkeys". This 16 mm. Kodachrome motion picture, taken through the microscope, records several scenes of normal unagglutinated blood and normal vessel walls and then traces one set of factors of pathologic circulatory physiology through lethal stages. The picture was made to make it possible to demonstrate some of our findings to physicians and medical scientists. It usually takes about sixty minutes to project the film. Copies of this film will be loaned free, except for transportation charges, to medical schools, medical societies, medical officers of the military services and research groups. Requests should be sent either to Dr. M. H. KNISELY, Department of Anatomy, University of Chicago, or to Dr. T. S. ELIOT, Department of Anatomy, University of Tennessee, Memphis, Tenn.

enough to normal so that no visible responses of blood or vessels to injury are present. Yet the blood and vessel linings are capable of the standard responses to injury. And the mechanical condition of the flowing blood together with the prolonged spasms of vessels which follow hemorrhage and the reactions of the leucocytes to the vessel walls provide continuous precise indicators which show if one has unknowingly permitted or caused experimental hemorrhage or trauma.

In these control studies and those of the liver which follow, magnifications from 32 × to 600 × have been used. Water immersion objectives make the higher magnifications possible. Bi-objective, binocular Leitz microscopes have been used to obtain true stereoscopic vision. They have 4, 6, and 12 × objectives and 8 × oculars and therefore give 32, 48 and 96 diameter magnifications. As is well, but not widely known, nor always remembered, bi-objective truly stereoscopic microscopes permit much more accurate perception of detail at the available magnifications than do monobjective microscopes of corresponding magnifications (cf. RAYTON 1944, p. 747).

The experiments described in the accounts which follow have been done upon frogs in which the brains were pithed and the cranial cavity immediately plugged with a wooden pin to prevent blood loss, and upon frogs which were kept under light urethane anesthesia. Most of them were opened with a cautery to prevent hemorrhage. Operations to be described later have been developed which make it possible to study circulation in several different organs in a single animal. One can move the prepared frog about and study circulation in one organ, then another, as easily as one moves a large slide around under the microscope.

The Problems.

The original purpose of the observations and experiments herein reported was to begin to determine exactly how one living phagocyte of spleen, bone marrow, liver, pituitary or adrenal gland removes one microscopically visible foreign particle from the circulating blood. But as the investigations proceeded it became apparent that the problem of how one phagocyte ingests one particle is but a part of a group of problems. Hence as the experiments progressed our purposes changed. Now the over-all purposes are to find out what anatomical, physiological, immunological, chemical and physical factors determine and limit the rates at which the single ingestion reactions occur. What factors determine that a particle will be ingested? What factors determine that it will not be? What factors prolong the period before it is ingested? What anatomical, physiological, immunological, therapeutic, chemical and physical conditions are simultaneously necessary to ensure the most rapid possible rates of selective phagocytosis?

In the spleen, liver and bone marrow of mammals there are enormous numbers of phagocytic cells which can remove particles from blood; by comparison there are only a few in the pituitary and adrenal glands. (The phagocytes of lung presumably are outside the vessel walls.) The method employed in this study has consisted of

transilluminating living internal organs of frogs *in situ* with fused quartz rods and studying particles of injected foreign materials, the blood and the vessel walls, with microscopes,

(a) at the moment the particles come in contact with flowing blood;

(b) while they circulate through organs whose vessels are lined with ordinary endothelium;

(c) while the particles pass into the sinusoids of the liver and come in contact with the phagocytic, sinusoid-lining von Kupffer cells. The conditions of these experiments have been varied, and accessory experiments performed to begin to analyze some of the processes observed.

An understanding of how one particle is removed by one living phagocyte, that is, how the single units taking part in this reaction behave, is necessary in order to be able to think with precision, inductively, about problems involving multiples of these single ingestion reactions.

Curiosity about a number of problems prompts one to want to determine how one phagocyte of liver, spleen or bone marrow removes one particle from the blood. By means of what biological, chemical or physical mechanisms does the phagocyte remove and ingest one particle? How does the phagocyte determine which particle to ingest and which to ignore? For example, these phagocytes ingest many kinds of inert foreign particulate matter and of bacteria from the blood stream, but not the animal's own normal white or red blood cells. How does the phagocyte distinguish between foreign particles or bacteria and the animal's own blood cells? Further, according to current concepts (see Löwit 1889; von Kupffer 1899; Hektoen 1906; Kyes 1914, 1915; Cary 1915; McNee 1923; Rous 1923; Dick 1925; and Higgins and Murphy 1928) these phagocytes ingest the animal's own "old" or "worn-out" or "damaged" red blood cells, but not its normal red cells. How does the phagocyte distinguish between old, worn-out or damaged red cells and normal ones? How does the phagocyte "select" what it takes?

But these phagocytes do not always distinguish accurately between those particles which, for the benefit of the animal, should be left in the blood stream and those which should be removed. For instance, Taliaferro and Cannon (1936) found in white throated monkeys infected with Plasmodium brasilianum malaria that during the earlier phases of the disease the phagocytes of spleen and liver ingest only those erythrocytes which contain parasites, but later on, during the "crisis", or during a super-infection they also take up unparasitized, apparently normal, red cells, sometimes in great numbers. How do these phagocytes at first distinguish between the red cells which contain parasites and those which do not? Why, through failure of what mechanisms, or because of what new mechanisms do they later lose their power to distinguish between parasitized and unparasitized red cells? Why do these phagocytes make mistakes?

Again, the phagocytes of spleen, bone marrow and liver sometimes fail to take up such particulate matter as pathogenic organisms which, for the benefit of the animal

should be ingested. As a specific example, PFEIFFER and TATUM, 1935, found in histological sections from untreated rats whose circulating blood was swarming with trypanosomes that the blood and blood sinuses of spleen and liver contained myriads of trypanosomes, but the phagocytic cells were, in their words, "practically devoid" of trypanosome nuclei. Why do these phagocytes fail to take up some kinds of noxious particles?

At times these phagocytes seem to fail or refuse to take up particles of the same material which, just previously, they have been ingesting. We say then that the phagocytes have been "overwhelmed", or that they have been "blockaded". But these are merely evasive, soothing words; they do not explain why the ingestion mechanisms fail.

Furthermore, the process of ingestion of particulate matter can, in some cases at least, be affected by therapeutic agents. When PFEIFFER and TATUM, 1935, treated rats whose circulating blood contained an astronomical number of trypanosomes (one for every 20 of the animals' red cells) with five mgm. of Mapharsen per kilogram, the trypanosomes disappeared from the circulating blood in from fourteen to fifteen minutes. Sections of liver and spleen taken from ten to fifteen minutes after administration of the Mapharsen showed the von Kupffer cells and spleen phagocytes to be engorged with trypanosomal nuclei, and the blood and blood sinuses of these organs to be almost entirely free of trypanosomes. Note the brief intervals from the administration of the Mapharsen until (a) trypanosomes were inside phagocytes and (b) the circulating blood was almost free of trypanosomes. This shows the astounding rates at which selective phagocytosis can proceed under apparently optimal conditions. As previously noted, one of our main problems is to determine the anatomical, physiological, physical and chemical conditions which are simultaneously necessary for the most rapid possible rates of selective phagocytosis. What did the Mapharsen do to cause the trypanosomes to be phagocytized? How do the currently available drugs affect the phagocytic mechanisms? What combination of specific effects do we want one or more drugs to produce in order (a) to accelerate the rates of selective phagocytosis and then (b) to maintain the maximum possible rates?

Can we learn enough about the mechanisms of selective phagocytosis, and the effects of therapeutic agents on the mechanisms, so that ultimately we can control the selective phagocytosis of particulate matter from the blood stream?

In summation, stated in biological terminology the problems are:

1. What mechanisms determine whether or not a particle suspended in the circulating blood is removed and ingested by a spleen, bone marrow or liver phagocyte? What mechanisms determine the selectivity of the selective phagocytic removal of particulate matter from the blood stream? Under any set of defined conditions what mechanisms determine the rates of selective phagocytosis from the blood stream?
2. How do the selective phagocytic mechanisms work?
3. Why do they make mistakes?
4. How do they fail?
5. How can these mechanisms be controlled?

Reflections about the Problems.

There are, of course, no valid *a priori* reasons for assuming that the mechanisms and processes involved in the selective removal of particulate matter from the blood stream are alike in the liver, bone marrow and spleen. In fact, there is enough evidence available so that it seems reasonably probable that selective phagocytosis has a number of factors in common in these organs but that in each organ there are anatomical and/or physiological factors specific to that organ.

Further, in immune animals the mechanisms of selective phagocytosis are not *a priori* necessarily the same, organ for organ, as they are in non-immune animals. Perhaps the rates, at least, at which these phagocytes accumulate particulate matter can be markedly accelerated as a part of the development of some specific immunities. For example, TALIAFERRO and CANNON (1936) and TALIAFERRO and MULLIGAN (1937), presented histological evidence from which they deduced that in monkeys with malaria the rates at which the phagocytes of the spleen accumulate and digest parasitized red cells are sharply accelerated as the monkey's "immune response" to the malarial parasites develops.

In thinking over these problems it seems obvious that a particle must come in contact with a phagocyte before it can enter that phagocyte. (Cf. FENN 1921 a, b, c, and MUDD, MCCUTCHEON and LUCKE 1934.) Which goes to which? As far as we now know, during the time when they are actually removing particulate matter from the blood the phagocytes of the spleen, liver and bone marrow are constituent parts of the organs they occupy. These phagocytes certainly are not at this time free white cells in the circulating blood. Some of them do, of course, move about, at least in local tissue areas during their development, or while ingesting particulate matter or after having ingested particulate matter (KNISELY 1936c, saw phagocytes in dying spleens moving about ingesting apparently normal red cells). But during the actual removal of particles from the blood, these phagocytes are a part of the organs in which they are located. Consequently, a trustworthy key fact is that these particular phagocytes cannot touch, and therefore cannot ingest, any particle from the blood until by chance that particle is carried into one of the blood vessels supplying the spleen, bone marrow or liver. If a particle suspended in the blood and one of these phagocytes are to come in contact with each other, the circulation must carry the particle to the phagocyte or to the general histological neighborhood in which the phagocyte is at that moment located.

There is good histological evidence that in the spleens of some species, at least under some conditions, foreign particles can be selectively separated from the circulating blood and selectively localized in the neighborhood of the phagocytes before being ingested by them. Hence to understand the selectivity of selective phagocytosis from the blood stream, we must be ready to recognize and analyse selective localization of particles preparatory to the selective ingestion of them.

As examples of selective localization before ingestion, the experiments of KYES 1916, and of TALIAFERRO and CANNON 1936, may be cited.

KYES injected pneumococci into the veins of pigeons, took histological sections at carefully timed intervals, and found great numbers of pneumococci within the walls and selectively localized along the outside of the walls of the arterioles emerging from the splenic follicles. This occurred some time before large numbers of pneumococci were inside phagocytes. After careful study of the structure of these specialized arterioles, the pneumococci in their walls, and the extravascular position of great numbers of the selectively localized pneumococci, KYES concluded:

"Surveyed from the point of view of function this distribution of the pneumococci within the spleen after various intervals leads to the conclusion (1) that pneumococci which enter the spleen by way of its closed vascular system rapidly leave this system, being transported into, and eventually through, the loose vascular wall of certain modified arterioles whose interstices allow the free outward passage of plasma but not that of the formed elements of the blood; (2) that the interstices between the cells constituting the great bulk of the vascular wall in question are of such minute size as to allow but a retarded transportation of pneumococci through them, the tissue thus operating as a partial filter accumulating the organisms within its area; and (3) that having thus gradually been washed through the vessel wall, the pneumococci are brought into contact with the hemophages[1] of the pulp cords and are ingested by these and digested as in the liver. Thus vast numbers of pneumococci are rapidly filtered from the plasma by a mechanism peculiar to the spleen, to be ultimately ingested and destroyed, however, by the same kind of phagocytic cell that we find in the liver."

TALIAFERRO and CANNON, 1936, deduced from histological sections of spleens taken from monkeys with malaria that parasitized red cells were selectively localized and concentrated in the spleen, perhaps for some time before being ingested by its phagocytes.

KYES accepted a concept of a closed circulation in spleen; TALIAFERRO and CANNON accepted a concept of an open one, saying: "This finding indicates that normal and infected cells enter the cords from the artery, but that only normal cells can pass into the venous sinuses. It seems probable that the parasitized red cells are held in the cords by some affinity with macrophages. . ."

According to the concepts of "open" splenic circulation the places in the spleen where particles are selectively localized before being ingested are passages through which whole blood flows. According to the closed circulation concepts, these spaces are extravascular tissue spaces. One strong resistance to the general acceptance of a closed splenic circulation has been the inability of some to see how a particle in the circulating blood could selectively pass through a "morphologically intact" vessel wall, this in spite of the fact that among morphologists it is common experience to find materials which have been injected into the vascular system outside the vessels in perivascular and connective tissue phagocytes almost all over the body (cf. FIELD

[1] The term 'hemophage' was used by Professor KYES to designate the phagocytes which ingest and destroy red blood cells. The term emphasizes this function of a large group of phagocytic cells.

and DRINKER 1936 who found by direct microscopic observation in living animals that some kinds of injected visible particles pass outward rapidly through intact normal ordinary vascular endothelium).

Hence, important problems which must be solved in order to understand selective phagocytosis from the blood stream (as well as to understand the microscopic structure and functioning of the spleen) are:

(1) By what mechanisms are particles selectively separated from the circulating blood?

(2) By what mechanisms are they selectively localized?

(3) By what mechanisms selectively phagocytized?

In spleen, liver and in bone marrow does the ultimate selective ingestion depend upon but one, or upon some combination of these three steps?

In summation then:

1. There are no valid *a priori* reasons for assuming that the mechanisms which determine the selectivity of selective phagocytosis from the blood are identical in spleen, bone marrow and liver.

2. In spleen, under some conditions at least, phagocytizable foreign particles are selectively separated from circulating blood before being selectively ingested.

3. The rates at which the phagocytes of spleen (a) accumulate and (b) digest red cells containing malaria parasites may accelerate as the animal's "immune response" develops.

This last point forces one to face the fact that there are no valid *a priori* reasons for assuming that the mechanisms remain constant during the course of a disease. And this in turn leads one to recognize that the mechanisms might not remain constant during different phases of normal physiology. Consequently, we can now ask, "How do the mechanisms of selective removal of foreign particles from the blood vary in spleen, liver and in bone marrow with different phases of physiological or immunological conditions or of an overwhelming infection?"

Preliminary Subdivision of the Problems.

At this point it becomes necessary to devise a method for subdividing the problems of selective phagocytosis into their component parts. Probably we can resolve any one of these biological problems into its anatomical, physiological, physical and chemical components by asking the following four basic questions about it:

1. What forces guide and bring the particle to the immediate neighborhood of the phagocyte? (And by extension, what factors determine the rates at which particles are brought to stationary phagocytes?)

2. What forces bring the particle to the individual phagocyte which ultimately ingests it?

3. What forces ensure that the particle and phagocyte actually touch each other?

4. After they are in contact with each other, what forces determine whether or not the particle enters the phagocyte?

The exact answer to each of these four questions may well be different from organ to organ, from species to species, in non-immune animals, and in animals with specific immunities, in untreated and in treated animals. The answers may vary with various common normal physiological states of the animal. The answers to these questions may change during the course of a disease as the reactions of parts of the body during the disease accelerate, interfere with, retard, or change the nature of the common physiological and/or biochemical processes of the animal. Consequently, one cannot hope to learn all about selective phagocytosis of particles from circulating blood simply by studying the mechanisms and processes by which it is accomplished in one organ of one species of animal under one set of physiological, immunological and experimental conditions. The answers to these four questions will have to be known for each organ and perhaps for each kind of particle under each set of specific conditions. Our ultimate concepts will have to be built up by the accumulation of observations and analyses of the mechanisms operating under many different carefully defined conditions.

Because the processes are undoubtedly complicated it has seemed wise to proceed by induction; study selective phagocytosis first in the simplest place under the simplest conditions possible, and thereby (1) learn how to study such processes, (2) lay a groundwork of observations and deductions against which to compare and contrast subsequent observations and deductions made during studies in the same and other organs, of other species, under similar and other, more complicated conditions, and (3) develop a group of testable hypotheses about the mechanisms and processes involved so that the analysis of mechanisms can proceed by several different sets of experimental techniques.

Materials and Methods.

Living frog liver seemed to be the simplest and easiest place to begin this type of study because:

1. Drinker and Shaw, 1921, found by quantitative methods that the liver was the main organ concerned with removing particulate matter from the blood stream.

2. The thinner parts, edges of frog liver are easily transilluminated for microscopic study. (Cf. Knisely 1939 and Bloch 1940.)

3. Its microscopic parts are comparatively large, and therefore easily studied. The capillaries and sinusoids of each species of animal are wide enough during the flow of blood so that the animal's own red cells pass through easily (Krogh 1929, p. 11, Knisely 1940, and Knisely, Eliot and Bloch 1945). Frog red cells, being flat ellipsoids, have three major axes; they are about 21 or 22 micra long, 14 or 15 micra wide, and 4 to 6 micra thick. Thus the width of the frog's red cell is about

twice the diameter of the red cell of ordinary laboratory mammals, and the frog's hepatic sinusoids are, during the flow of blood, much wider than those of small mammals. The microscopic structures of which the frog liver lobule is composed are built roughly on a correspondingly large scale, consequently, at each magnification one uses, more detail can be seen than in the livers of small mammals.

4. Because frogs are poikilothermic, not as precise control of the temperature of exposed frog liver is necessary as of mammalian livers. This was determined by experiment.

5. Living frog livers are much easier to hold still for study at high magnification than are mammalian livers. In many species of animal, the heart and liver press hard enough on, or are bound tightly enough to, intervening structures so that each heart beat moves the liver. In some frogs the heart beat moves the liver quite a bit, in others but little, in others not at all. In mammals the respiratory motions move the liver continuously. Frogs lightly anesthetized with urethane respire by swallowing air occasionally. Each swallow dilates the lungs and thereby moves the liver. By increasing the depth of anesthesia the frog can be made to respire so infrequently that the occasional respiratory gulps cause little difficulty.

6. In attempting to keep these first experiments on selective phagocytosis as simple as possible it seemed wise to try to a avoid having them complicated by special immune reactions which might occur in mammals. The reactions of frogs which constitute "immunity" are not yet known to be as specialized or as highly developed as those of mammals. Hence, the reactions of frog blood and of frog von Kupffer cells to the first injection of relatively simple non-antigenic test substances were chosen for study.

Two species of frog have been used. In Copenhagen Rana esculenta, excellent, well-fed, healthy ones which Professor KROGH very kindly imported directly from Austria especially for these studies; in Chicago and Memphis, well-fed, healthy Rana pipiens. When undernourished or starved animals have been used it was done knowingly; in some experiments intentionally. Diseased animals, and those having recognizable parasites, or microscopically recognizable tissue reactions to parasites, or tumors, have been discarded. Anything which was first learned in such animals has been repeatedly rechecked in animals in which we cannot, in our present state of ignorance, detect any abnormality. In the frogs used in this study:

(1) The circulating red cells were not agglutinated. Each was free from the others, and they tended to repel each other slightly (cf. AREY 1918 and ABRAMSON, MOYER and GORIN 1942, pp. 307—319.

(2) No white cells were sticking to the inner surfaces of the walls of small vessels. This agrees with and extends to frogs the observations of E. R. and E. L. CLARK 1935. The inner surfaces of the linings of uninjured small vessels were smooth and clean.

(3) The flow of the unagglutinated blood was laminar or streamlined (cf. POISEULLE 1841, fig. 6).

(4) The walls of small vessels lined with ordinary vascular endothelium did not

leak appreciable amounts of fluid, for no visible hemoconcentration of red cells passing through capillaries and post-capillary venules was occurring (cf. KROGH 1929, p. 14).

(5) The blood flowed so rapidly in most arterioles and venules which were from 60 to 120 micra in diameter that individual red cells could not be seen.

These five characteristics of normal blood and vessel walls have been presented in more detail by KNISELY, ELIOT and BLOCH 1945, and are illustrated in colored motion pictures taken through the microscope in monkey omentum in the Knowlesi Malaria film. This film is introduced by a paper by KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH 1945. Thus the concepts to be presented in this paper have been derived from studies of frogs which were as nearly normal as we as yet know how to select (cf. KNISELY, BLOCH and WARNER, 1946).

Attempts to see how one frog liver phagocyte removes one particle from the blood began by watching only in the liver where the particles come in contact with the phagocytes. But it soon became evident that a most significant step in this particular phagocytic process, namely the coating of the particle to be engulfed, by a visible precipitate derived from the blood, was occurring before the particle entered the liver. Hence particles were watched farther and farther upstream, searching for the points at which the coating of precipitate began to be visible, and it was found that some kinds of particles receive a coating of visible precipitate the moment they come in contact with the blood.

Hence it became necessary to prepare frogs so that one can:

1. Insert a minute injection needle into a vein, transilluminate the vein at that point, focus the microscope on the tip of the needle in the vein, and study the reaction between particle and blood, beginning the moment the injected material comes in contact with the blood.

2. Study the behavior of the blood, particles in it, and the vessel walls in various organs whose vessels are lined with ordinary vascular endothelium.

3. Study the actual removal of particles from the blood by the sinusoid-lining von Kupffer cells in the liver.

The preparation to be described was designed to make it possible to study in all these places in one frog. Because it has proved to be useful for many purposes, it will be described in some detail.

It was found that the animal had to be prepared in such a way as to satisfy the following seven conditions:

1. The whole operation must be made without losing any blood. The microscopic anatomical apparatus, to be described later, which controls the flow of blood through the sinusoids of the liver lobules, responds to hemorrhage from any part of the animal by forcibly discharging the blood contained in the sinusoids into the central veins and thence of course to the general circulation, and then shutting off almost all the flow into the liver lobules. Medium-sized Rana esculenta contain but four to six cubic centimeters of blood; Rana pipiens about two to four, and their

hepatic "autotransfusion apparatus", to be described later, begins to respond to loss of even minute amounts of blood. After it has responded maximally, very little blood comes into the liver. Consequently, if one wishes to study the phagocytic removal of particulate matter from blood flowing into or through the liver sinusoids, it is imperative that opening the frog be done with the minimum possible blood loss.

2. The operation must be made with the minimum amount of trauma in order to minimize and if possible prevent the formation of precipitates in the plasma of blood flowing through the small vessels adjacent to traumatized tissues and/or increases in the viscosity of the frog's blood plasma. (See KNISELY, ELIOT and BLOCH 1945.)

3. The injection apparatus must enable one to inject very small amounts of material slowly, sometimes into one of the roots of the portal vein, sometimes into the general circulation. The injection must be made very slowly so that the injected material leaves the needle tip orifice in droplets or particles small enough so that each cani be carried by the circulating blood down through the long, narrow cone-shaped arterioles into the narrow cylindrical capillaries or hepatic sinusoids (cf. DRINKER and SHAW, 1921).

4. It must be possible to move the prepared animal about so that one can study what is going on in one organ and then another, without disturbing any part of the animal. It is convenient to be able to leave the injection needle in the vein while the preparation is moved or studied, so that a new injection can be made at any time.

5. It must be possible to keep the animal quite still for long periods to make observations at the higher magnifications.

6. It is desirable to be able to keep each animal in good condition for long periods for repeated experiments or prolonged observations in one animal.

7. It is desirable to be able to prepare an animal quickly so that, when one wishes, many experiments can be made in a day.

To satisfy these requirements the frogs are prepared as follows:

For anesthesia, the animals brain may be pithed and the cranial cavity plugged immediately with a wooden pin to prevent blood loss. Or just enough 25 % urethane in water may be injected into the animal's dorsal lymph sac to anesthetize him deeply enough to permit the initial operation. For an average sized pipiens about 0.5 cc. is enough. After the animal is placed on the observation board his skin is kept moist or wet by pads of wet absorbent cotton. Usually a small trickle of plain water (never chlorinated tap water) is continuously supplied to the cotton to keep it wet. The overflow from this steadily washes the animal's sides, back and hind legs, diluting the Ringer's solution escaping from the body cavity, and prevents the skin from being subjected to undiluted Ringer's solution. (Ringer's solution on amphibian's skin dehydrates the animal. See KNISELY 1936a for more details.) Under these conditions a frog continuously absorbs water through his skin, and excretes dilute urine containing urethane through his kidneys. Hence by controlling the degree of moistness of the skin one controls the rate of water uptake and of urethane excretion, and hence the depth and duration of the urethane anesthesia. Many animals have been studied

while so lightly anesthetized that they made frequent regular respiratory motions. When an animal excretes enough urethane so that his respiratory motions become so rapid as to interfere with observations, a small additional dose of urethane (from 0.05 to 0.1 cc.) is injected into a subcutaneous lymph sac to compensate for that excreted.

The frogs must be, and can be, opened without losing any blood at all. Some have been opened with a cautery, some by cutting between clamped mosquito hemostats and then cauterizing the edges of the tissue before removing the hemostats, some by cutting with scissors and cauterizing the cut edges immediately. Knives are never used, for cuts made with knives leave many ends of small blood vessels open. Scissors crimp the edges of the tissue as they cut, especially if one cuts slowly, leaving the cut ends of most small vessels sealed. Most of them stay sealed long enough so that no blood is lost from them before the edges of the cut can be permanently sealed by cautery. It is easy to see that this is true by following the steps of an operation with a quartz rod illuminator and a low magnification stereoscopic microscope. Iridectomy scissors and forceps are most convenient for making these operations. No more tissue is cauterized than is absolutely necessary. The tissues being cauterized are carefully raised a little with a small forceps to prevent heat from the cautery, or steam from the cauterized tissues, from reaching the underlying organs. A quick stroke with a red hot iron seals the vessels tighter and burns far less tissue than longer cauterizing with a cooler iron. Care, of course, is taken to keep the zones of partially crushed and/or partially burned tissue as narrow as possible to minimize the volume of tissue releasing sludge initiator substances into the circulating blood (cf. KNISELY, ELIOT and BLOCH 1945).

The handle of an ordinary three-cornered laboratory file makes the best cautery we have used. The tip of the handle is ground down to a rounded blunt point and heated in a Bunsen burner. Being 4 or 5 millimeters thick the iron retains enough heat to cauterize a centimeter or two of incision edge before needing reheating. The cautery is rubbed from time to time on a piece of emery cloth to get rid of flakes of iron oxide which otherwise cling to the edge of the wound. Two or three of these cauteries are kept heating so that one can cut and cauterize uninterruptedly. We have tested a number of electrically heated cauteries and "electric knives", but we cannot yet be certain that these do not stimulate the animal in such a way as to cause reactions of small vessels or inhibit the reactions of vessels in distant parts of the animal. They offer but little if any technical advantage in cauterizing. We have thus far preferred not to use them. The iron cautery must be kept good and hot for as it cools it begins to stick to the tissues, and if one pulls it away when it is sticking, small vessels are torn and blood is lost.

The frogs are always opened in two separate steps. First, the skin is cut and turned back, then the body musculature. Attempts to save time by cutting both layers at once frequently lead to incomplete cauterization and subsequent hemorrhage. During the operation the frog is kept on an absorbent paper handkerchief or a piece

of filter paper. If blood is lost outside the animal, the white absorbent paper is certain to show a pink stain, and the animal can be discarded. When an operator is in practice, animals can be opened without leaving any blood stain on the paper. When an operated animal is first taken to the quartz rod illuminator a quick careful survey with a lowpowered dissecting microscope discloses any microscopic hemorrhages from the cut skin or body wall. (Cf. KNISELY, ELIOT and BLOCH 1945.) If there are small oozes they can be cauterized. If the microscope shows that blood has been lost into the body cavity, the animal is discarded.

The frogs are usually opened in such a way as to expose the left half of the liver, a part of the left lung, the stomach, parts of the intestines and their mesenteries, the urinary bladder, sometimes the left kidney, sometimes some striated muscle (usually a part of the intact right rectus abdominis and/or the submaxillares) (cf. ECKER and WIEDERSHEIM 1896, p. 136), and if injections are to be made, a length of the inner surface of the anterior abdominal vein.

In the frog the anterior abdominal vein (the V. abdominalis of ECKER and WIEDERSHEIM 1896, p. 410) runs just inside the body wall, in the midline in a groove between the two rectus abdominis muscles. Sometimes it is attached to the anterior abdominal wall by a very narrow ventral mesentery, sometimes it lies half in and half bulging out of the groove between the two rectus abdominis muscles. It is, of course, covered by peritoneum, which is very thin and transparent. Its anterior end leads into the portal vein; its posterior end is "Y" shaped, each branch of the "Y" is a ramus abdominalis of the corresponding femoral vein and as such is an extension of the femoral vein from a hind leg which also connects via the external iliac vein to the renal portal system of the corresponding side (see ECKER and WIEDERSHEIM 1896, p. 410 and fig. 126 for more details). Injections made forward into the anterior abdominal vein go directly to the portal vein and the liver. Injections made while one obstructs the vein anterior to the needle go backward, then laterally through the abdominal branches of the femorals and then through the external iliac veins to the renal portal system, and through the wide sinusoidal capillaries (which are interdigitated with and supply the tubules of the frog nephrons), thence into the renal collecting venules and thence to the vena cava. Thus the anterior abdominal vein is a convenient place to inject, for after placing one needle it is possible to inject either anteriorward into the portal vein or backward to the general circulation, and at will one can change the direction of the injection during any experiment.

To expose all these parts, the animal is laid on his back on a sheet of filter paper and the skin of his abdomen is opened with an incision shaped like a capital "L". The long arm of the "L" is cut parallel to and four or five millimeters to the right of the animal's ventral midline. (See fig. 1.) This exposes a part of the right rectus abdominis and a bluish midline under which lies the anterior abdominal vein. (See fig. 2.) This incision extends from about the level of the tip of the sternum, back nearly to the symphysis pubis. Thus its posterior end is just back of the level of the posterior end of the mesonephros. The shorter arm of the "L" is then led from the

posterior end of the long incision, at a right angle, to the left around the animal's body, to but not through the left lateral border of the dorsal lymph sac. This avoids the left Vena cutanea magna (see Fig. 2 and cf. ECKER and WIEDERSHEIM 1896, p. 399) which tears easily and from which hemorrhage is hard to control. The lateral end of this incision is close to the animal's back, and ensures, after the musculature is similarly opened and the animal is on his back, that Ringer's solution flowing over the exposed organs escapes freely, and does not accumulate in the body cavity (which is important). Next, a short incision through the skin is led from the anterior end of the long incision to the left about to the posterior border of the foreleg. The trapezoidal flap thus formed is reflected upward and to the left. (Fig. 2.)

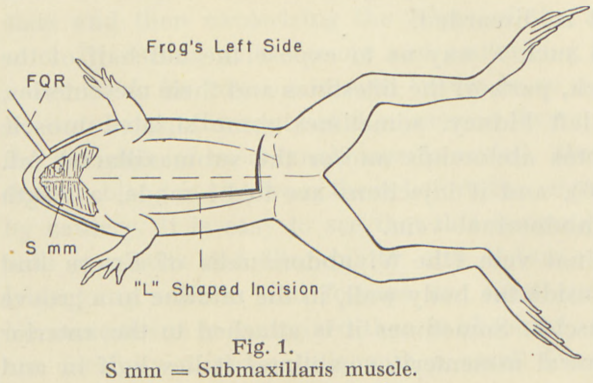

Fig. 1.
S mm = Submaxillaris muscle.
FQR = Fused quartz rod.

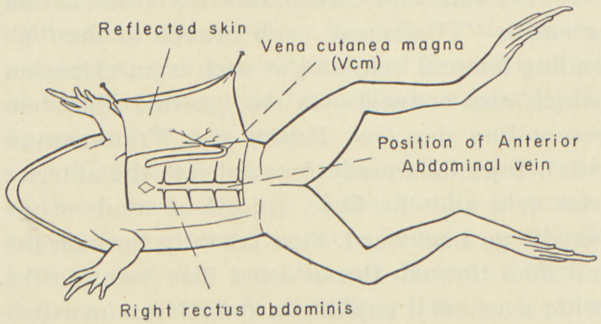

Fig. 2.

The animal's body musculature is then opened with an L-shaped incision; the long paramidline arm of which is made 2—3 mm. to the left of the midline, in order to leave the anterior abdominal vein intact. (Fig. 3.) This leaves a strip of the left rectus abdominis muscle 2—3 mm. wide on the left side of the anterior abdominal vein. If this is done carefully the anterior abdominal vein is not injured. With practice the incision can be made without stimulating the vein to contract. The first cut into the musculature is made into the middle of a segment of the left rectus abdominis. Here there are only small vessels which do not bleed easily. On the first cut the tendinous inscriptions are avoided. The vessels in them are cauterized before being cut with the scissors. Care must be taken not to run the long incision too far backwards lest one injures the ventral mesentery of the frog's bladder or the bladder itself. When the long incision is finished, a short incision is led from its posterior end to the left, again avoiding the Vena cutanea magna, nearly as far laterally as the corresponding skin incision had gone. The triangular flap of musculature on the left side, containing the Vena cutanea magna, is then turned back, exposing a part of the left lung, stomach, parts of the intestine and their mesenteries, the urinary bladder, and nearly half of the liver. (Fig. 3.)

Two or three fine sharp hooks made from insect pins are now inserted into

the narrow strip of rectus abdominis on the left side of the anterior abdominal vein. By gently lifting on these hooks and simultaneously pulling from left to right, a long stretch of the anterior abdominal vein is everted to the left, exposing its thin peritoneum-covered surface so that the vein can be transilluminated for microscopic study of the reactions which go on inside it while injections are made into it. (See fig. 4.) During the operation the vein itself is not touched with fingers or instruments. If all this is done carefully there is little interference with the blood flow through the anterior abdominal vein. Blood continues to flow at normal rates through the small vessels of the right rectus abdominis muscle, and drains by way of veins running medially in the tendinous inscriptions of the right rectus into the anterior abdominal vein. With good care the operation can be done with so little injury to the anterior abdominal vein that white cells do not begin to adhere to the inner surface of its lining. (Cf. E. R. and E. L. Clark 1935 and Knisely, Eliot and Bloch 1945.)

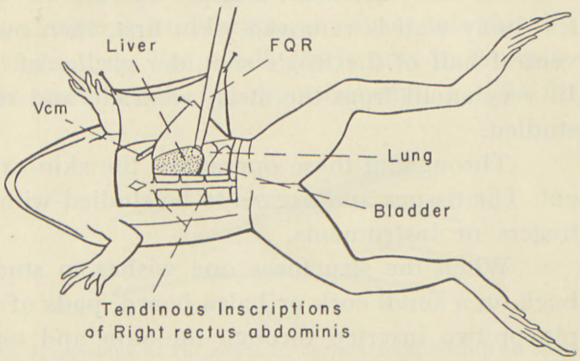

Fig. 3.

A part of the right rectus abdominis muscle has been exposed for study by having the first long paramidline skin incision four or five millimeters to the right of the midline.

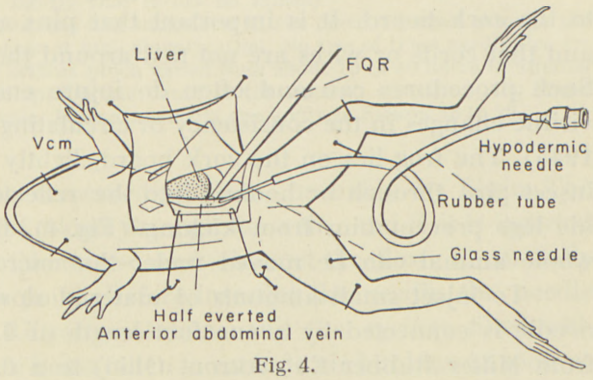

Fig. 4.

To study the submaxillaris muscles, the skin over the floor of the mouth is removed and the tip of the fused quartz rod put in the animal's mouth. See fig. 1. To study small toe muscles (for detailed anatomy see Ecker und Wiedersheim 1896, pp. 195—222), the skin over one or more of the toes of a hind foot is removed. Frog skin is not held down by strands of connective tissue; there are wide subcutaneous lymph spaces between skin and muscle, hence, the skin can be removed without injuring any of the underlying striated muscle fibers or the small vessels which nourish them.

To study the kidney the frog is prepared just as for study of the liver, with or without everting the anterior abdominal vein. A small retractor, about twice or three times the length of the nail of one's little finger, is made of soft annealed copper wire, coated with paraffin, and with this the stomach, liver and left lung are gently pulled to the right, exposing the left kidney. The best procedure consists in lifting the frog

by his left front foot and left hind foot so that his viscera fall into the right side of the body by gravity. After this, the retractors are put in place. The retracted tissues are not crushed if this is done carefully. The retractors we make have a small loop on the "handle end"; a pin through this into the frog board keeps the retractor in place, and thus the stomach, lung and liver retracted. With this preparation a living frog kidney can be studied for hours at a time.

To study the heart a larger operation is necessary. The anterior part of the ventral body wall is removed, skin first, then musculature, and the medial parts of the ventral half of the frog's shoulder girdle (cf. Ecker und Wiedersheim 1896, p. 60). In very small frogs the atria, ventricle and conus arteriosus can be illuminated and studied.

Throughout these operations the skin or muscle is cauterized after each scissor cut. The tissues and organs to be studied with the microscope are never touched with fingers or instruments.

When the structures one wishes to study are exposed the frog is laid on his back on a small cork or balsa board, pads of wet cotton placed on him, and an insect pin or two inserted through the skin and muscle flaps to keep the incisions open. (Figs. 2, 3 and 4.) The small retractor hooks made from sharp insect pins keep the anterior abdominal vein everted (Fig. 4). Other than this, the animal is not fastened to the cork board. It is important that pins are not put through the animal's legs, and that cords or wires are not tied around the extremities to hold the animal down. Such procedures can and often do injure enough tissue to initiate microscopically visible changes in the consistency of circulating blood (cf. Knisely, Eliot and Bloch 1945). The frog lies on the cork board, lightly held down only by three or four thin insect pins through or hooked into the reflected flaps of tissue. Pins placed beside his legs prevent him from kicking (Fig. 4). By moving the cork board about, the whole animal can be moved under the microscope like a large slide.

To inject small amounts of material slowly into the blood stream a fine glass needle is connected by a one foot length of 0.5 mm. bore rubber tubing (obtained from Miller Rubber Co., Akron, Ohio) to a 0.5 or 1 cc. tuberculin syringe, driven by a micrometer screw. The glass needle, having an outlet orifice of from 10 to 50 micra in diameter is inserted into the frog's anterior abdominal vein, the tip pointing toward the animal's head (see fig. 4). The tuberculin syringe and micrometer screw are mounted as a unit on a heavy but movable stand some five or six inches from the tip of the tissue illuminator. The one-foot length of fine rubber tubing easily permits the prepared frog to be moved about under the microscope without pulling the glass needle out of the vein, while the screw-operated syringe remains on its stationary stand.

The needle can easily be inserted in the vein freehanded, that is, without the use of a micromanipulator, if it be done while watching through the stereoscopic microscope at say 32 × magnification. The needle is inserted in the vein as follows: first it is pushed into the vein wall at a right angle to the vein, far enough to make

a little depressed "dimple" in the wall. Then the base of the needle is swung downward through an arc toward the hind legs of the frog, while simultaneously the needle tip is pushed deeper into the invaginating dimple. At the end of this maneuver the needle is pushing a cone-shaped invagination of the vein wall toward the head of the animal. When one makes these needles there is almost always a small, thin, sharp glass "lip" left on one side of its most forward part (cf. CHAMBERS and KOPEC 1937). As the needle is held pushing forward in the invaginated dimple of vein wall a slight rotation of the needle between one's thumb and forefinger causes this thin lip to cut quickly through the tip of the invagination. The needle tip is now in the vein, the vein wall slides up onto the cone-shaped needle tip, thus ensuring a tight fit,

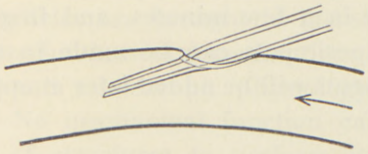

Fig. 5.

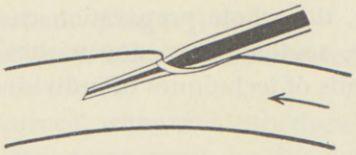

Fig. 6.

Figures 5 and 6 are diagrams and do not show the dimensions of the parts in correct relationship to each other. In different animals and at different degrees of contraction or relaxation in one animal, the anterior abdominal vein might vary from one half to two or two and one half millimeters in diameter. (The vein can of course contract so tightly that it has no lumen).
The glass tubing had an external diameter of about one half millimeter at the place it passed through the vein wall. The needle tip is shown as it might appear at high magnification. No two of these needle tips are ever exactly alike; the ones used have had outlet orifices which varied from about 10 to 40 micra in internal diameter. These diagrams are made to show the appropriate detail of the smallest structures, the larger structures are shown in a greatly reduced scale. These statements also apply to Figures 16 to 29.

(fig. 5) and the needle is almost instantly cemented in place, probably by fibrin. The needle is too narrow to stop the flow of blood through the vein, and if it is inserted carefully the vein does not constrict or thrombose and obstruct the flow. If carefully done, there is no hemorrhage from this point. Before inserting the needle, the needle and the rubber tube are filled with Ringer's solution, and the syringe filled with the material to be injected, rigorous care being taken to exclude air bubbles. As soon as the needle is in the vein a small injection of the Ringer's solution is made which prevents a clot from forming in the needle tip (Fig. 5). The fine rubber tubing contains so little Ringer's solution (about 6 cubic millimeters) that the injection of all of it does the frog no visible damage. When this Ringer's solution is all injected, the material to be tested has arrived at the orifice of the needle (see fig. 6).

Even with a small bore syringe (0.5 to 1.0 cc. syringe) driven with a micrometer screw, the screw had to be given barely a touch if the droplets of injected material were to be kept small enough to pass through the tips of the portal vein into the hepatic sinusoids.

With this setup then it is possible to watch the behavior of injected particulate matter at many places in the blood stream. Because the peritoneum and wall of the anterior abdominal vein together are very thin and transparent, the observer can focus

on the tip of the glass neddle in the vein, and watch the reaction between the injected material and flowing blood, beginning the moment the injected material comes into contact with the blood. He can move the whole frog about at will and study the behavior of particulate matter in the blood as it flows through the smallest vessels of organs, such as lung, stomach, intestines, mesenteries, urinary bladder, striated muscle or kidneys, almost as easily as a person moves from one part of a large microscope slide to another. Or he can move the preparation a little, transilluminate the edge of the liver and study the removal of particles from the blood stream by the phagocytic von Kupffer cells which line the frog's hepatic sinusoids.

The preparation described can easily be kept and studied for several hours. Many of them have been studied 4, 8, or 10 hours, some for as long as 12 to 18 hours. Moreover, the whole preparation can be made in a few minutes, and frogs are inexpensive, so that when one wishes, many experiments can be made in a day.[1]

Details of techniques of individual experiments will be added later at appropriate points.

The Structure and Mechanical Functioning of Living Frog Liver Lobules.

The experiments on selective phagocytosis to be described cannot be understood in terms of the morphology of histological sections of dead livers. The experiments were performed in living livers; the anatomy and mechanical functioning of living liver lobules, including that of the sinusoids, was a part of each experiment. During life, while they are storing blood and while blood is flowing through them, the hepatic sinusoids of frogs are smooth walled branching and anastomosing cylindrical tubes. The living sinusoids are not "narrow irregular tortuous spaces" originally described by Minot, 1900, and still commonly paraphrased in textbook descriptions of fixed histological sections. If during life the sinusoids were the narrow slits they are in sections of collapsed dead shrunken livers, no blood could flow through, no particles could be carried to phagocytes, no phagocytosis could occur. In living frog liver the von Kupffer cell is not a star-shaped cell suspended by processes like a spider sitting in a tube. The tubular sinusoids have a complete cellular lining, every portion of which is capable of selective phagocytosis.

The structure and mechanical functioning of living frog liver lobules have been studied more or less continuously since 1932. Whenever a frog's body cavity was opened for any purpose we studied the liver, which is the first organ encountered, before turning to the other organs. The living livers of more than 3500 frogs have now been

[1] These frog preparations are not very hard to make. Several graduate students and medical students have easily learned to make them. With the exception of the details of the structures in the interlobular spaces of the liver, the observations and experiments described in this paper are not hard to make; with a quartz rod illuminator and a little skill and practice many of them could be demonstrated anywhere to classes in medical histology or in microscopic physiology. The microscopic observations to be described were always carried out in a black dark room because when the observer's eyes are dark adapted he can see many small structures not otherwise perceptible, and all visible structures are seen with maximum clarity.

studied. We felt that if we took time to learn the frog liver lobule thoroughly, it would then be possible to see which structures and functions of other livers were like those of frogs, and which differed, and how they differed.

The structure and mechanical functioning of the living frog liver lobule have been studied with four purposes in mind: (1) to find out how the hepatic artery and portal vein terminals are distributed to and within the lobule, and how their activities may affect or control the chemical environment of the hepatic parenchyma cells; (2) to find out how the lobule stores and releases blood, thereby affecting or controlling the circulating blood volume and the cardiac output (cf. KROGH, 1912 a and b); (3) to find out how one phagocytic von Kupffer cell selectively removes one foreign particle from the circulating blood; and (4) to find out what factors determine the rates at which particles are brought to the stationary hepatic phagocytes.

Three fundamental assumptions have guided the studies of the microscopic living anatomy and microscopic physiology of the liver lobule:

(1) No mechanical function can be performed without a structure or combination of structures to perform it. Enough structures must be present to perform those mechanical functions already known. This keeps one searching for structures which the histological sectioning and staining methods have as yet failed to reveal.

(2) Those structures which are present must be so constructed both in arrangement and in magnitude that they can perform their own functions. The sum total of all the structures must be able to carry out all the known functions, some concurrently, some consecutively.

Thus it seems reasonable to assume that descriptions of arrangements and magnitudes of structures present in histological sections, which during life would prevent the carrying out of known functions, do not represent the structures as they exist during life. This assumption is a tool which can routinely be used to dissect morphological artefact from the real living anatomy.

(3) The smallest unit of any mechanical function must be performed by a definite small sized unit of structure, or by a group of small structural units which participate in the carrying out of an integrated reaction. By induction we may expect the total amount of a given function at any moment to consist of the summations of the actions of those individual small units which are at that moment carrying out that function. Consequently, a continuous consistent attempt has been made (a) to find the smallest anatomical unit which performs each function, (b) to study all the behavior patterns of each type of reacting unit, and (c) to study the coordinated reactions of various kinds of small units. This has been done in order to begin to lay a foundation for precise inductive thinking about problems involving summations of the various types of reactions.

Most of these studies have been made simply by watching the liver under the microscope while making every effort to keep the whole animal and the liver as undisturbed as possible. The purpose has been to study the coordinated reactions of the

tissue units while the reactions were integrated by the animal's own internal regulatory mechanisms.

The structure of the living liver lobule and of those activities of the vascular system of the lobule which control the rates at which particles are carried to hepatic phagocytes will first be described, in preparation for a description of the selective ingestion processes which take place in the lobule.

The Anatomy of the Vascular System of the Living Frog Liver Lobule: KNISELY, 1939, and BLOCH, 1940, briefly described the vascular anatomy and some of the mechanical functions of the living lobule. Figure 7 is a simple diagram

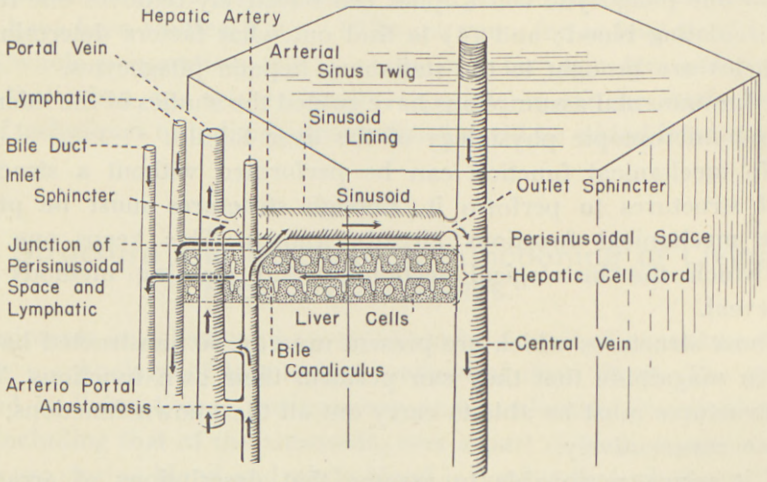

Fig. 7. Diagram. The diameter of the lobule can be 1—2 mm.

showing the anatomical relationships and interconnections of the various parts of the living lobule. This diagram shows four tubular structures, the interlobular hepatic arteriole, portal venule, bile duct and lymphatic (the so-called "portal triad") running parallel to one another in the portal space along one corner of a hexagonal lobule. In the diagram the four units have been separated somewhat to make it easier to depict the connections of each. In life, all four tubular structures are close together in the portal space.

The lymphatic has not been visible during life, but indirect physiological evidence proves it is present. This evidence is that under some physiological conditions, to be described later, large amounts of fluid continually pass for long periods of time, sometimes hours, out through every sinusoid wall into every perisinusoidal space. During these periods none of this fluid reappears in the central or sublobular veins nor in the bile ducts, and the liver tissue does not swell. Thus the fluid must be passing out into the only other exits from the liver, which are lymphatics. And from the literature based upon histological sections we know that each portal space of each species thus far studied contains lymphatics. See MALL, 1901, and MALL's figure 54, 1906, F. C. LEE, 1923, and BOLTON and BARNARD, 1931.

Mall, 1901 and 1906, and Bolton and Barnard, 1931, found the connections between the portal ends of the perisinusoidal spaces and the lymphatics in the portal spaces. (See Fig. 8) Lymphatics have also been found in the walls of hepatic veins, (see Gilbert and Villaret, 1909; Lee, 1923; Popper, 1931; and Pick, 1931); hence there may also be connections between the perisinusoidal spaces and lymphatics running in or along the adventitia of the central veins.

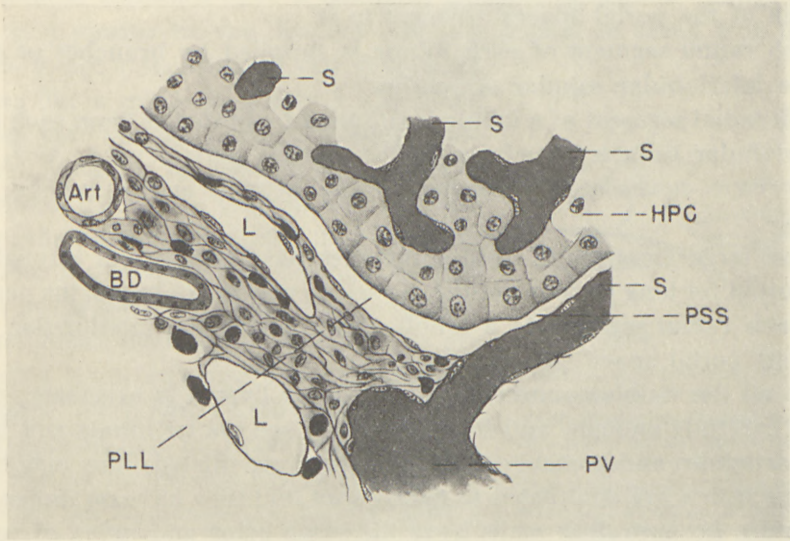

Fig. 8. The origin of the lymphatics in the liver. This figure is redrawn from Mall, Johns Hopkins Hospital Bulletin 12: 146, 1901. It is a section through the periphery of the liver lobule of a cat. The hepatic artery was injected with cinnebar gelatin, and the portal vein with Prussian-blue gelatin, stained with Van Gieson's' stain.
× 500. Art = hepatic artery; BD = bile duct; PV = portal vein; S = sinusoid; HPC = hepatic cell cord; PSS = perisinusoidal space, which is continuous with PLL, the perilobular lymphatic; L = interlobular lymphatic.
In this redrawing of Mall's figure, the perisinusoidal space and perilobular lymphatic have been left completely unstippled to demonstrate the connection between them clearly.

The bile duct system can be made visible by injecting sodium indigo disulfonate (Eastman) into the frog's dorsal lymph sac. From here the dye goes into the blood, where its high dilution makes it invisible. But it is secreted in concentrated form into the intercellular bile canaliculi of the hepatic parenchyma cell cords, staining their lumen blue green, and it slowly passes radially outward along the very narrow lumen of the cords to the interlobular bile ducts, staining their contents blue-green. The position of an interlobular bile duct along with an interlobular hepatic arteriole and portal venule positively identifies a portal space in the living liver. The position of the bile duct identifies the position of the portal space even when the interlobular hepatic arteriole or portal venule is contracted so tightly shut that it is invisible.

As Mall, 1906, showed, each lobule has at least two portal spaces adjacent

to it, most lobules have three, and some have four. (See MALL's figures 1, 46, and 48.) JOHNSTON, 1918, has shown some of the three dimension shapes of liver lobules.

In the thinner areas at the edges of frog liver which can be transilluminated for microscopic study during life we have found that:

1. All three of the visible interlobular tubular structures, the hepatic artery, portal vein and bile duct represented in Figure 7 are present in every interlobular portal space.

2. Each lobule is supplied by branches of each of the three visible tubular structures in each of the portal spaces adjacent to it.

3. Each radial segment of each lobule is supplied by branches of each of the three visible interlobular tubular structures.

4. Each radial segment of a lobule is supplied by branches from each of the three visible interlobular tubular structures in the nearest portal space. And,

5. We have never seen branches of the hepatic artery running along the central vein.

Because large amounts of fluid sometimes pass rapidly out through the walls of every sinusoid for long periods of time, we see no way to doubt that the perisinusoidal spaces of each radial segment of a lobule connect with the interlobular lymphatics in the nearest portal space (see fig. 8).

To see all the visible connections in the living liver it is necessary to study the preparation carefully enough, and long enough to see the terminals and branches of the hepatic arterioles and portal venules when they are dilated. This may take several hours. Vessels which are contracted so tightly shut that they have no lumen are almost always invisible because their walls have the same color and index of refraction as the surrounding tissues. Further, as stated above, it is important to be able, when in doubt, to confirm the identity of the portal space by causing the liver to secrete blue-green sodium-indigo-disulfonate into the interlobular bile ducts.

A motion picture has been taken through the microscope, various scenes of which show those features of the structure and functioning of the living frog liver lobule which are denoted in the next paragraphs by [1]. Some of the scenes were taken in Rana esculenta in Professor Krogh's laboratory in Copenhagen, some in Rana pipiens in Chicago. The picture was shown at the Boston Meeting of the American Association of Anatomists in March, 1939. One set of scenes shows Deysach's small sluice channels (cf. DEYSACH 1941); we had not recognized them, but did when DEYSACH and KNISELY studied the film together. This motion picture was shown at the May 4, 1944, meeting of the Ohio State Medical Association in order to show the factors which control the rates of blood flow through sinusoids, which are, of course, the factors which control the rates of supply of particles suspended in blood to hepatic phagocytes.

The hepatic arteriole runs along the portal venule like a vine on a tree[1] (BLOCH, 1940, see figs. 9, 10, and 11, confirmed by WAKIM and MANN, 1942). Both join the sinusoids.[1] Fig. 11 (This confirms MALL, 1906, and OLDS and STAFFORD, 1930.)

[1] Cinema recorded.

Every sinusoid is connected to a terminal portal venule.[1] Every sinusoid, or almost every sinusoid receives a branch, the arterial sinus twig, from the hepatic artery (see fig. 11). Contractile arterio-portal anastomoses (abbreviated "APA's" in fig. 10 to correspond with "AVA's" for arterio-venous anastomoses) interconnect the hepatic artery and the portal venule, as BLOCH 1940 said, "like rungs of a ladder"[1] (see figs. 9 and 10; confirmed by WAKIM, 1941, and WAKIM and MANN, 1942)

The tubular sinusoids have a complete continuous cellular lining. Each cell of this lining is a phagocytic von Kupffer cell (see p. 26). In living frog liver the von Kupffer cell is not a star-shaped cell suspended by processes like a spider in a tube.[1] The living sinusoids are not "narrow irregular tortuous spaces" as they often are in histological sections of dead collapsed livers. If, during life, the sinusoids were the narrow slits which they often are in sections, no blood could flow through; no particles could be carried in to phagocytes; no phagocytosis could occur. During life the sinusoids are smooth walled branching and anastomosing cylindrical tubes.[1] (See figs. 12, 13 and 14.) This finding agrees with that of ELLINGER and HIRT, 1929, who used a fluorescent dye to study living frog sinusoids. See ELLINGER and HIRT's figure 7 which is reproduced here as fig. 12; also see the figures of HIRT, ANSORGE, and MARKSTHALER, 1938. During life frog hepatic sinusoids are completely lined, smooth walled, branching and anastomosing cylindrical tubes. As previously noted the living transilluminated livers of about 3500 frogs have been studied at 32 to 500 × in this laboratory during the past 14 years. Not one star-shaped cell has ever been seen suspended between hepatic cell cords. Red cells have never been seen bumping against and turning, or squeezing past any transparent invisible obstruction. During the flow of blood all red cells pass through the sinusoids without meeting any visible or invisible obstructions. (Cf. PFUHL, 1926 and ZIMMERMAN 1928). All the living hepatic sinusoids thus far seen have been smooth walled branching and anastomosing cylindrical tubes. These factors of the living anatomy have been recorded in motion pictures taken through the microscope, (cf. KNISELY, 1939).

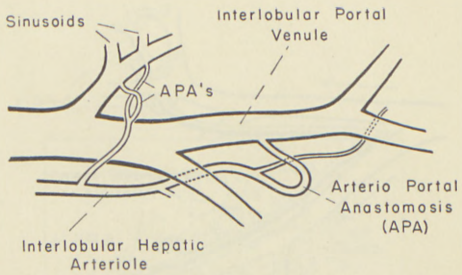

Figure 9, sketched from life, shows branches of vessels which were open at a given moment. These vessels had many more branches which are not shown because they were tightly closed while this sketch was made. The total horizontal length of the figure represents a portion of tissue approximately 800 micra long. The inlet portion of two sinusoids are shown at the upper left, the left hand one is approximately 50 micra wide, the right one approximately 40 micra wide. Note that the terminal portions of the hepatic arterioles including the arterio-portal anastomoses are much narrower. These vessels were constricted so tightly at this time that red cells passed through in single file. Because the pressure in these arterioles is high, red cells are pushed through even when an arteriole is contracted tightly enough so that each red cell folds as it traverses it.

Each sinusoid has an afferent inlet sphincter guarding its junction with the portal vein. See fig. 7. Each sinusoid has an efferent outlet sphincter guarding its junction with the central vein.[1] (KNISELY, 1939 and BLOCH, 1940.) (See figs. 7, 13, 14 and 15.)

[1] Cinema recorded.

The living lobules also have the "small sluice channels" described by DEYSACH in 1941. These consist of from a few, to several, to many confluent sinusoids having one single common outlet to a central vein or to a sublobular vein. See fig. 13. As DEYSACH showed, each small sluice channel has an outlet sphincter guarding its junction with the next larger-sized vessel.[1]

The portal end of the sinusoid, lined with phagocytic cells, and the arterial sinus twig usually come together at an acute angle (see figure 11). The point of junction is usually not less than $^1/_6$ nor more than $^1/_4$ and never more than $^1/_3$ of the shortest radial distance from the portal venule to the central venule. Centrally from these points of junction the sinusoids bifurcate at acute angles. All the way across to the central veins the sinusoids branch and reunite, almost always at acute angles.

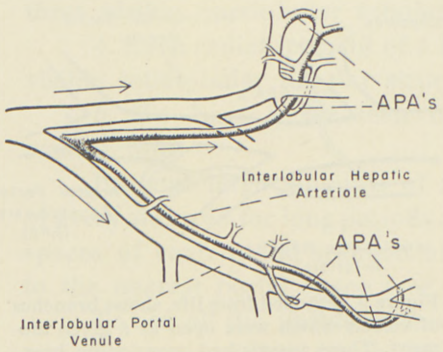

Figure 10. Arterio-portal anastomoses. (APA's). Sketched from life showing only those vessels which were open at a given time. If a diagonal line were superimposed on the lower combination of portal venule and hepatic arteriole, the maximum extent of the figure along the line would represent approximately 2.6 mm. of the living liver tissue. The arterio-portal anastomoses at the lower right had internal diameters at the points where they joined the side of the portal vein of about 40 or 50 micra at the time this sketch was made.

As one traces the patterns of the branching and anastomosing sinusoids across from the portal space to the central vein, it is obvious that for about the first half of this distance there are many more bifurcations than reanastomoses; for the second half of the distance, there are many more reanastomoses than bifurcations. That this is necessary in order to distribute sinusoids to all parts of the lobule adjacent to a given interlobular portal space, becomes obvious if one examines MALL's figure 1 (1906), which is a diagram of the horizontal relationships of adjacent lobules.

If to orient the description we speak of the lobule as though it were a regular hexagonal prism standing on one end (see the figures of JOHNSTON, 1918, for the three-dimensional shapes of liver lobules), then it is accurate to say that as seen from the top it would seem that most of the bifurcations and reanastomoses are in the horizontal plane. However, as seen from the side during life, it is obvious that there are also bifurcations and re-anastomoses in the vertical plane. As seen during life, at the edge of the liver, in three dimensions, it is obvious that groups of sinusoids leave the portal space at a slight upward angle, diverge, arch across, simultaneously branching and anastomosing, then pass horizontally, converge, still branching some but re-anastomosing more, and arch downward and join the central venule. And all the way across there are branchings and anastomosings which unite the sinusoid segments of each level with those above and below it.

The outlet sphincters of the sinusoids and of Deysach's small sluice channels are very short.[1] (Fig. 13.) During life they are contractile rings, each shaped about

[1] Cinema recorded.

like a doughnut. Those which guard the exits from sinusoids either lie tightly against the wall of the central vein or are ring-shaped structures embedded in the wall of the central vein. Either view could be defended by defining the "wall of the vein" appropriately. They lie outside the refractile endothelium of the vein, and during some phases of function seem to be embedded in the circular layer of smooth muscle which surrounds the endothelium of the vein.

Each of these outlet sphincters can contract tightly shut[1], dilate widely[1], or maintain any intermediate degree of tonic contraction[1]. They can act individually,

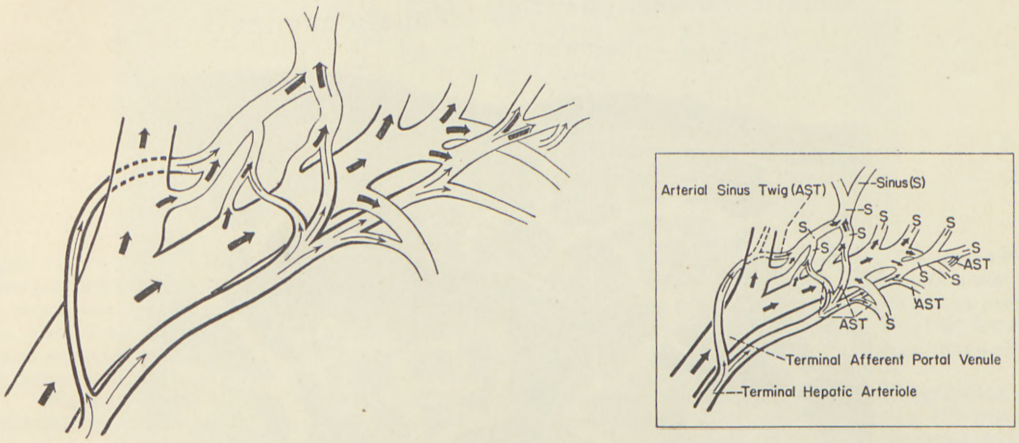

Figure 11 shows the details of the junctions of arterial sinus twigs with the sides of hepatic sinusoids at the edges of adjacent lobules. The arrows show mixing of the blood. The thick stemmed arrows represent portal blood, the thin stemmed arrows arterial blood, and the arrows with two stems represent mixed blood. The longest line which could be drawn on this figure, from lower left to upper right represents a distance of about 780 micra.

in groups, or all together (KNISELY, 1939). (Figs. 13, 14, and 15.) They guard the amounts and rates of exit of blood from each sinusoid, hence from the lobules and from the liver. Further, because of their anatomical location, they guard the exit, and regulate the rates of emission of blood from the whole anatomical area drained by the portal vein. We have named these the "efferent sphincters" (BLOCH, 1940) or the "outlet sphincters". (See Figures 7, 13, 14 and 15.) Taken altogether these outlet sphincters are the "sluice mechanism" of the living frog liver.

When the outlet sphincters are tightly contracted they are relatively opaque[1]; when widely dilated they are translucent, so nearly transparent in fact that there is no morphological evidence of their presence.[1] During life the smooth muscle of frog and mammalian gut and of mammalian uterus is also relatively opaque when contracted, more nearly transparent when relaxed. There are so many sinusoids joining each central vein that the outlet sphincters often seem to be contiguous with each other. (Figs. 13 and 15.) When all of the outlet sphincters along a stretch of the central vein are contracted tightly shut at once, they are all opaque at once, and their presence

[1] Cinema recorded.

makes the wall of the vein appear very thick[1]. At such a time the central vein wall looks like a thick, translucent to opaque membrane. (See Figure 13.) In any species of animal the presence during life of a thick translucent to opaque wall of a central vein, together with no, or but few, visible connections between the lumen of the adjacent sinusoids and that of the vein, are sufficient clues indicating the presence of sinusoid outlet sphincters, to warrant prolonged careful observations to see if semi-

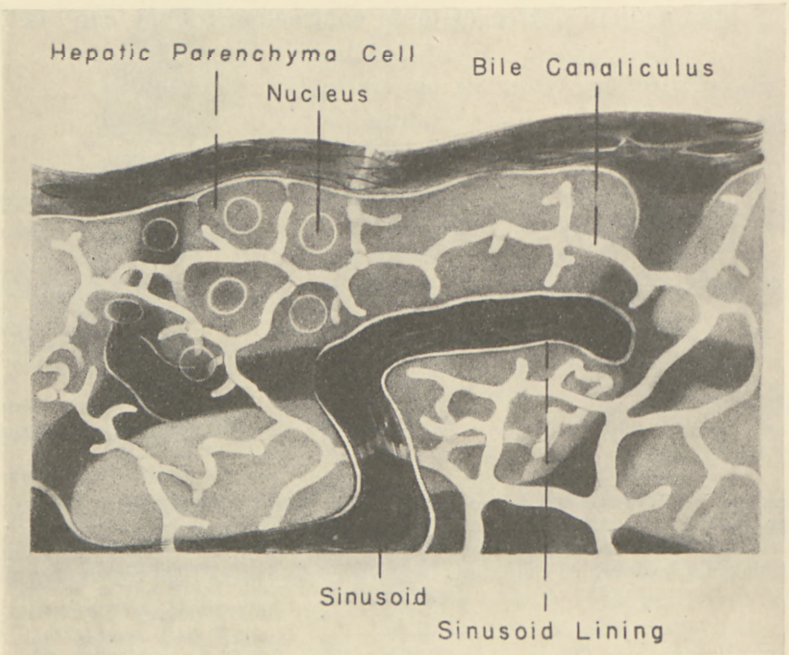

Fig. 12. Redrawn from ELLINGER and HIRT, 1929. This figure shows the sinusoids in living frog liver to be branching and anastomosing cylindrical tubes. The sinusoids are lined throughout, and there are no star-shaped cells suspended within the sinusoids. This figure is particularly valuable because ELLINGER and HIRT present it as evidence of what can be seen in living livers after the injection of a fluorescent dye. They were not studying the anatomy of the liver and had no anatomical concepts to present or defend.

transparent structures are present which encircle the outlet ends of the sinusoids and relax, contract, etc.

The Mechanical Functioning of the Lobule's Vessel System: The terminal hepatic arterioles[1], the arterial sinus twigs, the terminal portal venules, the arterio-portal anastomoses, the inlet sphincters[1], the tubular sinusoid linings, the outlet sphincters of the sinusoids[1] and of Deysach's small sluice channels, the central veins and the sublobular veins are all independently contractile[1]. By artificially obstructing the outlet venules (centrals or sublobulars), the pressure in the various vascular units of the lobule may be made to rise. Then by releasing the obstruction the pressure in these vascular units may be made to fall. We have seen units of each of the above

[1] Cinema recorded.

categories contract when the pressure within them was rising, and dilate when the pressure within was decreasing. This is evidence that these units have the power of active contractility. Each individual unit of each of the above categories is independently contractile. Each individual unit of each category can dilate widely, or contract

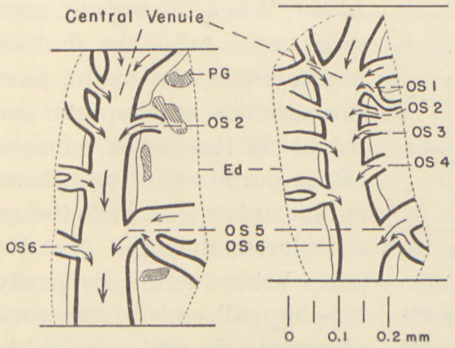

Figure 13. Two views of one living central venule. These are tracings from a motion picture. The scenes were photographed about three minutes apart. To obtain these line-drawing still-pictures from the motion picture, the scenes were cut apart and the ends of each scene spliced together forming a loop for continuous projection. Each loop of film was then projected continuously so that its image was on a large sheet of drawing paper. An artist then traced the outlines of the vessels on the paper. Thus these "ciné tracings" have about the same scientific accuracy as good camera lucida drawings.

The scale below the right figure is a ciné tracing of a Zeiss microscope scale made by photographing and projecting the scale through the same lenses and at the same distances as were used to obtain the tracings of the tissue. This method permits accurate measurements of the dimensions, and of the physiological changes in dimensions, of histological structures during life.

The dotted line "Ed" at the right and left of each scene marks the periphery of the area which was in sharp focus as the motion picture was taken. The heavy horizontal lines at the top and bottom of each scene mark the upper and lower borders of the motion picture frames. The right scene has six outlet sphincters marked OS1, OS2, OS3, etc.

The tissue moved after the first scene was taken, consequently the two scenes do not contain identical areas of the tissue and are not on corresponding areas of the film. The scenes are matched here so that OS5 is at the same horizontal level in each. This permits identification of corresponding points of anatomy in the two scenes.

OS5 is the outlet sphincter of one of Deysach's small sluice channels. The others are sinusoid outlet sphincters.

In the right scene outlets 1, 2, 3, 4 and 5 are open and outlet 6 is closed. In the left scene outlets 2, 5 and 6 are open, outlet 2 is more dilated than in the right scene, and outlets 1, 3 and 4 are each shut off so tightly that there is no visible evidence that any one of them has a lumen.

No attempt has been made here to sketch in the network of sinusoids which were distended with stored blood because outlets 1, 3, and 4 were closed.

PG is an area of black pigment which occurs in frog livers under some conditions.

The light line outside and parallel to the heavy central vein lining marks the outer surface of the thick translucent to opaque layer which is made up of contiguous closed outlet sphincters. There were many more outlet sphincters in this tissue area than were open in either of these scenes.

so tightly that it has no lumen, or can maintain any intermediate degree of tonic contraction.

Control of the Environment of Hepatic Parenchyma Cells: The degrees of dilation or of tonic contraction of the interlobular hepatic artery, arterioportal anastomoses and afferent interlobular portal venule determine the relative composition of the blood which is supplied to the sinusoids of each segment of each lobule, that is,

of the blood which nourishes the hepatic parenchyma cells. When the interlobular hepatic arteries shut off tightly throughout their lengths and the portal veins remain dilated, the sinusoids receive only pure portal vein blood[1]. (See Figure 7.) When the terminal interlobular portal venules are tightly constricted and the interlobular hepatic arterioles and arterial sinus twigs are dilated, the sinusoids receive only pure arterial blood. When the hepatic artery is dilated and the arterial sinus twigs are shut off, and the arterio-portal anastomoses are open, pure arterial blood passes through the arterioportal anastomoses into the terminals of the portal vein and is distributed by way of the portal vein tips to every sinusoid whose inlet sphincter is open[1]. With the arterio-portal anastomoses all closed, and the portal vein terminals and hepatic artery terminals partly open, the peripheral "Y-shaped" ends of the sinusoids receive a mixture of portal vein and arterial blood. Further, the arterioportal anastomoses at times are partly dilated, thus permitting various controlled amounts of arterial blood to be mixed into the portal blood passing along the interlobular portal venules[1]. See Figure 7. The kind of blood, or the ratio of the mixture, and the volume of blood supplied to a given set of sinusoids per unit of time, is continually controlled and may be maintained constant for hours at a time. From time to time the hepatic artery or portal vein may dilate or constrict a little, and the ratio of the mixture be changed, or the volume supplied per unit time be changed. The new ratio and volume supply may then be maintained for another period of time. Or, as has been stated, either the hepatic artery or the portal vein may shut off, while the other remains dilated, for long periods of time.

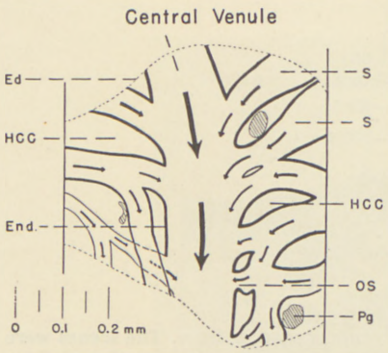

Figure 14. Ciné tracing of dilated sinusoids and dilated central venule. OS is a slightly constricted outlet sphincter. All the other outlet sphincters are so widely dilated that there is no morphological evidence of their presence. The flow through these sinusoids and the central venule was so rapid that it was necessary to photograph the area at 48 frames per second to make it possible to follow individual erythrocytes in the motion picture. This is near the upper limit of flow rates in all the capillaries and sinusoids we have thus far seen. The idea that the flow through hepatic sinusoids is always slow is not true. Note that the sinusoids are not narrow tortuous spaces, but are branching and anastomosing cylindrical tubes.
HCC is hepatic cell cord. Other abbreviations are the same as in Figure 13.

Thus the nervous and/or hormonal mechanisms which control the degree and ratio of the dilation and contraction of the interlobular hepatic arterioles and portal venules, the arterioportal anastomoses, the arterial sinus twigs and the inlet sphincters, determine one link in every one of the chains of cause and effect which control the chemical environment of the hepatic parenchyma cells. They sometimes force these cells to live in an arterial environment, provided with highly oxygenated arterial blood, and they sometimes force these cells to live in an environment whose concentration of oxygen cannot be higher than that in the portal blood. And they sometimes force these

[1] Cinema recorded.

cells to live in various environments maintained at intermediate levels between the two.

These observations have been confirmed by HOERR, 1944 b, p. 132, who says, "We have been able to confirm Knisely's (1939) statement that the afferent hepatic arterioles and portal venules are contractile thus permitting the sinusoid to receive mixed blood or blood from only one source." These observations demonstrate to us, beyond reasonable doubt, that in frogs the chemical environment of the hepatic parenchyma cells of each lobule is precisely controlled continuously, and that this environment is forced to vary within controlled limits.

Probably dogs also have a precise control of the environment of hepatic parenchyma cells, for SOSKIN, ESSEX., HERRICK and MANN, 1938, p. 561, found, using a thermostromuhr technique on dogs under sodium amytal anesthesia that "In different animals, the portal vein or the hepatic artery sometimes carried as much as 90 % or as little as 10 % of the total amount of blood entering the liver. Although such large differences in proportionate flow were the exception rather than the rule, smaller reciprocal variations frequently occurred during the course of the experiment, while the total outflow of blood from the liver remained constant."

It is, of course, now necessary to learn (1) all the differences in the chemical composition of the hepatic artery and portal vein blood during different physiological states, and (2) under what physiological conditions (such as rest, muscular exercise, during digestion, or during periods when the pancreatic islets are increasing or decreasing the amounts of insulin they are releasing) the sinusoids are supplied with one or the other or what proportion of arterial and portal blood. During our experiments on frogs, during most of which the anesthetized animal's striated muscles have been at rest for long periods, the sinusoids of most lobules have received mostly portal vein blood.

The Permeability Phases, Lymph Formation, and Blood or Blood Cell Storage: The tubular sinusoid lining membrane, which is contractile throughout its length, and each cell of which is a phagocytic von Kupffer cell (vide infra), has three distinct permeability phases. In one, the individual red cells enter the sinusoid separated by small volumes of plasma, and the distance between the red cells does not become visibly less as the column of cells passes along the sinusoid. During this phase not much if any fluid is passing out through the sinusoid wall into the perisinusoidal space and thence to lymphatics. The liver is probably forming but little if any lymph. MARKOWITZ and MANN, 1931, found that dog livers may not always be forming lymph. When outlet sphincters close at such a time, the sinusoids of these outlet sphincters store whole blood.

During another, extremely permeable phase the sinusoid lining membrane is probably permeable to all the colloids of the blood. As the red cells pass along the sinusoids they come closer and closer together until the central two-thirds, half, or third of the sinusoid contains only packed red cells moving, sometimes slowly, some-

[1] Cinema recorded.

times rapidly. If each outlet sphincter remains partly closed, the sinusoid systems remain in a "continuous filtration phase" such as sometimes occurs in spleen sinusoids (KNISELY, 1936 b and HOERR, 1944, p. 132), and almost all the blood plasma is continuously separated from the blood cells[1]. If outlet sphincters close at such a time the sinusoids of these outlet sphincters store highly concentrated blood cells. As the hydrostatic pressure exerted by the blood against the sinusoid wall is certainly not enough to force all the water and crystalloids out through the sinusoid wall while retaining and concentrating the blood proteins to nearly zero volume against these proteins' own inwardly directed osmotic attraction, it is necessary to conclude that when the red cells are packing tightly together, the blood proteins are all or nearly all passing out through the sinusoid wall. (Cf. KROGH, 1929, p. 14 and Chapter XIV, and KNISELY, 1936 b, p. 42.) During this phase, almost all the plasma of the blood passes into the perisinusoidal spaces, thence to lymphatics. The sinusoid linings sometimes remain in this most permeable phase when the flow into them is very fast, which is evidence that this permeability phase is not simply a result of stagnant anoxia. (Cf. LANDIS, 1928.)

When frog hepatic sinusoid linings are in this most permeable phase the rate of formation of lymph must be proportional to the rate of blood flow into the sinusoids. This agrees with the finding that dog and cat livers can and frequently do form large amounts of lymph which contains about as much protein as blood. (See STARLING, 1894; FIELD, LEIGH, HEIM and DRINKER, 1934; and MCCARRELL, THAYER and DRINKER, 1941.)

LAMSON, 1915, found and demonstrated that dogs could, after removal of the spleen, empty out enough concentrated red cells from the liver to raise the red cell count in all the circulating blood from (in his experiment No. 22, controlled by his experiment No. 16) 8.024.000 to 10.040.000 per cubic millimeter. The storage of concentrated blood cells is, of course, a necessary concomitant of plasma permeable sinusoid linings, lymph formation, and partly constricted or closed outlet sphincters (cf. BAUER, DALE, POULSSON and RICHARDS 1932, and DEYSACH 1941).

The frog hepatic sinusoids also exhibit a third "intermediate permeability phase" during which the linings probably are permeable to proteins of smaller molecular weight, such as albumen, but probably are not permeable to the larger ones, such as the globulins or fibrinogen. When the linings are in this phase the moving red cells come somewhat closer together, but do not come tightly together even when the outflow is artificially obstructed and the pressure in the sinusoids is artificially raised above its normal level. BLOCH, 1940, found that when certain concentrations of acetyl-beta methyl choline chloride were applied to the surface of frog liver, the sinusoid linings went into this intermediate permeability phase. Whenever outlet sphincters close while sinusoids are in this phase, the sinusoids of those closed outlet sphincters store partly concentrated blood cells.

We have traced literally hundreds of sinusoid systems from portal space across

[1] Cinema recorded.

to central vein during each of the three permeability phases. At magnifications up to and including 600 × no holes are visible in the tubular linings during any permeability phase. This agrees with BENSLEY 1923 and with BOLTON and BARNARD's 1931 finding in cat livers. If the liver has not been injured by rough experimental technique, red cells are not found outside the sinusoid linings (cf. WAKIM, 1944).

The perisinusoidal spaces are frequently visible at 400 to 600 × but are very narrow, even when fluid is passing rapidly out through the sinusoid walls. This confirms MACGILLAVRY, 1864; DISSE, 1890; MALL, 1901 and 1906; MANWARING, FRENCH and BRILL, 1923; and BOLTON and BARNARD, 1931, who were able to demonstrate the perisinusoidal spaces in histological sections. When fluid is not passing into the perisinusoidal spaces the sinusoid walls lie against the hepatic cell cords obliterating the perisinusoidal spaces just as the spaces between the leaves of a book are obliterated, becoming potential spaces when the book is closed.

In a given microscopic field under uniform experimental conditions the sinusoid linings may change from any one of these three permeability phases to any other, or may stay in one of these phases for several hours, or may go through rapid or slow cyclical changes from one phase to another. By combinations of the permeability phases and shutting and opening of the outlet sphincters various controlled amounts of blood cells and of whole blood are stored in the liver and various controlled amounts are released. The fact that all the permeability phases may alter while they are observed and that adjacent sinusoids may be in different permeability phases, and each change to another phase, and that they go through cyclical changes, are evidences that no one of the permeability phases results from conditions imposed by the anesthetic, the operation, or the transillumination technique.

In 1932, BARCROFT, NISIMARU and RAY reported some tests made to see if the livers of cats and dogs could store slowly moving or stationary blood. Their animals were anesthetized, usually with A. C. E. mixture, and then given an injection of carbon monoxide into the trachea. Then at short intervals parallel samples of blood were collected from the cut or torn surface of the liver and from the femoral artery. The concentrations of carbon monoxide hemoglobin in these samples were measured, and the changes in the concentrations of carbon monoxide hemoglobin in the blood from the two sources with the passage of time were compared.

The purpose of the tests was to compare the concentrations of carbon monoxide hemoglobin in the blood in the capillaries or sinusoids of the liver with those in the general circulation. A time lag in the rates of appearance or of disappearance of the carbon monoxide hemoglobin in the samples from the liver would be evidence that the liver stored blood. In all, they experimented upon only thirteen animals. They state, "In exceptional cases there seemed to be some evidence that the blood from the liver took a little longer to attain its maximum of CO than that in the general circulation, but we never obtained any certain reason for supposing that the blood in the liver retained its CO longer than that in the general circulation." Consequently, they concluded, "Although the liver is a store in the sense that it contains large quantities

of blood which can be transferred to some other site, it is not a store in the sense that blood is out of the circulation. . . In the vessels of the liver there are no considerable diverticula from the general current in which the blood can lie."

These conclusions disagree diametrically with the results of some of the microscopic observations of the activities of the sinusoid systems of frog and Rhesus monkey livers. For the blood backed up by each outlet sphincter when it is partly closed or tightly closed may be thought of as being in a small branched diverticulum. Consequently, we have been seeking for possible sources of experimental differences in the two sets of results. Thus far we have found two: (1) When one makes the smallest cuts or tears in the liver which can be made with small scissors or forceps, and then examines the cut area with a stereoscopic microscope, he invariably finds that almost all the blood being shed is coming from hepatic arterioles and portal venules. Most of it usually comes from portal venules because the cut ends of hepatic arterioles have a marked tendency to constrict tightly shut. At the instant the cut is made a little blood comes from sinusoids which may have been in storage phases. One can see this by watching the cutting itself under the microscope. But the cut opens some sinusoids so that blood flows rapidly through those sections of sinusoids which are between the afferent vessels and the cut openings. The cut itself seals or crimps off the ends of many sinusoids. And the smallest cut one can make with knife or small scissors opens afferent interlobular vessels in which the pressure is high, and central venules in which the pressure is low. Consequently, almost all of the shed blood comes from afferent vessels, but little comes from sinusoids or central veins. Larger cuts or tears than the smallest which one can make merely open greater numbers of the same kinds of structures. Thus it seems probable that Barcroft, Nisimaru and Ray did not obtain much sinusoid blood. Most of the blood they obtained probably came from cut intrahepatic portal venules, which probably would contain as much CO as blood taken from any other part of the general circulation.

(2) The sinusoids do not always contain slowly moving or stationary blood. In frogs and monkeys they are often dilated and contain large amounts of blood moving as rapidly, often more rapidly than blood in most other capillary systems. (See Figure 14.) When one selects an anesthetic, gives it to an animal, ties the animal down and operates upon it, he thereby puts the animal's circulatory system into one of its reaction states, and all tests made on that animal from that time on are tests which show only various factors of that reaction state, or of factors of those deviations from it which are possible under those particular experimental conditions. Consequently, even if these investigators did obtain blood mostly from sinusoids, there is no reason to believe that hepatic sinusoids are always in the condition they were in when those sets of samples were taken.

In the light of the microscopic observations described, we do not now believe that these particular conclusions of Barcroft, Nisimaru and Ray follow from the evidence they presented.

The "Sluice Mechanisms" of Living Frog Liver: The outlet sphincters

of the individual sinusoids and those of Deysach's (1941) small sluice channels are the outlet valve "sluice" mechanisms of the living frog liver. Each of these sphincters can dilate widely, contract tightly shut or maintain any intermediate degree of tonic contraction. They can open and close individually or in groups, or all may open or close, or any number may maintain any intermediate degree of tonic contraction. (See Figures 13, 14 and 15.)

They control the volume of whole blood and/or concentrated blood cells stored in the liver and backed up in the portal vein and its tributary veins and thereby continuously affect and are a major factor in the control of

(1) the peripheral red cell count
(2) the circulating blood volume,
(3) the venous pressure, and
(4) the cardiac output.

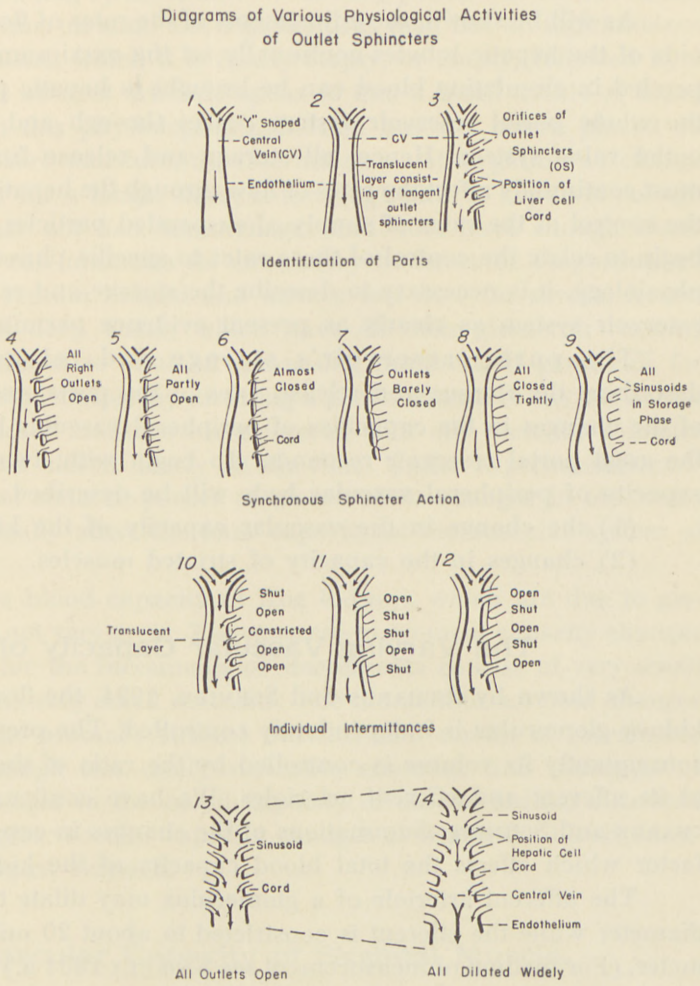

Fig. 15.

The maximum capacity of the great portal reservoir, which consists of the liver, spleen and portal vein bed, minus the minimum capacity of these structures, is equal to the maximum amount of blood which this great reservoir can store or release. The spleens of frogs are nearly spherical and but two to five millimeters in diameter. Under the conditions of our experiments they have seemed to play but little if any role in the blood or blood cell storage.

In frogs the maximum amount of blood which the liver and the portal vein with its tributary veins can store is probably as great or greater than the maximum capacity of the vascular beds of any other organ. It probably is as large as the total capacity of the vascular beds of all the animal's striated muscles. In frogs with a competent

blood volume, and a competent blood volume is indispensible, the liver and portal bed can, and under many experimental conditions do, make adjustments by accepting or ejecting blood, to the summations of the changes in capacity of all the other parts of the peripheral vascular system.

As will be shown, the adjustments of the rates of flow of blood through the sinusoids of the hepatic lobules continually set the maximum rates at which particles suspended in circulating blood can be brought to hepatic phagocytes. The outflow from the whole portal reservoir system passes through and is controlled by the hepatic outlet valve system. Hence, all storage and release from the great portal reservoir must continually affect the rates of flow through the hepatic sinusoids. Hence, to present the control of the rates of supply of suspended particles to hepatic phagocytes and to begin to relate the control of these rates to specific phases of normal and pathological physiology, it is necessary to describe the storage and release roles of the great portal reservoir system as clearly as present evidence permits.

The portal reservoir's storage and release roles: The first step in describing the storage and release roles of the portal reservoir will be to show some of the changes in the capacities of peripheral vascular beds to summations of which the great portal reservoir responds. To begin with, two examples of changes in the capacity of peripheral vascular beds will be described:

(1) the change in the vascular capacity of the kidneys, and

(2) changes in the capacity of striated muscles.

The Varying Vascular Capacity of the Kidney.

As shown by Richards and Schmidt, 1924, the flow of blood through each frog kidney glomerulus is independently controlled. The pressure in each glomerulus and consequently its volume is controlled by the ratio of the degrees of tonic contraction of its afferent and efferent arterioles. We have confirmed these observations of Richards and Schmidt. Summations of the changes in capacity of the glomeruli are one factor which affects the total blood capacity of the kidney.

The afferent arteriole of a glomerulus may dilate to about 30 micra in internal diameter while the efferent is constricted to about 20 micra or even 10 micra in diameter. (For method of measurement see Knisely 1934 a.) In such a case the glomerulus may be dilated until it nearly fills its Bowman's space, and the flow through its capillaries may be fairly rapid[1]. Or the afferent arteriole may constrict tightly shut while the efferent is dilated, in which case the glomerulus may decrease in size until it contains but a few stationary red cells and is but about one-half the diameter, that is one-eighth of the volume of its Bowman's space. During life the Bowman's capsules of Rana pipiens are nearly spherical and range from about 250 micra to about 325 micra in diameter.

Frogs which have been kept in deep water for some time before being opened

[1] Cinema recorded.

for observation, and which are anesthetized lightly with urethane and are opened by cautery with no blood loss often have all their glomeruli dilated with a very rapid flow through each. Frogs which have been sitting in air which has a low relative humidity, for several hours before being opened, and which are opened without blood loss often have almost all their glomeruli shut. Each glomerulus then has an intermittance consisting of a long period during which it is nearly empty, or empty, followed by a brief interval during which its afferent arteriole opens so that a small trickle of blood flows through it. If a frog in this physiological condition has wet cotton placed on its skin so that it begins to absorb moisture, the intermittances of its glomeruli change so that each has a larger flow for a longer time; if the frog's skin is kept wet enough for long enough, and if it has not had hemorrhages and does have enough blood stored in the liver so that it can maintain its circulatory homeostatic responses, all its glomeruli may open and remain dilated and have a fast flow for several hours. Conversely, frogs taken from deep water, which have all their glomeruli running at the beginning may be induced to shut off increasing numbers of glomeruli for longer and longer periods of time simply by directing a gentle stream of dry air across their back legs. As the frog loses moisture his glomeruli shut off, each for a longer period until nearly all may be closed nearly all the time. Under experimental conditions at least, summations of such responses of glomeruli to changes in the water balance of the animal continually affect the total capacity of the vascular system of frog kidney.

The total changes in the blood capacity of frog kidneys which are due to glomerular capacity changes are not very great. The moment-to-moment capacity changes are, however, very precise, for the increments or decrements consist of very small amounts and multiples of these very small amounts. The moment-to-moment changes in the vascular capacity of the animal's striated muscles also consist of increments and decrements of small though less easily definable amounts, and multiples of small amounts. The total changes in the blood capacity of all the animal's striated muscles are probably the largest vascular capacity changes to which the blood reservoir systems of the animal must respond.

The Varying Vascular Capacity of Skeletal Muscle.

The changes in the vascular capacity of striated muscles are related to the activities of the muscles. Microscopic observations of intact living frog muscles (submaxillaris, gastrocnemius, small toe muscles) transilluminated, stimulated by way of their nerves or by direct application of electrodes to the muscle surface (cf. E. Lange 1932), and studied microscopically in situ, show that the circulatory changes during several phases of muscle physiology are as follows:

When a striated skeletal muscle is rhythmically contracting and each contraction is strong, all of that particular muscle's arterioles, capillaries and venules are

[1] Cinema recorded.

relaxed; the walls of the capillaries and venules are flaccid. (For the anatomical distribution of the small vessels of striated muscle see SPALTEHOLZ, 1888 or KROGH's fig. 5. 1929) As is well known, all of a muscle's fibers do not contract each time the gross muscle contracts. (See ASMUSSEN, 1934.) Upon each contraction of the muscle those fibers which contract become shorter, straighter, taut, thicker and turgid. During strong contractions the laterally expanding muscle fibers press against each other so tightly that many of them change from nearly round cylinders to nearly flatsided prisms with sharply rounded corners, thus obliterating the tissue spaces between them. On each contraction of the gross muscle the capillaries and venules which lie between those fibers which do contract are suddenly compressed, squeezed, flattened out, and kept empty by the lateral expansion of the shortening thickening muscle fibers. The blood is forced out of the capillaries and venules, some goes backward into small arteries, more goes downstream into small veins, and, as long as the compressing pressure of the contracted fibers is greater than the local blood pressure, the capillaries and venules between contracted fibers are held squeezed out, flattened and empty. As seen under the microscope a muscle which is tightly contracted, as in the first part of a prolonged tetanic contraction, almost always is a translucent to opaque silvery-white color with but a few traces of the yellow-red of hemoglobin in it. While the muscle is tightly contracted it contains almost no blood.

During the period of the contraction the empty flattened capillaries and venules continue to relax, probably as a direct response to muscle metabolites already being released from the adjacent contracted fibers. (See ANREP and SAALFELD, 1935.) Thus at the moment the contracted muscle fibers begin to relax, the arterioles, capillaries and venules have a wide potential lumen, though at that moment each is compressed flat like an empty trouser leg. As the muscle fibers relax, the compression of the vessels is released, and the blood spurts from the dilated arterioles into the flaccid dilated capillaries and venules filling and distending and rushing through every small vessel in the muscle[1]. At such a time the muscle is bright red; under the microscope frog muscle may appear to contain almost as great a volume of blood as of muscle fibers.

A muscle may have as many as 700 capillaries per 200 muscle fibers (KROGH, 1929, p. 29) and many small venules (see KROGH's figure 5, 1929, from SPALTEHOLZ, 1888). Thus the total maximal physiological capacity of the vascular bed of a striated muscle is higher than one might at first suppose. KROGH, 1919b, calculated that "the maximum amount of blood which can be present in the capillaries of guinea pig muscles is not less than 750 times the minimum." During the relaxed phases between rhythmical contractions and for a time after the last contraction of a series, all of a muscle's arterioles, capillaries, and venules are simultaneously dilated and contain a lot of blood, all of which is moving very rapidly.

At the end of the last contraction of a series all the vessels remain dilated for a time, and then (probably as the muscle metabolites released during the contractions are carried away in the flowing blood, and the concentrations of these metabolites

[1] Cinema recorded.

around the small vessels progressively decreases) the arterioles, capillaries, and venules of the muscle progressively constrict. In very lightly anesthetized animals, opened with no blood loss, many arterioles shut off completely. A few remain open just wide enough to permit a small trickle of blood through each. Most of the capillaries and many of the post-capillary venules constrict tightly throughout their lengths.

Thus after the muscle has been at complete rest for some time, blood is slowly flowing through first one capillary and then another, but not through more than one-tenth to one-fourth of the muscle's capillaries at any one time.[1] (This confirms KROGH, 1919.)

The above observations agree with the experiments of ANREP, BLALOCK and SAMAAN, 1934; ANREP, CERQUA and SAMAAN, 1934; ANREP and SAALFELD, 1934; and of BÜLBRING and BURN, 1939, which were made to determine the effects of the contractions of the striated muscles of dogs on the flow of blood through the contracting muscles.

Further, the observations of ANREP and colleagues cited above and our own microscopic observations are confirmation of experiments originally performed by J. LINDHARD in 1920 on unanesthetized unoperated men. LINDHARD found in subjects doing static muscle work that (a) the uptake of oxygen during the work period was less than during rest, (b) the oxygen uptake increased remarkably little during the work and (c) immediately at the end of the work a significant increase of metabolism followed. From this he concluded that the statically contracting muscle presented a mechanical obstacle to the flow of blood.

When the arterioles, capillaries and venules of a muscle constrict at the end of a series of the muscle's rhythmical contractions, a few red cells are always left stranded here and there in one capillary or post-capillary venule or another and a few capillaries may remain dilated, filled with whole blood, or with plasma containing but a few cells[2]. But except for these, most of the capillaries are constricted tightly shut throughout their lengths, have no real lumen and contain no blood[2]. This is a most important point. Since KROGH's 1919a and 1919b studies of muscle circulation, it is common knowledge that in resting muscle but a few capillaries have blood flowing through them. It is, however, little known and seldom appreciated that those capillaries of muscles through which blood is not flowing do not contain stationary blood, but are almost all completely empty.

In a frog with an adequate blood volume, that is, in an animal which has enough blood stored in its liver so that it can maintain its homeostatic responses, the vascular system of a resting muscle is extremely sensitive to contractions of the muscle. Microscopically visible temporary tightenings of a few muscle fibers are followed promptly by temporary dilations of adjacent small vessels. After one or two weak contractions of a whole muscle many of its vessels begin to open up. After a few strong, rhythmical contractions all of its vessels are widely dilated again, and compressed flat empty on each contraction of the muscle.

[1] Cinema recorded. [2] Recently photographed.

When a muscle goes into a resting phase with most of its arterioles shut off for long periods, the endothelium of those capillaries through which blood is not flowing becomes progressively anoxic. After a time, probably because of lack of oxygen (see LANDIS, 1928), they become permeable to blood plasma colloids. Being empty they do not of course leak plasma during the period when no blood is entering them; they cannot lose fluid which they do not contain. But when the muscle makes one long, strong contraction, or when it goes into rhythmical contractions, the arterioles and capillaries dilate as a response to the muscle metabolites, and the first blood to enter the muscle flows into capillaries which are at that moment permeable to such large molecules that all or nearly all of the entering plasma goes out through the capillary walls. This is unmistakable under the microscope, because as the column of blood passes from arterioles to capillaries, the red cells, moving in a single file or double row, suddenly come progressively closer together, so that by the time they have traversed from $^1/_3$ to $^2/_3$ of the capillary's length the capillary contains a moving column of closely packed red cells. There is no space between the red cells nor between the red cells and the endothelial wall for more than very thin films of plasma. The best place to study this is in the ventral surface of the submaxillaris muscles of frogs which are so lightly anesthetized that they respire occasionally, thus using the submaxillares voluntarily. The loss of fluid from the first blood to enter muscle capillaries can most easily be seen in preparations which are covered with a bit of cover slip so that oxygen cannot come in through the surface of the muscle. However, it is frequently possible to observe this phenomenon deep in a muscle even when the surface has not been covered with a cover glass. (In the flat broad submaxillares this phenomenon can be studied at magnifications as low as 48 × stereoscopic. See fig. 1).

The blood pressure available in muscle capillaries certainly is not high enough to force all the water and crystalloids out through endothelium which is impermeable to colloids and force the retained colloids into almost zero volume of space against the inwardly directed osmotic "attraction" force of those colloids. This and the fact that almost the whole plasma volume goes out through the wall is inescapable proof that most of the plasma proteins go out along with the water and crystalloids. (See KROGH, 1929, p. 14 and chapter XIV.)

Probably, under most physiological and pathological conditions, but a small fraction of those proteins which leak out through the walls of small vessels returns to the vascular system through the walls of the same or neighboring small vessels. Probably under most conditions most of such lost proteins can return to the vascular system only by way of the lymphatics. Normal well-oxygenated endothelium acts like a semi-permeable membrane; it retains proteins (see LANDIS, 1928, and 1936). But when it is partly anoxic, as the downstream ends of some capillaries and post-capillary venules frequently are during certain phases of physiology, or when it is injured sufficiently, endothelium loses its ability to resist the passage of proteins (see ROUS, GILDING and SMITH, 1930; SMITH and ROUS, 1931a; ROUS and SMITH,

1931; SMITH and ROUS, 1931b; SMITH and DICK, 1932). If sufficiently anoxic or injured it becomes fully permeable to all of the osmotically active proteins of the blood; the red cells pack tightly together, completely filling the lumen of a small vessel (cf. LANDIS 1928 and KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH 1945). Whenever endothelium begins to leak proteins, the concentration of osmotically active proteins outside the leaking endothelium begins to rise, approaching as a limit the concentrations of the same proteins inside the vessel. Thus the difference between the osmotic "attraction" of the proteins inside and outside the vessel decreases, approaching zero. When ordinary capillaries and small venules are leaking whole plasma quantitatively, as they do whenever they are sufficiently anoxic or otherwise damaged, the concentration of osmotically active proteins inside and outside almost certainly are equal, in which case the osmotic attraction of the protein inside and outside are equal. At such a time the protein inside cannot assist by osmotic "attraction" in returning the lost fluid to blood vessels.

Perhaps even more important is the fact that the inward attraction force of the proteins inside a vessel can operate only when the endothelial membrane is impermeable to those proteins, that is when it is in the "semi-permeable" state. During those periods of normal and pathologic physiology when the linings of various sets of vessels are permeable to the blood proteins, the osmotic attraction of the proteins within the vessels cannot act toward returning the fluid lost to the lumen of those vessels with altered walls. Further, a rise in tissue pressure around vessels probably does not ordinarily act toward forcing lost protein-containing fluid in through the vessel walls, for whenever the hydrostatic pressure in tissues around small vessels becomes significantly higher than the local blood pressure, the small blood vessels collapse. Under the conditions defined most of the protein solutions lost from small vessels can return to the vascular system only by way of lymphatics.

A short time after arterial blood has begun to flow into previously anoxic muscle capillaries, the endothelial walls regain their ability to retain plasma. (See LANDIS, 1928.) From then on if the muscle rests, the red celles do not pack together as they pass along the open capillaries. During rhythmical contractions of the muscle many of its capillaries do, however, often remain partly permeable to some of the proteins, probably those of lesser molecular weight, for the red cells do come noticeably closer together, but not tight together, as they pass along the capillaries.

These direct observations of the loss of fluid from anoxic muscle capillaries as the muscle begins to contract and as it contracts rhythmically, made in frogs, agree with (1) BARCROFT and KATO's 1915 finding that there is an increased loss of fluid from the vessels of dog muscles during periods of rhythmical contractions and (2) with WHITE, FIELD and DRINKER's 1933 finding that there is an increase in the rate of lymph return from the legs of unanesthetized dogs when the dogs begin to exercise the muscles of their legs as they go from rest to walking or running. However, frog blood contains lower concentrations of proteins than mammalian blood, and it is commonly believed that under a number of conditions frog capillaries retain proteins

less well than mammalian capillaries, consequently, during muscular exercise the capillaries of mammalian muscle probably do not normally lose (leak) as large a proportion of the plasma of the blood flowing through them as do frog muscle capillaries.

One of the most striking aspects of the striated voluntary muscle system of any animal is the fact that one or two small muscles or many muscles or nearly all the muscles can go into or out of action at any moment, and almost any other conceivable proportion can be in action the next moment. Almost all of the resting animal's muscles may go into action at once as he begins to run or swim. Thus the total capacity of the vascular beds of muscles can and does change by rather small increments and decrements or by large amounts, and these changes in capacity often occur very rapidly.

For the present purposes the most significant points in the physiological changes in the vascular beds of frog striated muscles are:

(1) There are phases of muscle function during which the capacity of the vascular bed of a muscle is very low and the muscle contains almost no blood.[1]

(2) There are phases during which there is a rapid increase in the capacity of a muscle's vascular bed and at the end of which the muscle contains a considerable amount of blood.[1]

(3) There are phases of muscle function during which fluid, containing protein, is rapidly lost from the vascular system into tissue spaces and lymphatics.

(4) There are times when small numbers of small muscles are going in or out of action.

(5) There are times when larger numbers of muscles are going in or out of action.

(6) Voluntary, striated muscles can and frequently do go from rest to strong rhythmic contraction very suddenly.

The increase in the total capacity of the small vessels of a muscle, or of a group of muscles during certain phases of function is an actual direct increase in the total capacity of the vascular system. Blood is neither distensible nor compressible, hence the blood which fills this space must come from elsewhere; and when the space is obliterated the blood it contains must go somewhere.

The volume of the protein-containing fluid (as distinct from the total extravascular and extracellular fluid) which is at any moment in the tissue spaces and lymphatics is equal to the sum of the rates at which fluid has been lost from small vessels, minus the sum of the rates at which it has been returned to the circulatory system by lymphatics. During some periods of normal physiologic processes the sum of the rates of loss can be considerably larger than the sums of the rates of return. At the end of such a period the amount of protein-containing fluid in tissue spaces and lymphatics can be quite high. During succeeding periods the sums of the rates of return normally exceed the sums of the rates of loss, and the volume

[1] Cinema recorded.

of such fluid in tissue spaces and lymphatics decreases to insignificant amounts. According to DRINKER, 1946 p. 813, "Where the dimensions of lymph capillary areas have been measured and compared with similar figures for blood capillaries in the same tissue the results are quite similar, so that potentially the lymphatics can prove a fairly large fluid reservoir." And, "The total amount of lymph present at any one time cannot be approximated, but lymph volume must vary markedly and even fairly abruptly." Thus even though any small portion of protein containing fluid is only temporarily lost from the vascular system, the total amount present in the tissue spaces and lymphatics can, for short periods, be large enough to be worthy of attention.

A volume of blood equal to the volume of fluid temporarily lost from the vessels must come from somewhere within the vascular system, and when this fluid is returned to blood vessels an equal volume of blood must go somewhere in the vascular system. A certain extravascular space must be filled from and return blood to the blood reservoirs just as though the tissue spaces and lymphatics were a direct expansion of the vascular system itself.

The General Effects and Compensation of Capacity Changes in the Vascular Bed.

The above, reasonably detailed descriptions of capacity changes in the vascular beds of kidneys and striated muscles are sufficient to illustrate capacity changes of peripheral vascular beds. As is well known, in many organs, human skin for instance, there are direct vascular capacity changes as various numbers of arterioles, capillaries or venules change caliber during different phases of functions of the organs. Further, every movement of fluid in or out of the vascular system acts toward changing the capacity of the system.

If an animal's vascular system is to function as a competent hydraulic machine it must have blood depots which can respond to the summations of all the direct and indirect changes in the capacities of the peripheral parts of the system.

Blood from three large sources comes to the heart, a) the superior vena cava, b) the infra hepatic abdominal vena cava, and c) the hepatic veins. The cardiac output is governed by the venous supply to the heart. The heart cannot pump any blood it does not receive; therefore it cannot put out more blood per minute than it does receive. Hence at any moment the rate of supply sets the maximum cardiac output. Further, within wide limits the normal heart responds rapidly and quantitatively to increased rates of filling by increased rates of output. Consequently, within wide limits the output of the normal heart is equal to and determined by the amount of blood flowing into it. (See KROGH and LINDHARD, 1912; KROGH, 1912a and b; PATTERSON and STARLING, 1914; PATTERSON, PIPER and STARLING, 1914; BAINBRIDGE, 1915; STARLING, 1918; WIGGERS and KATZ, 1922; WIGGERS, 1923; and GREEN, 1944.)

The rate of supply of blood to the heart depends upon the pressure at which blood is supplied to the heart (the effective venous pressure, see WIGGERS, 1923, p. 105) and upon that alone. Consequently, the factors which determine venous pressure determine cardiac output and the summations of the moment-to-moment changes in these factors determine the moment-to-moment changes in cardiac output.

If the summations of the direct and indirect changes in the capacity of the vascular system were not compensated rapidly and quantitatively there would be wide uncontrolled fluctuations in venous pressure, rate of venous return and cardiac output. Many individual small changes in the capacity of vascular beds do, of course, cancel each other; the capillaries and venules of one set of muscles may be closing while those of another, or of kidney or of skin are opening, etc. And under many circumstances changes in total capacity are partly compensated by partial contraction of veins.

Sometimes it is assumed that the capacity changes in capillaries, venules and veins continually cancel each other. But this is certainly not always so. For instance, when human beings begin to run, there is (1) an increased flow through striated muscles (KROGH and LINDHARD, 1912), which must mean that the small vessels of the muscles are dilated, (2) the skin all over the body may become red and warm, which means that cutaneous vessels are dilated, and (3) the superficial veins are all dilated and have an increased pressure at the same time. (See HOOKER, 1911, 1914 and WHITE, 1924, and WHITE and MOORE 1925). An increase both in the capacity and pressure in the peripheral vascular system can occur only if blood is ejected from the reservoirs. The point is that under many conditions the algebraic summations of all the changes in capacity of the various vascular beds leaves a margin, a positive amount which must be stored, or a deficiency which must be supplied if there are not to be uncontrolled fluctuations in venous pressure, venous return and cardiac output.

In order that the venous pressure may be controlled, and thereby the cardiac output controlled, independent of moment-to-moment small or large changes in the total capacity of the peripheral vascular system, an animal must have one or more reservoirs capable of compensating the moment-to-moment changes in

(1) the total capacity of the system, and

(2) the venous pressure of the system.

To meet these necessities an animal's reservoir system needs

(1) the ability to retain blood, hold it back, prevent it from returning to the heart;

(2) a large total capacity to meet large changes in peripheral capacity;

(3) the ability to accept or eject small measured amounts and multiples thereof in order to permit precise control of circulating blood volume;

(4) to be able upon occasion to eject blood against pressure in order to elevate venous pressure, and

(5) needs to be able to make its responses rapidly to prevent fluctuations in venous pressure.

The portal reservoir system of frogs satisfies these five conditions. Thus far we have seen no evidence in living frogs that the capillaries or venules of skin (see FRANKLIN, 1937, p. 91, and MCDOWALL, 1938, p. 100), or of lung (FRANKLIN, 1937, p. 94; MCDOWALL, 1938, p. 100; and SJÖSTRAND, 1934) act as blood reservoirs. There are small capacity changes in these organs, but the capacity and flow in these vessels have not indicated that they act as reservoirs; they have exhibited no outlet sphincters, that is, no ability to retain blood; capacity changes in them have been among those to which the portal reservoir responds. In frogs, the present evidence indicates that the portal area, consisting in frogs of the liver and portal vein with its tributaries, is by far the largest and probably the only reservoir system in the animal.

There is some evidence (reviewed by FRANKLIN, 1937, p. 91 and by MCDOWALL, 1938, p. 100) that in man the venous plexuses of the skin can act as a reservoir. And in normal mammals, including man, we know, from the investigations of BARCROFT and his school and others, that the spleen acts as a reservoir for concentrated blood cells. (See BARCROFT, et al., 1921—23; BARCROFT, et al., 1925; HARGIS and MANN, 1925; BARCROFT, 1926; BARCROFT and STEPHENS, 1927; BARCROFT and POOLE, 1927; SCHEUNERT and KRZYWANEK, 1926; YANG, 1928; YANG and CHANG, 1930[1]; BINET, 1930; PAFFENHOLZ and SCHÜRMEYER, 1931; BAUMANN and SCHILLING, 1931; KNISELY, 1936b; MACKENZIE, WHIPPLE and WINTERSTEINER, 1941; PECK, quoted by HOERR, 1944; the excellent reviews by MCDOWALL, 1938 and BJÖRKMAN, 1947). As far as we now know in normal mammals the blood from the spleen cannot reach the vena cava without passing through the hepatic outlet valves. (Guinea pigs and Rhesus monkeys have hepatic outlet sphincters anatomically like those of frogs; see p. 58.)

It is sometimes assumed that the collecting veins and larger veins of the body act as blood reservoirs. Certainly they undergo changes in capacity. Further, the venous pressure is the resultant of the volume of blood in the large veins and the degree of tonic constriction of the large veins. And the large veins can constrict to nearly zero lumen during severe hemorrhage or when an animal has a low blood volume. But in contradistinction to true blood reservoirs the larger veins have no outlet valve mechanisms which can constrict and cause the veins to retain blood. The valves of the veins are flap valves and prevent back flow. They do not hinder forward flow. As previously pointed out, blood returns to the heart from three great sources, the superior vena cava, the infra-hepatic abdominal vena cava, and the hepatic veins. Of these, only the hepatic vein system is as yet known to have throttle valve mechanisms which can retard or prevent forward flow and thereby cause controlled retention of blood.

In frogs there are times when most of the outlet sphincters of the liver lobules are tightly constricted, the sinusoids are widely dilated each full of blood[2], and blood is backed up in the portal vein and its tributaries. At such a time the blood retained in the liver and portal vein is not in the general circulation. The fact that it is out

[1] YANG and also YANG and CHANG found that human spleens can store and release concentrated red cells.
[2] Cinema recorded.

of the general circulation is a large factor acting toward maintaining a physiologically low circulating blood volume. When increasing amounts of blood are released from the liver the increasing rates of release increase the rate of supply of blood to the heart. The amounts released increase the circulating blood volume and act toward increasing the venous pressure and maintaining a higher rate of supply to the heart and cardiac output. As the amount of blood released from and kept out of the liver and portal vein tree can be maintained at any level, the circulating blood volume can be maintained at any level. And insofar as the amount released and excluded from the liver contributes to an increased venous pressure it helps first to accelerate and then maintain an increased venous return to the heart.

Thus far the sinusoid and small sluice channel outlet sphincters are the only contractile structures which we have seen cause the liver to store and release blood. (See Arey, 1941.) We have never seen the blood stand still in the sinusoids and central veins when the afferent vessels, sinusoids and outlet sphincters were open, as would happen if sublobular veins or larger hepatic veins were shut off. The sublobular veins and larger veins do dilate and contract as a part of coordinated reactions. But thus far they appear to be regulating their own capacity, as somatic veins do, rather than opening and shutting like valve mechanisms.

Each one of the outlet sphincters controls the storage or release of a small amount of blood, or if the linings of its sinusoid or sinusoids are in one of the colloid permeable phases, of concentrated cells. One, two or any number of the outlet sphincters may shut down, causing the storage of precisely measured amounts of blood or of blood cells. When they all shut at once they do not remain tightly shut for an indefinite period of time. A few intermittently open and close, thus permitting enough flow through the lobule to keep the lobule alive even when large amounts of blood are being stored. One, two or more or any number may open slowly or suddenly, releasing blood or blood cells into the central veins and thence to the vena cava. Thus the amounts of blood and of blood cells which are stored can be and are increased or decreased by very small measured amounts, and by controlled multiples of these very small amounts. And the storage or release reactions can occur rapidly. Thus in the frog, under experimental conditions at least, the red cell count of circulating blood, the circulating blood volume, the venous pressure, the volume of blood supplied to the heart, and hence the cardiac output are precisely controlled from moment to moment continuously.

The above observations are in accord with the following seven concepts which have been derived from many earlier experiments on the blood reservoir and cardioregulatory roles of the splanchnic reservoir systems of mammals:

1. The liver itself can and does change volume because of increases and decreases in the amount of blood it contains. (See Stolnikow, 1882; Johansson and Tigerstedt, 1889; Thompson, 1899; Macleod and Pearce, 1914; Mautner and Pick, 1915; Edmunds, 1915; Weil, 1917; Bainbridge and Trevan, 1917; Hunt, 1918; Lamson and Rocca, 1921; Lampe and Mehes, 1926a and b; Clark, 1928;

McLaughlin, 1928; Mattson, 1929; Grab, Janssen and Rein, 1929; Gollwitzer-Meier, 1930; Dock and Tainter, 1930; Tainter and Dock, 1930; Paffenholz and Schürmeyer, 1931; Pick, 1931; McMichael, 1932 and 1933; Bauer, Dale, Poulsson and Richards, 1932; Snyder, 1938; Deysach, 1941.)

2. The portal vein and its tributaries, including the great number of small venules of the intestines, can change capacity and thereby act as a blood reservoir. These vessels can dilate, and under the influence of the splanchnic nerves they can constrict and thereby eject blood against pressure. (Cf. Mall, 1896; Thompson, 1899; Donegan, 1921; Jarisch and Ludwig, 1927.)

3. In normal mammals the spleen can dilate and contract; it acts as a reservoir for concentrated red cells. (See authors cited on page 51, and the review Chapter VI of Franklin, 1937.)

As far as is now known in normal mammals blood from this great splanchnic reservoir (consisting of the liver, portal vein plus its tributaries, and spleen) can return to the vena cava only by passing through the hepatic veins, that is, only by passing through the hepatic outflow control mechanisms[1].

The anatomical mechanisms which control the outflow from the liver may be different from species to species; smooth muscle has been described on veins of various orders from the sublobulars up to the large hepatic veins, and these muscles have been assumed, usually with but little and poor physiological evidence, to be the throttle veins of the great reservoir system. (See the reviews by Benninghoff, 1930; Franklin, 1937; Tischendorf, 1939; and Arey, 1941.) It has frequently been assumed, purely on the basis of negative evidence, that some species, such as the cat, do not have outlet control mechanisms. Deysach, 1941, has shown that cats and several other species of mammals do have anatomical apparatus capable of regulating the outflow from the liver.

4. The liver can withdraw blood from the general circulation and thus reduce the work of the heart. (See Stolnikow, 1882; Johansson and Tigerstedt, 1889.) The latter found that when saline or defibrinated blood is transfused into rabbits large quantities of blood are stored in the liver. They say (translated) "The second fact to which we would draw attention is the great distension of the liver which we have observed in all our transfusion experiments and which has also been noted by earlier workers. After the injection of large quantities of fluid the liver becomes almost as hard as a board. If after the death of the animal the liver is cut out, fluid streams from it in great quantities. We see then a considerable quantity of fluid is taken up by the liver and thus withdrawn from the general circulation." (See also Thompson, 1899; Dock and Tainter, 1930; Tainter and Dock, 1930; Pick, 1931; and Roberts and Crandall, 1933.)

[1] Not enough is yet known about the collateral circulation of the portal bed to permit a careful analysis of its functions during health. (For the anatomy in human beings see standard textbooks of gross anatomy.)

It seems possible-to-probable that these collaterals may be under strict vaso-motor control and that they may be controlled safety-valve overflow pathways for blood in case the reservoirs become over filled.

5. So much blood can be retained in the liver and backed up in the portal vein and its tributaries that the animal has a sharp fall in arterial pressure or even dies in one of the shock states, from failure of venous return to the heart. (See Thompson, 1899; Voegtlin and Bernheim, 1911; Mautner and Pick, 1915; Weil, 1917; Elias and Feller, 1931; Arey and Simmonds, 1920; Simmonds and Brandes, 1925a, 1925b and 1927.)

Consequently, under ordinary physiological conditions the hepatic outflow control apparatus must be so regulated as to prevent too much blood from being stored in the great splanchnic reservoir.

6. In the absence of the hepatic outflow control apparatus and controlled portal reservoir, dogs with normal blood volumes die from overwork of the heart. Stolnikow, 1882, made Eck fistulas and removed the livers of dogs, taking care to lose as little blood as possible. This (a) short circuited the portal circulation, (b) prevented the hepatic outlet valves from preventing an oversupply of blood to the heart and (c) retained a nearly normal volume of blood in the animal. This last is an absolutely essential part of the experiment.

Under these conditions, the abdominal veins became widely dilated, they pulsated, and spurted blood when injured, and the animals' hearts became greatly dilated before they died. Stolnikow said, "Bei der Section findet sich an der Stelle der exstirpirten Leber und überhaupt in der Bauchhöhle kein Blut, folglich hat keine Zerreissung der unterbundenen Lebergefässe stattgefunden. Das Herz ist im höchsten Grade dilatirt, — ein klassisches cor bovinus; so grosse Herzen haben wir kein einziges Mal bei Hunden getroffen. Die grossen Venenstämme sind blutüberfüllt."

Consequently the hepatic outflow control mechanisms must normally be so regulated as to prevent prolonged oversupply of blood to the right heart.

7. In mammals the regulation of the volume capacity of the great portal reservoir continually affects and acts toward controlling the circulating blood volume, circulating red cell count, venous pressure, and cardiac output. (See the spleen references on page 51 and Krogh and Lindhard, 1912; Krogh, 1912a and b; Dock and Tainter, 1930; Tainter and Dock, 1930; Pick, 1931; Rein, 1933; Eppinger, 1933; Wollheim, 1933; Nissen, 1933; Barcroft, 1934; Franklin, 1937; and Green, 1944.)

Summarized without the references the seven concepts are:

1. The liver itself can and does change volume because of increases and decreases in the amount of blood it contains.

2. The portal vein and its tributaries, including the great number of small venules of the intestines, can change capacity and thereby act as blood reservoirs. These vessels can dilate and under the influence of the splanchnic nerves they can contract and thereby eject blood against pressure.

3. In normal mammals the spleen can dilate and contract; it acts as a reservoir for concentrated red cells. As far as is now known, in normal mammals blood from the great splanchnic reservoir, consisting of the liver, portal vein, plus its tri-

butaries, and the spleen, can return to the vena cava only by passing through the hepatic veins, that is, only by passing through the hepatic outflow control mechanisms.

4. The liver can withdraw blood from the general circulation and thus reduce the work of the heart.

5. So much blood can be retained in the liver and backed up in the portal vein and its tributaries that the animal has a sharp fall in arterial pressure or even dies in one of the shock states, from failure of venous return to the heart.

Consequently, under ordinary physiological conditions the hepatic outflow control apparatus must be so regulated as to prevent too much blood from being stored in the great splanchnic reservoir.

6. In the absence of the hepatic outflow control apparatus and controlled portal reservoir, dogs with normal blood volumes die from overwork of the heart.

Consequently the hepatic outflow control mechanisms must normally be so regulated as to prevent prolonged over supply of blood to the right heart.

7. In mammals the regulation of the volume capacity of the great portal reservoir continually affects and acts toward controlling circulating red cell count, circulating blood volume, venous pressure and cardiac output.

The mammalian experiments which lead to the development of the above seven concepts were for the most part of such a nature as to permit the demonstration of moderate to large changes in the capacity of the great portal reservoir, but not small changes. The microscopic observations outlined in the preceding sections show that the control of the exit of blood from the liver lobules of frogs is much more precise than had previously been supposed. The volumes which are stored and released are precisely controlled, and the times when and rates at which storage and release occur are also under continuous precise control.

The Autotransfusion Reactions: When a lightly anesthetized frog, which has a moderate to large percentage of its hepatic sinusoids storing blood or concentrated blood cells, has a moderately rapid hemorrhage from any part of its body, the terminals of the hepatic arterioles and portal venules and the inlet sphincters constrict tightly shut, the outlet sphincters open, the sinusoid linings constrict (often peristaltically from the periphery of the lobule toward the center), thus ejecting the contents of the sinusoids into the central veins. Thus the animal "gives himself a blood transfusion." The outlet sphincters then close, which prevents backflow into the sinusoids. The central and sublobular veins may also constrict somewhat (but usually not tightly shut) thus ejecting a little more blood. The whole autotransfusion is a precisely coordinated reaction of the individual structures which participate. In well nourished lightly anesthetized animals the blood is actively ejected by contraction of the sinusoid linings. The evidence is that the sinusoids may be completely empty and contracted so tightly that they have no lumen at the end of the ejection. GILBERT and VILLARET 1909 did perfusion, injection, and histological sectioning experiments from which it is at least possible and perhaps necessary to conclude that the sinusoid linings of mammalian livers have the power of active contractility. When a hemorrhage occurs

in a heavily anesthetized frog, the blood merely runs out of flaccid sinusoids, and irregular amounts may be left in various relaxed flaccid sinusoid systems.

If not too much blood has been lost from the animal, then from time to time after the autotransfusion reaction one afferent vessel or another of the liver lobules may open a bit, and a few sinusoids dilate some, letting an irregular trickle of blood through a small percentage of the sinusoids. This appears to be entirely comparable to the irregular intermittent opening of a few small vessels in resting striated muscle in response to accumulations of local tissue metabolites.

When a well nourished lightly anesthetized frog which has a moderate to large percentage of its hepatic sinusoids storing whole blood or concentrated blood cells has a very small continuous hemorrhage from any part of its body, individual sinusoids and small groups of sinusoids go into individual autotransfusion reactions one after another causing small amounts of blood to be continuously ejected from the liver. During a long slow hemorrhage the circulation in brain, connective tissues, smooth muscles of GI tract, lung, striated muscles and kidney usually show little or no changes in numbers of capillary systems open, or rates of flow through them until the blood stored in the liver is nearly gone. The circulation in each of these organs or tissues proceeds and the vessels react in accordance with their own reaction patterns (see the descriptions of the reaction patterns of the vessels of striated muscles and of kidney glomeruli, pages 42 to 49) just as though no hemorrhage had occurred or was occurring. That is, these progressive autotransfusion reactions compensate the slow losses from the vascular system as rapidly as they occur for almost as long as there are reserves of blood with which to do the compensating. As the hepatic reserves are beginning to be depleted the arterioles of striated muscles and of connective tissues go into prolonged spasmodic contractions, and increasing numbers of kidney glomeruli shut off for increasing periods of time.

All of the above descriptions apply to animals which have a competent blood volume—that is, animals which have enough blood to fill their various capillary beds, conducting vessels, and heart, and have enough left over so that various moderate-to-large amounts are stored in the liver. There are, however, particularly in the late winter or early spring, frogs which have a very low blood volume when they are first opened, even though no blood has been lost in the preparatory operation. Animals which have a low blood volume to start with, or from which blood has been lost in the preparatory operation, may have but little blood stored in the liver, and have flow through but a small percentage of the sinusoids, the rest being empty and contracted shut, or almost shut, throughout their lengths.

It is not possible to get blood out of an empty liver. Animals which have a low blood volume to start with or from which too much blood has been lost in the preparatory operation do not perform the autotransfusion reaction. Livers cannot autotransfuse more blood than they contain. In response to severe hemorrhage, a maximal autotransfusion reaction is not followed by a second one.

A Discussion of Literature Concerning Mammalian Livers. Loeff-

LER and NORDMAN, 1925, transilluminated living livers of mice, rats, and rabbits and studied the vascular reactions at 38 × and 55 × magnifications. They saw the interlobular portal venules, sinusoids, central and sublobular venules and found that each of these vessels is contractile. Occasionally they also saw a hepatic arteriole. They saw the sinusoids change capacity. The magnifications they used obviously were too low to permit precise studies of the anatomy, or of the co-ordinated reactions of the parts of the living liver lobules of the species they studied.

Dr. KAHIL G. WAKIM, who spent two weeks in our laboratory in March, 1939, studying our methods, preparations and motion pictures, has since published partial confirmations of some of these observations in frog livers, and has extended some of them to some of the smaller laboratory mammals (see WAKIM, 1941; WAKIM and MANN, 1942; MANN, 1941 and 1942; and WAKIM, 1944).

WAKIM and MANN have published several concepts which we believe to be in error. These and the sources of the errors seem to us to be as follows:

1. They concluded that some of the radial segments of some lobules do not receive branches of the hepatic artery, and some do not receive branches of the portal vein. (See WAKIM and MANN's 1942 Figure I.) This concept is a result of having failed to observe carefully enough or long enough to see or recognize the respective branches to these areas when the branches were dilated. The concept is based solely on negative evidence, and is a result of having studied but a few livers, each with insufficient care.

2. They concluded that "About 75% of the hepatic circulation is in an inactive state under ordinary conditions when the liver is not under the influence of either excitatory or inhibitory agents." Here they have introduced an ambiguous and misleading term, for "inactive" does not distinguish which of the activities in which sinusoids participate may or may not be going on during the "inactive state." Further, as they use the term, it implies that the sinusoids alone control the rates of flow through sinusoids. But aside from this, the concept that 75% of the sinusoids are ordinarily in any one state is quite wrong. As has been pointed out, the circulation in the liver lobule is continuously co-ordinated with the other parts of the circulatory homeostatic adjustments of the animal. Sometimes all sinusoids are in storage phases, sometimes 75%, sometimes none are, at other times other proportions. In co-ordination with various circulatory homeostatic reactions almost any proportion of the sinusoids may perform any one of their visible functions. The concept that 75% are "ordinarily" in any one state probably came from studying too few animals, all more or less in the same state of experimental physiology, and that state probably was a result of similar degrees of hemorrhage at operation, and almost complete rest of the animal's striated muscles during the observations.

3. WAKIM and MANN, 1942, concluded that in frog livers there were places where hepatic arterioles run along branches of the hepatic venules and are interconnected with these hepatic venules by arterio-venous anastomoses. This is a very important point for if such shunts existed they would permit hepatic artery blood to pass through the liver without passing through the sinusoids—that is, without nourishing the hepatic tissues. We

do not believe that any hepatic arterioles run along central or sublobular venules or that any such shunts exist. This concept of WAKIM and MANN is based on a failure to identify the portal venules, mistaking them for central or sublobular hepatic venules.

There are times, usually short in normal animals, when the arterio-portal anastomoses between interlobular terminal hepatic arterioles and the corresponding interlobular portal venules (see Figure 7 Page 28) are dilated so widely that arterial blood flows into the sides of the portal venules so rapidly and at such high pressures that blood flows backwards in the stems of the interlobular portal venules. At such times the tips of the portal venules distal to the arterio-portal anastomoses carry blood forward into the lobules and the stems of the portal venules proximal to the arterioportal anastomoses contain blood moving backward away from the lobules. Under such conditions, if one first saw the blood flowing backward in the portal venules, he might mistake these for efferent central venules interconnected with closely adjacent hepatic arterioles.

However, many times we have watched while arterio-portal anastomoses between vessels which were easily identifiable as hepatic arteriole and portal venule opened slowly while portal blood flowed forward in the portal venule. Each time the sequence of events was as follows: As the anastomoses begin to open, the arterial blood mixes into the portal blood and is carried forward along with it. When the pressures are just about balanced the flow may jet forward from the anastomosis into the side of the portal venule on each systolic phase of the arterial pulse and all flow through the anastomosis stop for a moment on each diastolic phase. (We have a motion picture taken in the liver of Rana esculenta which shows this.) If the anastomosis opens more widely (1) the pressure at the junction of arterio-portal anastomosis and portal vein rises, (2) distal to that junction the tip of the portal vein then conducts pure arterial blood toward the lobules, and (3) the blood flow reverses in the stem of the portal venule. At this time the portal venule stem carries blood backward away from the lobule.

As the arterio-portal anastomoses close again, the flow in the stem of the portal venule again goes forward toward the lobules. And this is evidence that this vessel is really a portal venule, for if it were a central or sublobular venule, the flow would continue to be backward (efferent) when the anastomoses closed.

Further, whenever we have found such a place and given the animal sodium indigo disulphonate, the arteriole and its accompanying venule were found to be running along an interlobular bile duct in the portal space. This we take to be definite evidence which identifies these vessels as hepatic arteriole and portal venule.

For these reasons we do not believe that any hepatic arterioles run along central or sublobular hepatic venules, or that there are any shunts between hepatic arterioles and efferent vessels of the lobule.

LOUISE WARNER, 1941, working under Dr. SYLVIA BENSLEY, was able to stain certain cells which encircle the outlet ends of the hepatic sinusoids of guinea pig livers, in such a way as to reveal fibrillar structures within the cytoplasm of these encircling cells which were indistinguishable from myofibrils.

BLOCH has been studying the livers of living Rhesus monkeys. With the ex-

ception of the arterio-portal anastomoses, the intermediate permeability phase, and the reactions to hemorrhage and low blood volume which he has not yet studied, he has found that the anatomical pattern, and the behavior of the vascular system of the monkey liver lobule is almost identical with that of the frog liver. In the monkey KNISELY has seen the outlet sphincters, the tubular sinusoid lining, and that the von Kupffer cells are constituent cells of that lining, and frequently has seen the sinusoids receiving pure portal blood, and once saw all the hepatic artery tips dilated widely in lobule after lobule (while the portal vein tips were shut off), sending torrents of arterial blood jetting through all the arterial sinus twigs into and through all the sinusoids. Hence it seems inescapable that in the monkey, as in the frog, the particular states of those circulatory homeostatic reactions in which the liver is participating, from moment to moment continually under all normal and pathologic conditions, set the maximum rates at which particles suspended in the blood can be brought hepatic phagocytes. (Cf. KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH, 1945.)

The Significance of the Variable Blood Flow through the Liver for the Rate of Phagocytosis.

As frequently stated one of the main purposes of this paper is to show the relationship of the rates of phagocytosis of particles from the blood stream to circulatory phenomena. We have now described enough of the anatomy and circulatory physiology of the living liver lobule to make it possible to begin to take up the selective phagocytic removal of particles from the blood by stationary hepatic phagocytes.

Foreign particles suspended in the circulating blood are carried into each hepatic sinusoid at whatever rate blood is flowing into that sinusoid. Hence, the rate of supply of particles to hepatic phagocytes is faster when many inlet and outlet sphincters are open than when the outlet sphincters are closed during periods when the sinusoids are in blood storage phases or when many sinusoids are empty and shut off after hemorrhage or when an animal has a low blood volume. And the fastest rates of supply of suspended foreign particles occur when the hepatic arterioles are widely dilated sending blood in jetting pulses into, through, and out of the sinusoids. A stationary phagocytic sinusoid lining von Kupffer cell cannot come in contact with a foreign particle suspended in the blood until that particle is carried to the phagocyte. Consequently, the rates at which blood flows through the hepatic sinusoids from moment to moment, continually set the upper limit on the rates at which particles can possibly be brought to these phagocytes. As has been shown, the rate of flow through sinusoids is controlled in the uninfected frog by the activities of the parts of the lobule which control the environments of the hepatic parenchyma cells and control the storage of blood or blood cells, and thereby control peripheral red cell count, circulating blood volume, venous pressure and cardiac output. Thus the maximum rates at which particles can be brought to the hepatic phagocytes are forever limited by the circulatory homeostatic reactions of the animal. This also holds true when the homeostatic reactions are out of order, or undergoing special reactions in infected animals, or in animals which have

had hemorrhages or which have a low blood volume. Thus the particular states ot those circulatory homeostatic reactions in which the liver is participating from moment to moment continuallyunder all normal and pathologic conditions set the maximum rates at which particles suspended in the blood can be brought to the stationary hepatic phagocytes.

Selective Phagocytosis by the Frog's Hepatic Phagocytes.

The anatomical and physiological factors which determine the rates at which particles suspended in circulating blood are brought to frog hepatic phagocytes have now been described. But the sinusoid lining cells do not ingest every particle which comes into the hepatic sinusoids. What factors determine the selectivity of the phagocytic removal of particles from the blood stream? Why do the sinusoid lining cells ingest some particles and miss or ignore others?

India ink was the first test substance used because (1) it is well known that the hepatic phagocytes ingest it, (2) it is not known to be antigenic, and (3) it is very black, which makes it possible to identify and study very small particles of it easily in many places in the circulatory system. Higgin's American India ink, undiluted, direct from a new bottle, was always used.

The reactions between ink, plasma, and von Kupffer cells are by microscopic standards massive and therefore can be studied easily. As the reactions with ink are typical for those with a class of substances, they will be described in detail.

The Coating of Foreign Particles Introduced into the Blood. When fine droplets of undiluted India ink are injected with a micrometer screw driven syringe into the blood stream, each droplet immediately receives a visible coating of a clear glassy precipitate, probably protein, derived from the frog's plasma (vide infra). The coatings on the ink droplets often, but not always, have long feathery streamers of newly-formed glassy precipitate attached to them. The coating, which entirely surrounds the ink, changes the ink droplet into a coated semi-solid particle. (Cf. Lewis, 1925, p. 363.)

Sometimes, if the injection is made very slowly, one can see the glassy coating already formed on the surface of the ink when the emerging droplet is no more than a hemispherical meniscus bulging out of the orifice of the needle. (See Fig. 16.) On ink this material frequently, but not always, forms a relatively thick layer which can easily be seen at but 48 to 96 × magnifications. Thus the outer diameter of the coated particle is greater than the diameter of the injected ink droplet. The coating is a clear relatively transparent material, sometimes slightly straw colored. It has an index of refraction enough different from that of plasma to make it visible, which is evidence that there is a real interface between the outer surface of the coating and the fluid plasma. Often the outer surface of the coating can be seen very sharply. A most significant point is that from the moment this coating is formed, no part of the surface of the ink itself is ever again in contact with fluid plasma.

The precipitate exhibits two quite different morphological forms. One is the

smooth rounded coating. After it is formed the ink no longer behaves like a fluid droplet but like a somewhat-rubbery semi-solid particle. The coated particle may be nearly spherical, or it may consist of several portions of rough spheres or spheroids like a potato with large knobs on it. The glassy coating frequently follows the contours of the knobs making the whole look like a minute black cumulus cloud with a tight glassy coating which has a smooth refractile outer surface. (See Figs. 17 to 29.) When such a coated particle turns over in the blood its various bumps and knobs do not move around on each other nor flow in and out of each other like pseudopods of an amoeba. The coating holds the subdivisions of the particle together tightly as though they were embedded in a rather stiff, but somewhat flexible, gel. Thus the coated ink behaves like a semi-rigid particle.

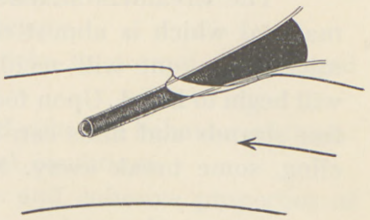

Figure 16. India ink bulging from needle tip into flowing plasma. Note cap of precipitate already formed on meniscus of ink.

Thick coating layers are visible under the microscope. Thinner coatings are less easily visible and the thinnest ones are not visible. But the behavior of particles can be used as one step in the evidence for determining whether or not they are coated. Those particles which we have studied which have not had coatings while in the blood stream have had no tendency to stick to each other. This included the animal's own normal red cells, which, as was pointed out in the first part of this paper, have no tendency to stick together. (See also Reel I of the Knowlesi Malaria Film.) Visibly coated particles in the blood stream have in these frog experiments exhibited a strong tendency to stick together whenever they touched each other. Sometimes upon contact they stick together tightly becoming in effect one particle. Thus the tendency of particles in the blood stream to stick to each other is strong presumptive evidence that they are coated.

The Streamers: The visible precipitate also forms long fine flexible streamers attached sometimes singly, sometimes in tufts, at odd points on the particle's smooth coating. The streamers are harder to see than the coatings; consequently they cannot be as accurately described. Further, no single description fits all forms of the streamers. They may be long fine feathery strands, fibrils or wisps, each almost independent of the others, or they may be matted together in little networks. Frequently they appear like shredded cotton waste, or like old badly mutilated lace. Quite often adjacent to the coated particle there is a hillock (like the axone hillock of an anterior horn neurone) or a matwork of fine strands which tapers off into one or two long whip-like wisps; and the ends of these frequently taper until their tips are too fine to see. (See Figs. 17 to 22.)

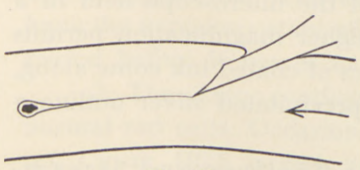

Fig. 17.

Sometimes it is possible to see the streamers formed from the material of the coating. As the ink droplet emerges from the needle tip one can see the coating already formed on it. One can see the coating stuck to one side of the orifice of the needle,

and see it drawn out into long fine strands as the blood stream pulls the coated particle away from the needle tip. (See Fig. 17.) But it is not possible to be certain that all of these strands are formed by being drawn out from the coating material. Frequently the streamers are formed, or perhaps come to one's attention, suddenly, without any evidence that they were drawn out from a previously precipitated coating.

The streamers attached to the coating on an ink particle look very much like material which is almost certainly fibrin. Often in studying living organs under the microscope, one will accidentally cut or tear a small vessel, or a cauterized vessel will begin to bleed. Upon focusing on the point where the blood escapes from the vessel, fine strands and films can be seen forming. Many form on the edge of the cut, some cling, some break away. Many form in the escaping blood. The streamers on the

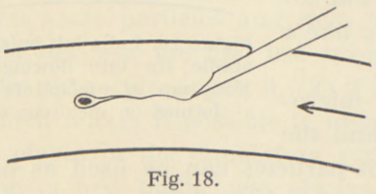

Fig. 18.

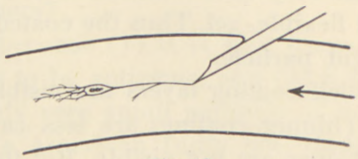

Fig. 19.

coated ink are morphologically similar to the strands and films which form at the edges of cuts and in escaping blood. The morphological similarity does not, of course, prove that the streamers or coatings are chemically identical to fibrin; the similarity indicates only that fibrin should be considered when attempting chemical identification of them.

The streamers are very flexible and surprisingly strong and tough. Often one sees a coated ink particle tethered by a thin visible streamer to the needle tip, or to a bit of injured endothelium, the full force of a rapidly flowing blood stream whipping and beating the particle back and forth for quite some time before the wisp of streamer is broken. (See Figs. 18 and 19.)

It is often possible to be certain that a coated particle has a long strong streamer without being able to see it. One frequently sees a coated clump of ink whipping and beating back and forth in the blood stream, somewhat downstream from the needle tip, or from a bit of injured endothelium, without being able to see the strand which tethers it. This is like watching a kite while being too far away to see the kite cord. The coated particle would be carried downstream out of the microscope field in a moment if it were not tethered. Sometimes a shift to a higher magnification permits one to see the tethering strand. Sometimes very fine particles of coated ink come along, stick to the waving strand and outline it just as bits of precipitated silver outline a nerve fiber in a Golgi preparation.

The Adhesive and Cohesive Properties of the Coatings and Streamers: The outer surfaces of the glassy coatings and streamers have some very special adhesive and cohesive properties. An important part of microscopic physiology and of microscopic pathologic physiology is knowledge of what structures stick to what within the vascular system. (Cf. Knisely, Stratman-Thomas, Eliot and Bloch, 1945, and

KNISELY and BLOCH, 1942, and 1945) Under carefully defined physiological, pathological, immunological, therapeutic, and experimental conditions, what sticks to what? Under the most nearly normal conditions which we have achieved experimentally in frogs and mammals:

1. Red cells do not stick to each other.
2. White cells do not stick to white cells of any category.
3. Red cells do not stick to white cells of any category.
4. Neither red cells nor any type of white cell sticks to normal undamaged ordinary vascular endothelium.

We have not yet attempted to study the adhesive and cohesive behavior of mammalian platelets or amphibian thigmocytes under normal conditions.

Because of the importance of the special adhesive and cohesive properties of structures present within the vascular system under normal, specific pathological, and/or experimental conditions, these properties of the outer surfaces of the coatings and streamers formed on India ink in frog blood are here stated in detail:

1. The outer surfaces of these coatings and streamers do not stick much, if at all, to the frog's normal undamaged ordinary vascular endothelium. They slide along undamaged endothelium for long distances, and, like mercury droplets on paper, show no signs of sticking.

2. These surfaces are, on the other hand, rather sticky to blood vessel linings which have been injured mechanically. Frequently when inserting the needle into the anterior abdominal vein one will scratch the lining of the vein with the needle tip. Sometimes one accidentally rubs the needle around on (by microscopic standards) quite large areas of the inner surface of the anterior abdominal vein. On all such injured surfaces coated particles accumulate, some by direct contact of the coating, some by having a wisp of streamer catch and stick, in which case the tethered particle may stick tightly or whip in the stream a while and then break loose and go downstream. The inner surfaces of injured vessel linings are also, of course, sticky to white cells (cf. E. R. and E. L. CLARK, 1935, and KNISELY, ELIOT and BLOCH, 1945), thus the injured lining accumulates a mixture of white cells and bits and shreds of coated ink. Sometimes, if the injury is not too severe, the white cells and shreds of coated ink both accumulate, giving the inner surface of the vein wall a mottled gray and black appearance. The sticking of coating material to injured vessel linings may have the very important effect of delaying the ingestion of the particle, prolonging the period before it passes to phagocytes. This will be taken up later.

3. The glassy coatings and streamers are not very, if at all, sticky to the frog's normal red cells. Occasionally, one sees red cells trapped in a mass of this material (cf. LEWIS, 1925, p. 364, and KNISELY, ELIOT and BLOCH, 1945), but hour after hour one can watch red cells carried along the surfaces of streamers and coatings which are tethered in the axial stream, without seeing the red cells show any tendency whatever to hesitate or stick to the surfaces of streamers or coatings.

4. When coated particles are being carried along in rapidly flowing blood, it is

quite difficult to determine whether or not the coatings and streamers are sticky to circulating leucocytes. But leucocytes in circulating blood which are carried against tethered streamers and particles, frequently stick to them. It is possible but not yet known with certainty that both white cells and coatings or streamers may have altered surfaces during various pathologic conditions which may make them much more sticky to each other. However, in frogs which have been opened with a minimum of trauma and use of the cautery, in those tissues which have not been traumatized at all, at least some white cells stick to some coated particles. The stickiness of this material to various types of the frog's circulating white cells and the ingestion of coated ink by those cells was studied by MARGARET R. LEWIS in 1925. Factors which may affect the adhesiveness of such coatings to the various types of circulating white cells of mammals, factors such as burns, trauma, and diseases (KNISELY and BLOCH, 1942, 1945, 1946) and drugs now obviously need rigorous study.

5. The outer surfaces of the coatings and filaments are quite sticky to themselves. When the coatings or streamers of one particle suspended in plasma touch those of another, they tend to stick together, and frequently do stick tightly, thus building up aggregates in the circulating blood. This frequently proves useful for the observer. Long strands of filaments which are so fine and nearly transparent one can hardly be certain of their presence, accumulate bits and shreds of coated ink which outline the strand so clearly it is unmistakable even when it is moving.

6. The filaments and coatings which are precipitated from frog plasma on India ink are very sticky to the von Kupffer cells which line the frog's hepatic sinusoids. They are so sticky to the sinusoid-lining cells that the moment a coated particle touches the surface of one it sinks into the cytoplasm of the sinusoid lining, apparently as quickly as a small globule of mercury rolling along a laboratory table top touches and merges with a larger one.

The Source of the Coatings and Streamers: These observations immediately bring up a number of questions. Do the glassy coatings and filaments come from the ink alone or are they a result of a reaction between ink and blood, or do they come from the blood alone?

One often hears it said that India ink "tends to agglutinate," or is "agglutinated in the bottle" or "agglutinates in the blood." (Cf. LEWIS, 1925, p. 363; LANG, 1926, p. 45; and KROGH, 1929, p. 375.) What does the word "agglutinate" mean here? Does the ink "agglutinate" itself, or does something "agglutinate" it? To try to find answers to such questions the following simple experiments were done:

A thin drop of the HIGGINS ink was placed on a carefully cleaned slide and examined, using various intensities of light and magnifications of 100 × and 400 ×. The ink was spread out thin by drawing a hair through the main droplet, dragging a thin meniscus along the slide. As is well known, the smallest visible particles in India ink are a dirty gray, larger ones browner, and the largest quite black (cf. LANG, 1926, p. 45). The finest in rapid Brownian motion, the larger slower, the largest being barely jostled. Although the larger particles were obviously flocculated aggregates of smaller

ones (one could see small ones continually coming together forming larger ones, and large ones separating into smaller), the particles themselves were not surrounded with any coating of precipitate. Nor were any wisps or strands visible, nor did any particles tend to line up in any pattern which could indicate that invisible sticky strands were present. The fact that the particles exhibited Brownian movement is also evidence that they were not held together by an invisible precipitate. These are good evidences that the ink is not already agglutinated in the bottle, and does not agglutinate by itself on the slide.

Could the precipitate be formed by diluting the ink? Higgins India ink is probably kept in suspension by some type of protective colloid. Putting the ink into the blood might be diluting it. Could it be that upon simple dilution, the ink's protective colloid precipitates, forming the glassy coatings and filaments, or that upon dilution of the protective colloid, the ink particles agglutinate? To test this a droplet of ink was placed on a slide beside a drop of distilled water and a hair drawn from the ink over into the water, thus joining the two by a hair-width strait of ink. The exact points at which the ink dispersed into the water were immediately studied, with bright illumination and with decreasing light. Oblique illumination was used in an attempt to reflect light off otherwise invisible strands and precipitates (cf. KNISELY, ELIOT and BLOCH, 1945). The ink particles sometimes dispersed freely, sometimes flocculated and then dispersed, but no glassy or filamentous precipitates were ever seen. Nor did particles line up in patterns suggesting the presence of invisible precipitates, nor did the particles cease their Brownian movement.

The same experiment was done using the ink and frog Ringer's solution. The results were the same, no visible precipitates, no evidences of invisible precipitates, and the Brownian movement of small particles did not stop. These are evidences that the precipitated coatings and filaments seen when ink is injected in blood are not formed simply by diluting the ink with the water and/or electrolytes of the blood.

If the precipitates do not come from ink alone or from ink plus water and electrolytes, do they result from a reaction between constituents of ink and of blood, or can the precipitates come from constituents of blood alone?

To test this, a drop of freshly drawn frog blood was placed on a clean glass slide, a drop of ink was placed near it, and a hair drawn from the ink into the blood. A mass of thick coatings and strands formed on and around the ink as soon as it entered the blood. But perhaps the precipitates here formed resulted in part at least from coagulation of the blood on the clean glass slide. Consequently, the experiment was repeated, using a paraffined glass slide, and blood drawn from a frog's heart with a carefully oiled syringe. When the hair was drawn from ink to blood, glassy wads and strands of precipitate were formed just as happened when ink was injected into flowing frog blood.

This is evidence that one or more constituents of blood are necessary to form the precipitate, but does not indicate whether the precipitate can be formed from blood alone.

As noted above (p. 62) the filaments and wisps of the streamers look very much like the filaments, films, and strands which form as a clot begins to form in blood escaping from a cut blood vessel. Are the coatings and filaments which form on the injected ink related perhaps to fibrin?

To test this, frogs were prepared without blood loss as outlined above, the needle mounted in the anterior abdominal vein and 2 cc. of Roche Organon heparin (each cc. of which was sufficient to keep two liters of recalcified citrated beef plasma liquid for four hours at 37° C.) injected into the frog's anterior abdominal vein, immediately before the ink was injected. As the ink was injected into the heavily heparinized frog, while one watched the point of the injection needle with 96 × magnification, the ink and blood mixed without forming any visible precipitates (either glassy coatings or filaments) for some time. Later glassy coatings did begin to form on circulating ink particles. Thus heparin in high concentration did inhibit or retard, but did not prevent the formation of the coatings and filaments, just as it inhibits or retards, but does not prevent the formation of fibrin.

This suggests (1) that the glassy coatings and filaments may be in some way related to fibrin and (2) if so, can be derived from the blood alone without chemical union with constituents from the ink.

The composition of Higgins ink is a trade secret. It probably contains a number of substances, some of which might combine with constituents of blood. This suggested using test substances consisting of particles as nearly insoluble as possible. Kaolin and graphite have been tried. Each has been suspended in Ringer's solution and under a microscope injected (a) into untreated frogs, and (b) into frogs which previously received 2 cc. of Roche Organon heparin intravenously. In each case the precipitate formed as the material touched blood in untreated frogs; it did not form for quite some time in the frogs which had first received the heparin.

From this one may deduce that a precipitate having special adhesive and cohesive properties, perhaps fibrin or related to fibrin, can be formed from constituents of frog blood on insoluble particles injected into the blood stream.

However, it must be borne in mind that this is the extreme case. Here we have completely insoluble particles. When substances which are soluble or partly soluble and/or which can combine chemically with blood constituents are injected and do receive or form precipitates and coatings, these precipitates and coatings will not necessarily have the same composition as those which form from blood alone on insoluble particles. The exact composition of the coatings and precipitates will have to be determined for each substance injected and for each species of animal into which it is injected and perhaps for each immunological state of each species into which it is injected. It is worth while, however, to know as a point of departure that precipitates and coatings having special adhesive and cohesive properties can be formed within the living vascular system from constituents of blood alone.

The formation of coating precipitates on particles in the blood stream has a number of aspects important for pathologic physiology and immunology. (1) If the

coatings are formed from dissolved proteins, then, because only dissolved proteins can be osmotically active, the changing of dissolved protein molecules to precipitated proteins must be a factor acting quantitatively toward reducing the total number of osmotically active protein molecules within the vascular system. Precipitated proteins cannot assist osmotically in the retention of water within the vascular system (cf. STARLING 1896). (2) The formation of coating precipitates on particles must, regardless of what they consist, reduce the numbers of ions and/or molecules of dissolved precursors of these precipitates within the vascular system, thus decreasing their concentrations, thus (because of mass action effects) decreasing the maximum possible rates at which such precipitates can be formed in a long series of such precipitate-forming reactions. These two aspects may not seem important when one is considering the formation of coatings on but a few particles. But they may be very important when coatings are being formed on astronomical numbers of particles, as for instance when coatings are being formed on parasitized red cells in monkeys with malaria. (Cf. KNISELY, STRATMAN-THOMAS, ELIOT, and BLOCH, 1945.)

Utilization of the Streamline Flow Patterns of the Portal Vein System to Study Particles Being Ingested. To study particles while they are being ingested it is necessary (a) to know the branching and anastomosing pattern of the sinusoids of the particular small area of the liver into which the particles are to be carried, (b) to be able to watch the particles carried in the blood stream for a few moments before they are carried against the sinusoid lining, and (c) to be able to watch the ingestion of individual particles at high magnifications. A single microscope field includes but a small area, and the higher the magnification, the smaller the area, yet the area of one microscope field is all that can be studied at any one time. The chance or probability that any given injection of a few minute particles made into the anterior abdominal vein, will be carried into any randomly selected microscope field of the living liver, is practically nil. Hence a problem in technique is presented, namely to pre-select the microscope field in the liver to which a minute injection into the anterior abdominal vein will be carried. To do this, the edge of the liver of a freshly prepared animal is transilluminated and studied at low magnifications while a minute injection is made in the anterior abdominal vein. The coated particles are carried down the vein, into the portal vein trunk, out some intrahepatic branch, to some one set of interlobular portal venules, into the contiguous edges of some pair of adjacent lobules, into the sinusoids of one lobule and there ingested. The ingested particles now mark the position to which the stream line flow patterns now present in the portal system are now carrying particles from the present position of the needle tip in the anterior abdominal vein. If the stream line flow patterns are not disturbed, a second injection will be carried to the same microscope field.

Hence the experimental procedure consists of (a) transilluminating the edge of the liver, (b) making one small injection into the anterior abdominal vein, (c) moving the frog board about gently under low magnification until the injected material is found phagocytized in the lining of the sinusoids of some lobule, (d) adjusting the

illuminator, frog board and microscope so that this lobule and its afferents can be studied, (e) shifting to a higher magnification, (f) learning the precise anatomy of this area, (g) making a second injection and watching the injected particles carried into the afferent vessels of the pre-studied microscope field. When one is in practice, all this can be done in a few moments.

The theory of this is very simple; the practice requires skill and patience. The stream line flow patterns are amazingly precise as long as no part of the animal moves. But the slightest movement of the animal, even a respiratory movement, or the movement of a leg, or even a few small peristaltic motions of the G.I. tract, establishes a new stream line flow pattern, so that a new initial injection must be made.

It is possible to tie an animal down so that the stream line patterns carry blood from one root of the portal vein to a specific region of the liver, but during the free life of unanesthetized animals, particularly mammals which have a series of abdominal pressure changes with each respiratory movement, the stream line flow patterns of the portal vein must be changing from moment to moment continually. (Cf. COPHER and DICK, 1928; HENSCHEN, 1932; LICHTMAN, 1942, p. 39 to 41.)

The Role of the Coatings and Streamers in Selective Phagocytosis: It is now possible to describe in some detail the steps in the selective removal of one ink particle from the blood stream of the frog by one stationary hepatic sinusoid-lining von Kupffer cell.

Most of the coated particles whose ingestion we have studied have been from 15 to 30 micra in diameter. The streamers attached have had variable lengths, from 50 to 100 micra up to a millimeter or more. The widths of those hepatic sinusoids of frogs into which blood is flowing may vary from 15 to 30 micra; occasionally they are as wide as 60 micra. (See dimensions of sinusoids in figs. 13 and 14.) The injection must be made very slowly through a fine glass micro-needle tip (about 20 to 50 micra in diameter cf. CHAMBERS and KOPAC 1937) to keep the size of the coated particles small enough to be carried into the sinusoids. The particles must be small enough to pass through the finest tips of the afferent vessels at the periphery of the liver lobule, and pass into the hepatic sinusoids. If an injection is made too rapidly, two different results may occur either of which ruins the experiment:

(a) The material may leave the needle tip in large droplets, receive a coating of precipitate from the blood and thus form large irregular masses which embolize the intrahepatic tips of the portal vein before reaching the sinusoid-lining cells.

A small embolus in the portal vein tip or in the outer end of a tubular sinusoid obviously prevents blood from passing into a small area of liver tissue, hence prevents particles suspended in blood from being carried to the phagocytes of the small area. This obviously prevents these phagocytes from acting to remove particles from circulating blood. The point is that each embolus, hence multiple embolization of areas of the liver, acts directly toward decreasing the number of phagocytes which can receive particles. Multiple embolization thus has the direct effect of decreasing the total capacity of the hepatic phagocytic system. (See p. 81).

(b) If the injection is made quite a bit too rapidly, a whole column of the injected material fills the vein completely with only the ends of the column touching the blood. Such a column of Higgins India ink goes down stream and passes through the liver, turning the lobules so black that the processes going on in the sinusoids cannot be studied.

1. When injected into the blood stream the ink droplet immediately receives a coating which completely separates it from the blood. Some coated particles have streamers and some do not.

2. The outer surface of the coating thus formed is not sticky to red cells, nor to normal undamaged ordinary vascular endothelium.

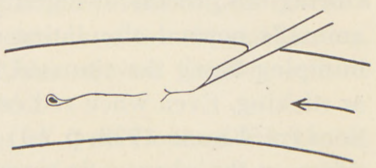

Fig. 20. Particle breaking loose from needle tip.

3. The coated particle is swept along downstream into the portal vein trunk, from there to the liver and out along an intrahepatic portal venule and through to an intralobular branch not sticking at any point as long as it is outside the liver lobule. Thus the flowing blood, and it must be flowing, brings the particle to the immediate neighborhood of the phagocyte. (See Figs. 20 to 29.)

As a coated particle which has streamers passes from an intralobular portal vein tip lined with ordinary endothelium into the branching and anastomosing sinusoids lined with phagocytic cells any one of several similar but slightly different things may happen to it.

1. At the first or any other sinusoid bifurcation the coated particle may go one way, the tail of one of its streamers the other. (See Fig. 22.) The streamer tail is thus whipped around the sharp point of the bifurcation and there forced into contact with the sinusoid lining. There is sticks instantly. The coated particle is now tethered to the sinusoid lining at one side of the blood stream like a boat tied by a rope to a river bank. (See Fig. 23.) The force exerted on the tethered particle by the flowing blood develops two components, one longitudinal in the direction of the stream, one lateral toward the sinusoid lining. The longitudinal force pulls the streamer taut; the lateral component slaps the particle against the sinusoid lining, and at whatever point or points the coating material and the streamer touches the sinusoid lining, the clear glassy coating with its contained ink droplet and the streamer pass instantly into the cytoplasm of every sinusoid lining cell or cells they touch. (See Fig. 24.)

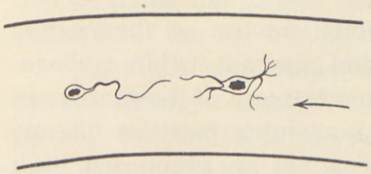

Fig. 21. Particles being carried down a root of the portal vein.

2. A streamer hanging from a coated particle may drag a short distance anywhere along the sinusoid lining, suddenly stick and become attached. The flowing blood instantly pulls the streamer taut and pushes the tethered particle against the wall, and, wherever the coating or streamer touches the lining, the streamer and the coating with the ink it contains are instantly engulfed by the cytoplasm of the lining.

3. The glassy coating of the particle rather than a filament may first come in

direct contact with a lining cell, frequently at the point, or just at one side of the point of a sharp prow-shaped sinusoid bifurcation. In this case the coated particle, streamers and all, sinks into the cytoplasm at the point it touches the sinusoid lining.

The process of ingesting coatings which contain ink and streamers was selective. During the process of ingesting coated ink the phagocytes did not take up any of the animal's normal circulating red cells or white cells. These could be seen sliding or bumping along the sinusoid linings without showing any sign whatever of hesitating or sticking. Even when red cells "saddle bagged" across the points of sinusoid bifurcations (cf. KROGH 1929, p. 10) and were thus forced tightly against and battered up and down on the phagocytic sinusoid lining for as long as several minutes before slipping off, the normal uncoated red cells were not ingested. (As previously stated no coatings

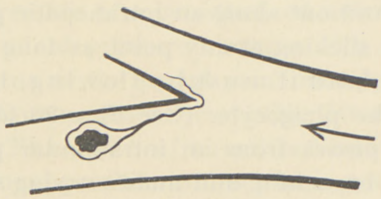

Fig. 22.

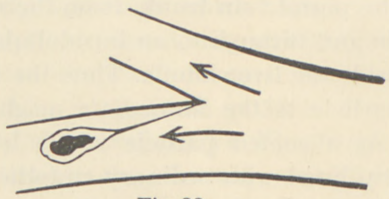

Fig. 23.

or filaments have been seen on these normal frog red or white cells, nor do they have any tendency to stick to each other as they might if coated with an invisible precipitate.) The process of ingesting coated ink selectively removed the ink, and left the normal uncoated blood elements entirely alone.

A coated particle which has no streamers may pass some distance along the sinusoid system before striking the wall hard enough to make firm contact with the lining. (See Figs. 25 to 27.) But at whatever point it bumps or rubs firmly against the lining, it passes instantly into the sinusoid-lining cell cytoplasm. (See Figs. 28 and 29.) Again, the ingestion of the coated particle is selective. Coated particles are selectively removed from the moving stream of normal uncoated erythrocytes, while the uncoated erythrocytes are completely "ignored" by the phagocytes.

Each ingestion of a coated particle removes a finite amount of the coating material, as well as the particle, from the blood stream, and places it within a phagocyte. Thus each ingestion must act toward depleting the blood stream of the precursors of the coating materials. Thus the ingestion of coatings containing particles directly and quantitatively removes the materials of which the coatings are composed from the blood stream. This must hold true for the ingestion of all chemical and immunological types of ingestible coatings which are formed on particles within the vascular system. Again this may not seem important when one is considering the ingestion of but a few particle-containing coatings, but it may be very important when coatings containing particles are being ingested by spleen, liver and bone marrow phagocytes in astronomical numbers, or for instance when coated parasitized red cells are being

ingested in Rhesus monkeys with Knowlesi malaria (cf. KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH 1945 and KNISELY, BLOCH, ELIOT and WARNER, 1947).

A sinusoid lining (von Kupffer cell) can ingest particles without being in Pfuhl's hypothetical "Fangstellen" catching position. The inner surface of the sinusoid is the inner surface of the cells which have the ability to ingest particles. This refutes HAVET's 1925 concept that the phagocytic von Kupffer cells were outside of the sinusoid lining. The history of concepts about the morphological positions of von Kupffer cells is reviewed by HIGGINS and MURPHY 1928 and PFUHL 1932.

The Time Taken for the Selective Ingestion of One Coated Particle: When ink was injected into a root of the portal vein the droplets (a) received a coating and became coated particles, (b) passed to the liver, and (c) were ingested, in not more than 5 to 15 seconds. After the coated particle touched the phagocytic sinusoid

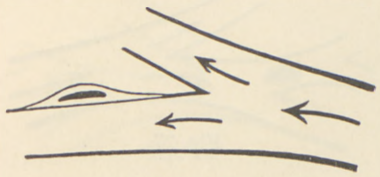

Fig. 24.

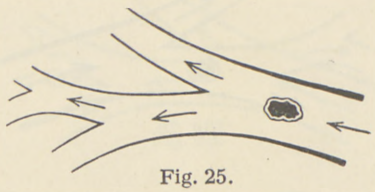

Fig. 25.

lining, the ingestion process took less than a second, probably not more than one-fourth of a second. These phagocytes have not been seen to put out pseudopods. Their observed ingestion processes have been much too fast for that, and thus far, appear rather to have the characteristics of simple strong surface tension phenomena. (See RHUMBLER 1898 a, b, 1910, 1914; FENN, 1921 a, b, c; MUDD, MCCUTCHEON and LUCKE, 1934.) Electrokinetic phenomena may, of course, also be involved. (Cf. MUDD 1933; ABRAMSON 1934; and ABRAMSON, MOYER and GORIN 1942.) The coated particles appear to enter the sinusoid lining cells as quickly as two mercury droplets which are rolled against each other merge into one. The actual time required for the process probably can be measured by taking high speed, i. e. "slow motion" motion pictures and counting the frames during which each step in the process takes place. (For an example of the use of this method, see KNISELY, ELIOT and BLOCH 1945.)

In the hepatic sinusoids which we have watched coatings containing ink were always removed from the columns of flowing blood cells so quickly that the flow through the sinusoids was not impeded at all (cf. KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH 1945.)

It seems probable that the conditions of these experiments were nearly optimal for rapid selective ingestion of each coated particle for: (a) Very few sinusoid lining cells contained visible previously-ingested material before the ingestion of ink began; hence, presumably the von Kupffer cells were not partially "blockaded". This may be most significant for, as Professor BENSLEY often points out to his students, "The properties and abilities of the cells studied in an experiment depend upon the previous

experiences of the cells as well as upon the circumstances provided by the experiment." (b) The interfaces between coatings and plasma, and plasma and sinusoid linings were sharply refractile, hence real interfaces, not intergrades of substances partially dissolved in each other. (c) The plasma was normally fluid, not abnormally viscous (as it becomes after traumatizing injuries to frogs) and (d) the coated particles were small enough and far enough apart so that each could enter the sinusoid lining cytoplasm easily. Under less favorable conditions the selective ingestion of each particle may take more time. It is, however, important to know as a point of departure, that under optimal conditions each selective ingestion of this type of coating from normally fluid plasma by this type of phagocytic cell can proceed from contact to complete ingestion in but a fraction of a second. (Cf. WERIGO 1892 and 1894.)

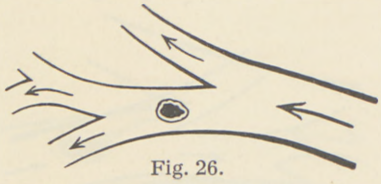

Fig. 26.

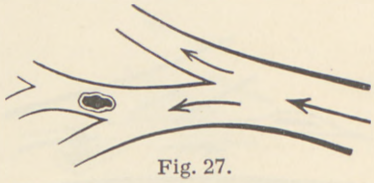

Fig. 27.

Some Changes in the von Kupffer Cells During and After Ingestion: As a small region of sinusoid lining ingests a coated particle, its volume increases. During a short interval after ingesting a particle some sinusoid-lining cells have been seen (at various magnifications up to and including 600 ×) to swell a little more, but it is difficult to be certain that this occurs every time. When it does occur the total increase in the volume of the von Kupffer cell is, of course, greater than the original volume of the ingested coating and its contents. The increase in volume after ingestion almost certainly indicates that the cell takes up additional fluid during this period.

After ingestion the swollen sinusoid lining cell may bulge, depending upon the summations of the pressures on each side of it (blood pressure and tissue pressure) either into the sinusoid lumen or outward indenting the adjacent hepatic parenchyma cell cord. During the flow of blood through the sinusoid these swollen cells usually bulge outward much more than inward. See Figs. 24 and 29. We have watched many individual cells at 400 × for as long as an hour after they have ingested coated particles. Neither before nor for one hour after ingesting particles have we seen von Kupffer cells suspended by processes within the lumen of the sinusoid like a spider in a tube; at all times during life they have been a part of the sinusoid lining. (Cf. PFUHL, 1926 and ZIMMERMANN, 1928.)

The details of all the above processes have been watched many times at 48, 96, 240 and 400 ×, and a few times at 600 diameters magnification.

Accessory Analytical Experiments.

Are the Coatings Sufficient and Necessary to the Ingestion Process? These questions were arrived at as follows:

The coated ink was selectively ingested by the hepatic sinusoid lining cells; these cells did not take up normal uncoated red cells. But the ink itself was completely coated with the glassy material. Thus no part of the ink itself ever touched the sinusoid wall; only the coating touched the lining. Thus the sinusoid lining cells did not take up ink; they took up coatings, and inescapably also the ink within the coatings.

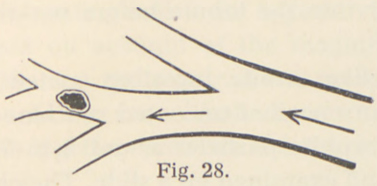

Fig. 28.

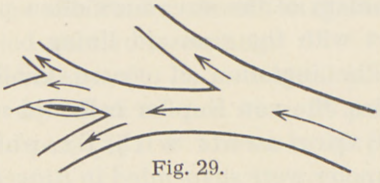

Fig. 29.

This raises two basic questions: (1) Will the sinusoid lining cells take up any substance enclosed in this particular coating material? Will these phagocytes ingest the coating no matter what is in it? Is the presence of the coating sufficient to permit ingestion of the coated particle? (2) Will the phagocytes ingest an uncoated particle? Is the coating necessary to permit the particle to enter the phagocyte?

This is the crux of this research. Does the presence of the coating, and the nature of its outer surface provide a selective factor for the selective removal of particulate matter from the blood stream by the sinusoid lining cells of frog liver?

To find answers to these questions the following experiments were done: Kaolin and graphite particles were suspended in Ringer's solution, studied on slides and then injected into untreated and into heavily heparinized frogs.

Experiments with Kaolin: According to the MERCK Index, 1940, kaolin is, "essentially a hydrated aluminum silicate, approximately $H_2Al_2Si_2O_8.H_2O$." Under the microscope, particles of kaolin are a yellow-gray brownish colo, hencer not quite easy as ot watch in the blood stream asbl ack India ink. On slides kaolin particles in Ringer's solution showed no tendency to stick to each other.

When injected into untreated frogs the larger particles of kaolin received a visible precipitate which exhibited the two familiar forms, the smooth coatings and the streamers. The coatings were thinner than those usually formed on corresponding sized particles of ink, and were therefore somewhat more difficult to see. The streamers formed on the kaolin particles were shorter, perhaps not quite as flexible, perhaps more brittle, than those which formed on ink. In the blood stream the smallest particles of kaolin always behaved like coated particles; they stuck to each other,

to injured endothelium and to the long fine strands on particles with visible coatings. These are evidences that the smallest particles received coatings which were too thin to see while moving, at the magnifications used. The smallest particles of kaolin probably did not have streamer filaments attached. The coatings and streamers on kaolin exhibited exactly the same adhesive and cohesive properties as those which form on India ink. Frog liver sinusoid linings selectively ingested the coatings containing kaolin particles from moving blood by exactly the same processes and just as rapidly as they did the coated ink particles.

There was one significant additional observation. Compared to ink, the kaolin injections provided a smaller proportion of large particles without streamers. The large particles with streamers tend to catch and stick and be ingested at the periphery of the lobule. The small particles which lacked the extraefficient contact ensuring mechanism of the streamers often passed farther into the lobule before making firm contact with the sinusoid lining.

The ingestion of coated kaolin was selective; while ingesting coated kaolin particles, the von Kupffer cells did not take up any normal uncoated red blood cells.

Experiments with Graphite: Next, graphite particles about 3 to 7 micra in diameter were suspended in Ringer's solution and examined on a slide. They showed no tendency to stick to each other, they had no coatings and no filaments. When injected into untreated frogs all graphite particles received coatings. Very few received visible filaments. The coatings were very thin, on most particles invisible. But in the blood of untreated frogs all graphite particles exhibited the same cohesive and adhesive properties as the visible coatings on kaolin and ink. In frogs which had received 2 cc. of heparin before receiving the graphite, no graphite particle received a visible coating and none exhibited the special adhesive and cohesive properties of coated particles. This is evidence that in untreated animals the coatings were present even when too thin to be visible.

In untreated frogs, when any coated graphite particle touched firmly against the hepatic sinusoid lining, the coated graphite was instantly ingested. While ingesting coated graphite particles, the sinusoid lining cells did not take up any normal uncoated erythrocytes. Thus the sinusoid lining cells selectively ingested coatings which contained graphite.

In summation, when finely powdered graphite or kaolin suspended in Ringer's solution entered the blood stream of untreated frogs, each particle or small group of particles received a coating. Usually in our experiments these have had smaller streamers than the ink particles, or no streamers. When coatings containing graphite or kaolin rubbed against the sinusoid lining they were instantly ingested. And this ingestion process was selective, the uncoated normal frog red cells on each side of the coated particles were entirely ignored by the phagocytes even though they rubbed on, or were rubbed tightly against and along the sinusoid lining. Hence, it is permissible to deduce that the hepatic sinusoid lining phagocytes of frogs can take up coatings which contain different kinds of materials.

Microscopic Observations of the Selective Ingestion of Coated Masses of Submicroscopic Particles; Experiments with Colloidal Mercuric Sulphide: This research is primarily a study of the selective ingestion of microscopically visible particles. In using India ink, kaolin and graphite we have descended a scale of particle size and visibility. As one descends this scale less dependence can be placed upon seeing the coatings and filaments to determine whether or not the particles are coated; more dependence must be placed upon a careful study and comparison of the behavior of the particles while they are on a microscope slide and after they are in the blood stream.

There is no reason, of course, for assuming that the size of a particle determines whether or not a coating will form on it in the blood stream. There is no reason for assuming that particles which are visible at one magnification or another would receive a coating and those beyond visibility at some arbitrarily selected magnification could not on account of the magnification selected receive a coating.

The next test material was selected precisely because (1) its individual particles are too small to see with the lenses used, (2) it is very black so that when its particles are close together their mass behavior can be followed, (3) it is known to be phagocytizable.

The material is a commercial preparation (Hille) of colloidal mercuric sulphide probably suspended in a protective colloid; for it must be used when fresh or kept in a refrigerator to prevent molds from growing on it and throwing the HgS out of suspension. It is insoluble in body fluids and therefore relatively non-toxic. After intravenous injection in pigeons and monkeys it is accumulated in large amounts in the phagocytes of spleen and liver and bone marrow. In vitro it is a rather clear-looking fluid whose exact color depends upon the thickness of the layer one is looking at and the intensity of the light used to transilluminate it. Very thin layers transmit a yellowish light. With increasing thickness the color is yellow-red, red-brown, to brown-black. The layer need be only a few tenths of a millimeter thick to appear jet black.

Undiluted on the slide the material has no particles visible at 400 × magnification. It is a clear homogeneous looking fluid, having no precipitates or strands in it. At 1000 × minute particles in Brownian movement are visible in it.

When injected into flowing blood in untreated frogs great broad veils of precipitate are formed on this material either instantly or a few moments after the material touches blood. Some simple strands are formed, too. In rapidly flowing blood one seldom can see the precipitate which holds the material together. By obstructing the anterior abdominal vein down stream from the injection point, one can stop or greatly retard the blood flow in it, and then watch the reaction between injected HgS and stationary or slowly moving blood. If large amounts of HgS are injected into slowly moving or stationary blood one can often see good thick coatings and strands formed around masses of the injected material. This shows that this mixture and untreated blood does, and therefore can, form the coating and streamer precipitates.

As noted, when the injection is made into rapidly flowing blood, one seldom can see the precipitate itself. What one does see are fine, thin delicate yellow-gray or brownish membranes whose various portions and folds maintain continuity with each other while they wave in the flowing blood. When first injected one might mistake these for mere streamline effects where one fluid enters another, but a moment later one can be sure that these veils resist stretching forces; and they never mix homogeneously with blood as an injection of Ringer's solution does.

One of these films is a but slightly stretchable yet very flexible membrane. As with ink, once the precipitate is formed, the injected material and precipitate no longer behave like a fluid, but like a solid. When the HgS is injected into blood which is not moving too fast, often the delicate membranous lacy veils form on and around the HgS and become attached simultaneously at several points on injured endothelium. Then the veil can be seen undulating gently in the slowly flowing blood. Frequently under these circumstances particles of the sheet break away or wisps and shreds tear loose. Sometimes it seems to disintegrate into many small fragments almost all at once.

These films or veils appear to be very thin sheets of the precipitate from blood, containing enormous numbers of submicroscopic particles. The outer surfaces of the films or veils exhibit the same adhesive and cohesive properties exhibited by the coatings on ink, kaolin and graphite.

If one makes very small injections of this HgS into the anterior abdominal vein while watching in the liver, all possible forms of fragments of these sheets come down stream. There are patches, strands, and folded masses. They touch the sinusoid wall and are instantly engulfed. Sometimes when injecting as slowly as possible while watching hepatic sinusoid linings at a point near where the last previous injection had been phagocytized, one can see clear transparent sinusoid linings slowly turn gray-brown as they accumulate fragments of this material, without ever being able to see one microscopically visible particle enter the cell. During the ingestion of coated colloidal HgS no normal uncoated red or white cells are ingested. Thus the sinusoid linings selectively ingest coatings which contain submicroscopic particles of this colloidal mercuric sulphide.

In summation, four substances, India ink, kaolin, graphite, and HgS (Hille), each receive coatings when injected into the flowing blood of frogs whose brains have been pithed or which have been anesthetized with urethane. As far as can be determined by the methods thus far used, these coatings have similar cohesive and adhesive properties while they are within the frog's vascular system. In all the experiments we have done, these particular coatings have been selectively ingested by the frog's hepatic sinusoid lining phagocytes regardless of what material was contained within the coatings. Hence it seems reasonable to assume, as a working hypothesis, that the presence of a coating of this material on a particle is sufficient to make that particle ingestable by the hepatic sinusoid lining regardless of the nature of the material contained within the coating.

Are the Coatings Necessary for the Ingestion Process?: Is it necessary to have a coating on a particle in order to have that particle selectively ingested by a hepatic phagocyte? Under the microscope the coatings and streamers look and act as though they might be fibrin. Hence frogs were given large, one or two cc., intravenous doses of heparin. Roche Organon Liquaemin was again used, each cc. of which was sufficient to keep two liters of recalcified citrated beef plasma liquid for four hours at 37° C. One or two cc. of this heparin is an enormous dose for a mediumsized frog, for the untreated frog's total blood volume is but 4 to 6 cc. After giving the heparin, the above experiments were repeated except that HgS was not used. In the presence of high concentrations of heparin coatings did not form on the injected ink, kaolin or graphite particles for a least five to ten minutes, and during the periods when the particles had no visible coatings, the particles did not have a tendency to stick to each other as coated particles do. And the naked particles slid along, bumped against, and rubbed along the inner surfaces of the sinusoid linings without being ingested. But as the heparin effect wore off, circulating particles began to stick to each other, coatings began to be visible and coated particles began to stick to and be instantly ingested by whatever sinusoid lining cells they touched.

These observations are evidence that (1) the phagocytes did not take up uncoated particles, and (2) that when the particles did receive their coatings, the phagocytes took up these particles in the presence of a high concentration of heparin.

Two more observations were made; once the coatings were on the particles the injection of more heparin into the animal did not (1) cause the disappearance of the coatings which had been formed, nor (2) stop the ingestion of previously coated particles.

Further Tests to See if the Coatings Are Necessary to the Ingestion Process: It seemed possible that the very high concentrations of heparin, which were necessary to inhibit the formation of the coatings for even short periods of time, might also have had a temporary effect on the phagocytes; the failure to take up the uncoated particles might have been due to an effect of the high concentration of heparin on the phagocytes rather than, or in addition to, the fact that the particles were uncoated.

Hence, it was necessary to try to find some particles which when injected into untreated frogs would not receive coatings. When amber particles (made by filing a bit of amber with a jeweler's file) or fine (6 to 12 micra) droplets of viscolized cream (obtained from the Wanzer Milk Company, Chicago) were injected into untreated frogs, the particles or droplets did not receive a coating for at least five or ten minutes, and during the period when they were not coated, they slid along or rubbed against the sinusoid lining without being ingested. When animals in which the amber or cream droplet tests had been made were immediately afterwards injected with India ink, the ink immediately received a coating, and that coating, including the contained ink, was ingested when- and whereever it made firm contact with hepatic sinusoid

lining. Hence, the hepatic phagocytes in these frogs did not fail to take up amber or cream because the phagocytes were inactive or indisposed; the evidence seems clear that hepatic phagocytes of these untreated frogs selectively ignored the uncoated particles of viscolized cream and amber.

Uncoated Frog Red Cells Are Selectively Rejected by von Kupffer Cells: Throughout all these experiments, the normal red cells of the frog have had no visible coatings. Nor have they exhibited any tendency to stick to each other as coated particles and coated red cells often do. (Cf. KNISELY, STRATMAN-THOMAS and ELIOT, 1941; KNISELY and BLOCH, 1942, 1945; KNISELY, STRATMAN-THOMAS, ELIOT and BLOCH, 1945; KNISELY, ELIOT and BLOCH, 1945.) The uncoated normal red cells have been seen to slide, bump, and grind along the inner surfaces of the sinusoid linings but were not ingested. Even when they "saddle bagged" (see KROGH, 1929, Fig. 3, p. 10) across the sharp point of a fork in the sinusoids and were forcibly pushed and pounded against the phagocytic lining for several minutes, the normal uncoated red cells were not ingested.

In summation, in heparinized frogs naked particles of India ink, kaolin, and graphite were not ingested. In untreated frogs viscolized cream and amber were not ingested. And in no experiment have we seen naked normal red cells ingested by hepatic sinusoid lining cells. Hence it seems reasonable to assume as a working hypothesis that some kinds of particles will not be ingested when they come in contact with frog hepatic phagocytes unless they are coated. The outer surfaces of some kinds of particles are not themselves ingestible. For particles having such outer surfaces, the formation of a coating on the particle must be a necessary preliminary to the actual ingestion process.

These observations are in agreement with the experiments of MANWARING and COE 1916; VON JANCSO 1929; HÖBER and TITAJEW 1929; FERRARI and HÖBER 1933; and HÖBER 1940, who found that protein constituents of the blood must be present to permit hepatic sinusoid lining cells to ingest foreign particles.

These experiments in the frog also agree with those of VON JANCSO, 1931. VON JANCSO gave mice and rats heparin, or novirudin, or germanin, which hinder or retard, but do not stop, blood clotting, and then gave them colloidal gold intravenously. Animals killed ten minutes after receiving the colloidal gold did not have colloidal gold in the phagocytes of liver and spleen. Control animals which had not received anti-coagulants did have colloidal gold in the spleen and liver phagocytes at the end of ten minutes. (See VON JANCSO's table I for dosages and details.) VON JANCSO emphasized that the hindering effect of these agents on this phagocytic ingestion lasts only a short time, just as their hindering effect on blood coagulation is only of short duration.

This type of experiment is not particularly easy to do, especially if one cannot watch and thereby control, each step of each experiment in the living animal; for there are a number of methods of failing to find the effect of the heparin. First, if the amount given is insufficient to inhibit the formation of thin coating films, or is only

sufficient to inhibit their formation for a brief interval, one might assume that heparin can have no effect on the phagocytic processes. It is obvious from the experiments described above that much higher concentrations of heparin are necessary to inhibit the formation of films on injected colloidal aggregates or on small visible particles than to inhibit gross clotting of drawn blood. Second, if one uses histological sections as indicators for estimating the amount of the injected material which has been ingested by the phagocytes, one must be certain to obtain the tissues to be studied either before, or just as soon as, this effect of the heparin begins to disappear and the films begin to form on the particles. Precise methods must be used in each experiment to determine if and when the coating films begin to form. For histological sections are summation indicators; they show the summation of the amounts of material ingested by individual phagocytes during the ingestion period. They do not show when the ingestion reactions began. Further, spleen and liver phagocytes are capable of accumulating material at truly amazing rates. For instance, PFEIFFER and TATUM, 1935, found that spleen and liver phagocytes of rats could remove an astronomical number of trypanosomes (see page 12) from the blood of Mapharsen treated rats in but 10 or 15 minutes. Hence, if one uses histological sections as indicators for estimating the amount of injected material which has been ingested by phagocytes, one must be certain to obtain the tissues to be studied either before or just as soon as this effect of the heparin begins to disappear and the films begin to form on the particles. If one waits long enough after the films have formed, before taking the tissues for sectioning, there will be no differences distinguishable by crude inspection-estimation methods, between the amounts of material in the phagocytes of untreated and heparin treated animals. These factors were uncontrolled in the experiments reported by RIGDON and WILSON, 1941, and by RIGDON and SCHRANZ, 1942, and RIGDON, 1944, and therefore from their experiments it is not possible to conclude that heparin has no effect on the ingestion processes. Although the experiments by RIGDON and colleagues do not help analyze the factors and mechanisms of selective ingestion, they do demonstrate the fact, which may be very important clinically, that considerable amounts of heparin do not necessarily inhibit phagocytosis for more than short intervals. As has been shown, VON JANCSO's experiments and ours also support this concept.

Materials May Exist which can be Ingested without Coatings: Professor KROGH pointed out, in 1939, that it is quite possible that some kinds of particles, perhaps some kinds of bacteria, may exist which when injected into the blood stream will not receive coatings, but whose own outer surfaces are of such a nature that the phagocytes can selectively ingest them. Obviously, none of the above experiments rule out this possibility. Obviously, the possibility must be kept in mind for such particles may be found at any time. As yet we have not found particles which fit this category.

Coated Particles are Selectively Removed from Moving Blood: Coated ink, kaolin, or graphite particles intermingled between naked red cells in

blood flowing slowly or rapidly through the sinusoids are selectively ingested while the naked red cells are entirely ignored by the sinusoid lining cells. We have never seen blood flowing so rapidly through frog hepatic sinusoids that the coated particles suspended in it could not be ingested by the sinusoid lining cells. The actual ingestion process is very rapid, not slow like the pseudopodial ingestion by amoebae or leucocytes (see LEWIS, 1925, pp. 365—366). When the hepatic arterioles are widely dilated, the blood flow through hepatic sinusoids is as fast or faster than it is through any other capillary system of frogs which we have yet studied. But no matter how fast the flow through the sinusoids up to the maximum rates we have seen, the phagocytes selectively remove these particular coatings with whatever they contain, from the flowing blood. Conversely, these phagocytes have never been seen removing particles from stationary blood. This particular selective ingestion process is so fast that a particle carried in blood which is decelerating because some contractile structure is closing, is selectively removed from the blood before the flow in the sinusoid stops. While the flow in the sinusoid is stopped, no more particles are brought into the sinusoid.

From this it seems reasonable to generalize as follows: Rapid flow is necessary into a phagocytic organ such as the liver, spleen, or bone marrow in order to permit rapid selective ingestion of particles from the blood by stationary phagocytes. The rates of taking up particles can be no faster than the rates at which they are brought to stationary phagocytes. However, rapid flow through a phagocytic organ may not result in rapid removal of foreign particles from the blood, for rapid flow alone is not sufficient to cause or permit the ingestion of suspended particles. It is of course still possible that under some circumstances as yet unobserved, particles whose own outer surfaces or the outer surfaces of whose coatings may be ingestible may still, for some as yet unknown reason, be carried through a phagocytic organ so fast that the particles cannot be ingested. As noted, this possibility has not yet in our experience been encountered.

The Factors of Chance and of Probability: Some small coated particles may pass through the sinusoid system over into the central veins without ever really touching the sinusoid walls. (They must of course touch the wall to be ingested.) They are carried along in an axial stream, a thin peripheral layer of plasma separating them from the sinusoid lining. Three factors affect the probability that a coated particle will touch the sinusoid wall. First, the size of the particle. The larger the particle, up to a size that would embolize an afferent vessel, the more probability that the particle will touch the sinusoid wall. Second, the streamers on the particles tend to catch on the walls or whip around sinusoid bifurcations. The streamers provide an extra-efficient contact-ensuring mechanism. Other things being equal, a particle with a streamer has more chance of touching the wall than one without. Third, the repeated branching and anastomosing of the sinusoid system increases the probability that a particle will touch the wall. A particle being carried in the center of a sinusoid may be forced directly against the lining at a fork in the sinusoid. Histological sec-

tions of livers from animals which have received small intravenous doses of ingestible particles have more particles in the phagocytes at or near sinusoid bifurcations than elsewhere; and more near the periphery of the lobule than toward the center. This last is true because the phagocytes toward the center of the lobule have less chance of being bumped by a particle than those toward the periphery. For at whatever point a particle is phagocytized its ingestion automatically deprives all of those phagocytes farther toward the center from a chance to ingest that particle.

Interim Summary: The observations and experiments reported above may be summarized as follows,

In the frog:

1. Some kinds of particles will not be ingested by hepatic phagocytes unless they are coated.

2. The tendency of particles in the blood stream to stick to each other is strong presumptive evidence that they are coated.

3. The hepatic phagocytes selectively ingest these particular coatings regardless of what is in them.

Thus the coatings are necessary on some kinds of particles to permit them to be ingested by hepatic phagocytes, and when the coatings are present the coatings are sufficient to make the particles ingestible.

4. Uncoated red cells are not ingested.

5. Coated particles are ingested in the presence of high concentrations of heparin. Heparin does not dissolve the coatings. The presence of heparin does not stop the phagocytes from ingesting coated particles.

6. Thus far, the presence of the coating has determined the selectivity of the selective ingestion of particles by these stationary phagocytes.

7. The sinusoid lining cells can selectively ingest coated particles from blood which is flowing rapidly. The idea that blood must be stationary or flowing slowly to permit phagocytosis is not true.

8. Thus far particles have never been seen being ingested from stationary blood.

9. The particular states of those circulatory homeostatic reactions in which the vascular apparatuses of the liver lobules are participating, continually from moment to moment, under all normal and pathological conditions, set the maximum rates at which particles suspended in blood can be brought to the stationary hepatic phagocytes.

Significance of the Number of Cells Capable of Ingesting Particles, The Capacity Factor: We have now described the control of the rates at which particles suspended in blood are brought into hepatic sinusoids, and the coatings which cause or permit the phagocytes to select what they ingest.

The number of particles which can be removed from blood per hour or day depends upon these two factors and the chance that a particle will touch the sinusoid wall, plus one other factor—the number of phagocytes which at any one time are ready to ingest particles. (See also p. 68)

The literature contains the idea that some of the sinusoid lining cells are phagocytic and some are not. This is based upon the well-known fact that in histological sections from animals which have received injections of phagocytizable material, such as India ink, some of the sinusoid lining cells contain ink and some do not. (Cf. NATHAN, 1908, SCHILLING, 1909; ZIMMERMANN, 1928; PFUHL, 1926 and 1932; HIGGINS and MURPHY, 1928.) Two evidences show that this concept is erroneous; the same two evidences show that each and every sinusoid lining cell is a mature phagocyte instantly ready to ingest: (1) In our experience in normal, apparently healthy frogs, coated particles enter whichever sinusoid lining cells they touch or rub tightly against. Sinusoid lining cells do not contain particles until they have been bumped or rubbed by coated particles. And immediately after an empty cell is rubbed or bumped by a coated particle, it contains that particle. (2) If repeated small injections are made, all the sinusoid lining cells of an area can be made to take up coated India ink as fast as one can get it to them, say within half an hour. Hence, it is no more logical to say that those sinusoid lining cells in living livers or in histological sections of dead livers which do not contain particles are not phagocytes, or are not von Kupffer cells, than it is to say that a mouse-trap which does not contain a mouse is not a mouse-trap. The fact that one of these sinusoid lining von Kupffer cells does not contain particles is not evidence that it was not capable of ingesting particles.

The literature also contains the idea that these phagocytes can be "blockaded," that is, that they can become so full of particles that they cannot take up any more. But we personally do not yet know what the mechanical "blockading" of one single phagocyte really means. We have seen a single hepatic phagocyte take up two or three or four coated particles within a few minutes. But we do not know how many, or what quantity, it may take up before it refuses more; or how soon thereafter it might be ready to ingest again. Nor do we know the life span of one phagocyte; nor how long it takes the cytogenic processes to develop a new one; nor how rapidly upon adequate stimulus large numbers may be formed; nor how long it might take to form any large number of new phagocytes; nor what rate of production of phagocytes might be maintained over any long period of time; nor what physical, chemical or dietary factors determine and limit the rate of production of these phagocytes. This knowledge, which is seriously needed, must await the development of histological methods which will permit determination of the actual number of phagocytes in an organ, and determination of changes in the number of phagocytes in short precisely measured periods of time.

But we can say that the rates at which particles suspended in frog blood are carried into the hepatic sinusoids determine the maximum rates at which particles can be brought to hepatic phagocytes; and this rate together with the selective surface factors which determine that a particle is phagocytizable, and the factors of chance which cause a coated particle to touch a phagocyte, determine the rates at which particles are removed from the circulating blood by those hepatic phagocytes of frogs which are at any one time capable of ingesting particles.

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(4) The heparin used was generously donated by Roche-Organon, Inc. of Nutley, New Jersey.

(5) A part of this work was done while one of us was a Fellow of the General Education Board of the Rockefeller Foundation at the Zoophysiological Laboratory of the University of Copenhagen.

(6) It is a privilege to express appreciation and gratitude to Professors Robert R. Bensley, William H. Taliaferro, Preston E. Kyes, Paul Brandt Rehberg and August Krogh for suggestions and criticisms which have guided these experiments.

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