White Thromboembolism in the Hamster Cheek Pouch after Trauma, Infection and Neoplasia

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White thrombosis and embolism were investigated by cinephotomicroscopy in the transilluminated cheek pouch of the hamster in an experimental study of factors which may be involved in the febrile hemolytic syndrome in man, characterized by a finding of generalized platelet thrombosis of arterioles, capillaries and veins at autopsy. Endothelial trauma resulting from vascular occlusion produced platelet thrombosis and embolism. Dicumarol prevented the formation of platelet thrombosis and embolism after trauma. Heparin produced spontaneous platelet emboli and both heparin and dicumarol resulted in a leukocytic thrombosis. Platelet and leukocytic coatings and embolization were photographed during staphylococcus infection and malignant neoplasia. Sludged blood was not found in trauma, infection or malignant neoplasia.

An experimental investigation of the production of intravascular agglutination and the factors involved is significant because of the frequency of thromboembolism, both spontaneous and postoperative, and the practice of administering the anticoagulants heparin and dicumarol as therapeutic agents. In recent years, the intravascular agglutination of circulating erythrocytes bound together by a sticky glassy precipitate to form "sludged blood" has been reported by Knisely and co-workers.1-5 These workers 1, 3 described the formation of sludged blood in traumatic areas and proposed that sludged blood may form thrombi in the capillaries, resulting in the production of traumatic shock. Laufman, Martin and Tanturi6 occluded the mesenteric vein in the dog and reported that heparin and dicumarol caused a diminished adherence of erythrocytes to the vessel wall but did not prevent the formation of sludged blood. The authors concluded that anticoagulants prevent thrombosis by preventing agglutinated erythrocytes from adhering to the endothelial wall. Laufman, Martin and Tuell,7 using Knisely's quartz rod apparatus with magnifications of × 25 and × 48, found a clumping of blood elements which they interpreted to be the blood sludge described by Knisely. Odell, Aragon and Pottinger,8 using Knisely's technic for observing, at × 48, the blood flow in the conjunctiva of man, found sludged blood in a small group of pathologic cases and in pregnancy. Timonen and Zilliacus9 reported sludged blood in patients with skin allergies. Bloch10 reported sludged blood of the "soft clump" type in the conjunctivae of 262 patients suffering from poliomyelitis.

Except for a single in vitro experiment1 in monkey malaria, erythrocyte masses have not been probed or otherwise tested for adhesiveness. Blood flow examined under the usual binocular magnifications frequently shows that the erythrocytes are arranged in groups simulating the condition referred to as "sludged blood." This may account for the widespread occurrence of sludge reported in the literature under conditions where it would hardly be expected. For example, Fowler11 described "blood sludge" in the small venous vessels of the conjunctiva of the cat on electrical stimulation of the cervical sympathetic nerve and after injections of epinephrine. Since the procedures of Fowler produced vasoconstriction, sometimes rhythmic, the observations of Fahraeus12 that contractions of blood vessels may cause the appearance of aggregated clumps of erythrocytes in the blood stream provide a physiologic explanation.

The importance of white thrombi, especially the intravascular agglutination of blood platelets, is not recognized generally, although 20
or more fatal cases have been reported in the last 25 years, characterized by febrile hemolytic anemia, thrombocytopenia, and marked generalized platelet thrombosis of arterioles, capillaries and veins. Zucker reported platelet thrombi limited to the openings cut in the walls of the rat mesenteric blood vessels. Smith and co-workers observed the presence of a white coating, probably platelets, on the walls of blood vessels in the wing of the bat after irradiation. Youngner and Algie and Schlegel found circulating white emboli (not identified as platelets) in the blood vessels in a transparent skin flap preparation in the mouse. Copley and Robb found that heparin did not prevent the clumping of platelets in vitro in the dog and mouse. Copley demonstrated, in vivo, the presence of circulating platelet emboli of leukocytic size in the cheek pouch of the hamster following the administration of heparin. Copley and Fleck have speculated that hyperheparinemia, producing platelet agglutination, may be an important factor in the production of various types of shock. Furthermore, Copley suggested that heparin and platelet agglutination may account for the thrombocytopenia and hemorrhagic events of radiation sickness. However, nowhere in the literature has fragmentation of platelet thrombi to form circulating emboli been reported in connection with anticoagulant administration. Neither has the method of resolution of platelet thrombi been described.

During the past 15 years, we have been investigating vascular phenomena by direct microscopic observation and motion picture recording of the blood flow in the transilluminated retrolingual membrane of the frog, Rana pipiens, and the cheek pouch of the hamster, Mesocricetus auratus. In view of the controversial significance of "sludged blood" and the need for more direct microscopic investigations on thrombosis and embolism, we have investigated the characteristics of blood flow under pathologic and traumatic conditions similar to those in which sludge has been reported.

**Methods**

Hamsters were anesthetized with urethane administered intraperitoneally (0.15 Gm. per 100 Gm. body weight) or with intraperitoneal injections of Nembutal (0.15 cc. pentobarbital sodium per 100 Gm. body weight). At least one cheek pouch, and often both, were transilluminated in 75 hamsters by the method described previously for investigation of the peripheral vascular neuromotor mechanism. The anesthetized hamster was placed in a spun stainless steel operating dish and the cheek pouch was everted over an optical glass transilluminating block, covered with Ringer's solution, and maintained at 37 C. during observations by means of a thermostatically controlled electronically heated microscope stage. In the frog with brain and medulla pithed, the retrolingual membrane was prepared and transilluminated as described previously. Simultaneous observations and motion picture records were made by means of a light-splitting prism mounted on the body tube of the microscope in place of the three way nosepiece.*

Our methods for transilluminating the cheek pouch of the hamster and the retrolingual membrane of the frog permit clear visualization and photography of the characteristics of blood flow, the nature of the intravascular elements, and the structural components of the blood vessel wall. Microscopic magnifications varying from $\times 200$ to $\times 1200$ have been used. The methods used by other investigators for observing sludged blood in man, and in animals for the most part, have been limited by the necessity of examination by reflected illumination and relatively low magnifications ($\times 54$, $\times 90$). Furthermore, we have tested erythrocyte groups by compressing the blood vessel with a microneedle as an indication of intravascular agglutination.*

The pathologic and traumatic conditions selected for investigation were of three principal types: first, physical and chemical injury; second, infection; and third, malignant neoplasm. These conditions correspond with the various circumstances under which Nissely, Block, Eliot and Warner reported the presence of sludged blood. The sixty or more diseases in which sludged blood has been reported may be classified similarly.

Physical injuries were produced in 24 hamsters and numerous frogs by occlusion of the main blood vessels of the transparent membranes with small rubber-tipped serrefines or with hemostatic forceps for a time interval of 20 to 45 minutes. Slight physical injuries were made by burning small areas in the membranes with a hot needle, by probing the small blood vessel with a microneedle, and by injurious faradic stimulation with a microelectrode.

Limited chemical trauma was produced by the topical application of croton oil and mustard oil from a micropipet. Extensive trauma resulted from massive limb crushing, visceral manipulation, and a combination of both procedures.

Direct microscopic observations of intravascular conditions have been made in 11 dicumarolized hamsters (1.25 mg. suspended in gum acacia per 100 Gm. body weight administered by stomach tube daily from one to five days) and in 4 heparinized hamsters (1.5 mg. per 100 Gm. body weight injected into the femoral vein) with and without vascular occlusion. Blood platelet counts were determined in anesthetized and also unanesthetized hamsters from cardiac blood by a modification of the Nygaard method. A sample of 0.1 cc. of cardiac blood was drawn into a 1 cc. tuberculin syringe containing 0.9 cc. of sodium oxalate (1.1 per cent) and a minute amount of brilliant cresyl blue. Erythrocyte sedimentation was permitted for one hour in a paraffin-lined serologic tube containing 1 cc. of sodium oxalate. Counts were made by means of a Spencer Bright Line Haemacytometer from a sample of the supernatant fluid taken just above the erythrocyte sedimentation line by means of a red cell pipet.

For the investigation of peripheral blood flow during infection, 18 hamsters were inoculated intraperitoneally with Staphylococcus aureus (beta hemolytic). The inoculations consisted of 0.4 cc. bacterial suspensions prepared by adding 10 cc. of 0.5 per cent sterile sodium chloride to 24 and 48 hour agar culture slants. Hamsters were selected for blood vessel microscopy at 24 hour intervals over a period of five days after inoculation. Blood counts (red cell, total and differential white cell, and platelet) were made at daily intervals for seven days. Bacteriologic cultures were prepared from blood and peritoneal fluid. The characteristics of blood flow were investigated also in frogs with the infectious disease known as "red leg."

The characteristics of blood flow associated with malignant neoplasms were studied both by reflected light and by transillumination in the cheek pouches of 160 hamsters with advanced sarcomas of carcinogenic origin and with cheek pouch transplants of carcinogen-induced sarcomas. Hematologic studies, including platelet counts, were made on 13 of these animals.

Results and Implications
Platelet Thrombosis and Embolism Resulting from Trauma

Traumatic injury of the vascular endothelium and complete stasis in the blood vessels of the cheek pouch were produced by complete occlusion of the supplying arteries and veins for a period varying from 20 to 45 minutes. After release, the blood flow resumed slowly during the next several minutes. Recovery from stasis occurred first in the venules by the formation of anastomosing intravascular pathways within the masses of static erythrocytes until the channels coalesced to fill the lumen of the vessel. Within the next 10 to 15 minutes translucent granular aggregates were found adhering to the walls of numerous venules, particularly at vascular junctions but also at points remote from junctions. Each white aggregate increased in size, forming a thrombus which frequently filled the lumen and blocked completely the blood flow in the venule. Such thrombi were observed in venules varying in size from 10 to 200 microns in diameter. As a result the venous blood leaving the capillary network was rerouted through adjacent unthrombosed venules, resulting in reversals in the direction of blood flow in the capillary network. Under high magnifications, the white thrombi proved to be acellular. In a number of cases a portion of a venule containing a thrombus was excised by means of a microknife and the intravascular elements were suspended in the diluting fluid of Rees and Ecker and stained with brilliant cresyl blue. Leukocytes, erythrocytes and fibrin could not be demonstrated in the thrombus. The primary component of the thrombus was identified as the blood platelet.

Blood platelet thrombi frequently occluded venules completely, producing stasis in the corresponding capillary network for variable and indefinite periods. The platelets comprising a thrombus were strongly adherent to each other and to the vessel wall, as determined by probing with a microneedle mounted in a micromanipulator. However, circulating erythrocytes were observed cutting a maze of anastomosing channels which coalesced, underlined the platelet mass and set free emboli varying in size and shape. Platelet thrombosis and embolus formation have been observed consistently after release from vascular occlusion (figs. 1A, B, C and D; 2C, D and E). Some emboli were smaller than the hamster erythrocyte (7.0 microns) and others were large enough...
Fig. 1. Enlargements from cinephotomicrographic sequences showing thrombosis and embolism in cheek pouch of hamster following release from vascular occlusion. Magnification × 100. A and B. Blood platelet thrombus (t) in a venule (88 microns in diameter). In B, circulating platelet embolus (e) formed by fragmentation of the thrombus. C and D. Fragmentation of a platelet thrombus (t) in a venule (90 microns in diameter) of the cheek pouch opposite to that occluded. Individual platelets (p) were swept from the thrombus to join the embolus shown turning the corner at the vascular junction. E and F. Movement of a large platelet embolus (e) in a venule (100 microns in diameter). Progression is shown in F taken 1.5 seconds after E.

to contact opposite walls of blood vessels 50 to 100 microns in diameter (figs. 1E and F). At times vast numbers of platelets were swept off a thrombus individually, like "drifting snow," into the circulating stream (fig. 1C and D). Platelet emboli resulting from vascular occlusion have been detected and photographed in the arterioles of the occluded cheek pouch.
Fig. 2. A–E. Enlargements from cinephotomicrographic sequences taken after occlusion. A, circulating platelet emboli at x and y in an arteriole (34 microns in diameter), photographed at 64 frames per second. B, taken one-sixteenth of a second after A, shows progression of emboli. Magnification X 100. C, D and E. Successive stages in the formation of an embolus. Platelet thrombus (t) almost stops the blood flow in a venule (67 microns in diameter) in C. In D, fragmentation of the thrombus forms an embolus (e) which is carried away in E. The vessel wall (w) opposite the thrombus is coated heavily with platelets. Magnification X 520. F. Infection with Staph. aureus. Mixed thrombus composed of platelets, leukocytes and trapped erythrocytes at a venous junction (vessel diameters 72, 86 and 101 microns). Note platelet coating (p). Magnification X 260.

(fig. 2A and B) and also in the blood vessels of the opposite unoccluded pouch (fig. 1C and D). Consequently, unresolved platelet masses must enter the heart, lungs, and other vital organs. Many large platelet emboli traverse the vascular pattern of the cheek pouch, and presumably that of other organs without forming thrombi, undoubtedly because of the presence
of arteriovenous anastomoses. We have frequently followed emboli shunting across from the arterial side. This is direct evidence for the phenomenon demonstrated indirectly by Prinzmetal and co-workers using glass beads of various diameters.

Platelet thrombi and emboli were produced at will by slight endothelial injury caused by inserting the point of a microneedle obliquely through the vessel wall, abrading the lining, and then withdrawing the needle. A platelet thrombus was immediately formed, seemingly platelet by platelet, partially occluding the vessel until it was swept away as an embolus. Local injury was also produced by the topical application from a micropipette of a minute amount of desoxycorticosterone glucoside (Per-Corten, Ciba) in a nonphysiologic concentration. Platelets and white cells accumulated temporarily at the point of application. The conditions of blood flow were examined in the cheek pouches of hamsters during severe trauma produced by massive limb crushing, visceral manipulation and a combination of both procedures. In these drastic experiments emboli in the cheek pouch vessels were few. The reason for this is not known. However, because of the severity of the injury, blood flow through the damaged tissues was probably negligible. How efficient the liver may be in removing agglutinated platelets from portal blood is not known to us.

Platelet counts were made on cardiac blood both before and after vascular occlusion, using an appropriate modification of the Nygaard method. The normal values for the cellular components of hamster blood have been reported by Stewart and co-workers, but not for blood platelets. We have found no record in the literature of platelet determinations in the hamster. In the normal hamster (anesthetized and unanesthetized) the count ranged from 420,000 to 460,000 per cu. mm. of blood in 5 animals. Determinations made during platelet thrombus formation did not vary significantly.

In the mesentery of the frog, intravenous platelet thrombi were observed after vascular occlusion of the large mesenteric vessels. However, embolus formation comparable to that of the hamster was not seen. Microinjuries were produced on the walls of small blood vessels in the retrolingual membrane by probing with a microneedle, stimulating with an injurious faradic current by means of a micro-electrode, and by applying topically croton oil and mustard oil from a micropipet. Leukocytes accumulated at the injured portion of the blood vessel and persisted only for several seconds to several minutes after the stimulus was discontinued. Slight local injuries did not produce thrombus formation or stasis. Small droplets of croton oil deposited on the epithelium of the retrolingual membrane were surrounded by groups of ciliated squamous epithelial cells and carried away. Minute droplets of croton oil and mustard oil (5 to 15 microns in diameter) applied to the epithelial surface directly above small arterioles produced marked vasodilation resulting from stimulation of the perivascular nerve plexus, since vascular segments not exposed to contact with the irritants were involved. Vasodilation persisted during the period of exposure to the irritant. Prolonged exposure was characterized by the adherence of white cells to blood vessel walls in the exposed area and ultimately by the development of stasis.

The extensive platelet thrombosis and embolism resulting from vascular occlusion in the hamster has significant surgical implications and suggests that blood platelets may be more important than previously recognized in the etiology of thrombosis.

The Effect of Dicumarol and Heparin

The effectiveness of dicumarol in the prevention of platelet thrombi and embolism resulting from vascular occlusion was investigated by direct microscopy. Cheek pouches were everted for transillumination on the first, second, third, fourth and fifth days following administration daily of 1.25 mg. of dicumarol by intubation. Normally, relatively large blood vessels may be severed with practically no bleeding during the minor surgery involved in preparing the cheek pouch. After dicumarolization, preparation of the cheek pouch resulted in considerable bleeding and a greatly prolonged clotting time. In several animals
petechiae formed. Portions of certain arterioles were constricted markedly, alternating with dilated sections, thus forming sausage-shaped vascular segments filled with erythrocytes. Considerable hemoconcentration was noted, probably due to an increase in permeability, but stasis did not predominate. Spontaneous platelet emboli and thrombi were not seen.

Blood platelet counts made at intervals between 24 to 96 hours after administration of dicumarol were 30 per cent greater than the normal count. The reason for the increase is not known. Several factors which may possibly be involved are as follows: first, a decreased adhesiveness of platelets to each other and to erythrocytes so that fewer platelets were carried down by sedimenting red cells in the counting technic; second, an increased production of platelets; third, a decreased destruction and removal of platelets; and fourth, an increased mobilization of platelets from possible storage depots.

Trauma was produced in the cheek pouch of dicumarolized hamsters by vascular occlusion for 20 to 45 minutes. The blood vessels were released from occlusion and examined at X 200 and X 520 for the presence of platelet thrombi and emboli. No platelet thrombi and no platelet emboli were found, except for small emboli in 1 hamster five days after administration of a single dose. Consequently, dicumarol prevented the formation of platelet thrombosis and embolism after trauma. This is direct microscopic evidence to support the contention of clinicians that dicumarol is of therapeutic value in preventing the propagation of a thrombus and in decreasing the recurrence of coronary thrombosis.46–50

Irrespective of vascular occlusion, vast numbers of leukocytes were observed consistently after 24 to 48 hours coating the walls of venules and capillaries in dicumarolized hamsters. This might conceivably impair the oxygen, carbon dioxide and nutrient transfers in vital tissues and organs. It must increase the peripheral resistance. Aggregates of white cells were observed breaking away from the vascular endothelium to form leukocytic emboli which subsequently lodged at vascular junctions to form thrombi blocking the blood flow. This phenomenon may be of possible significance in cases where controlled dicumarol administration has been implicated in fatalities.41 No reference to the tendency of dicumarol to cause plating of leukocytes on the lining of small blood vessels has been found in the literature. It may be important to screen anticoagulants for leukocyte aggregating properties. We are investigating the characteristics of other anticoagulants.

The effectiveness of heparin in the prevention of platelet thrombosis and embolism was also investigated. The cheek pouch was everted for transillumination within 30 minutes after the administration of heparin. Preparation of the cheek pouch in heparinized hamsters resulted in excessive bleeding and prolonged clotting time. From the moment of initial observation, spontaneous platelet emboli were seen moving in the vessels of the cheek pouch. This confirms the report by Copley32 that heparin administration in the hamster produced circulating platelet emboli. The size of the emboli varied from that of a leukocyte (upper limit reported by Copley) to a mass 20 microns or more in diameter.

In view of the fact that heparin produced platelet emboli spontaneously, only a few vascular occlusion experiments were attempted, and the results of these were inconclusive. Presumably the platelets were agglomerated by heparinization and unavailable for agglutination following trauma by vascular occlusion. In heparinized hamsters leukocytes were observed consistently coating the walls of small blood vessels, even forming attached clumps, but no leukocytic emboli were seen. This confirms, in original, physiologically mature blood vessels, the increased adhesiveness of leukocytes and the absence of leukocytic emboli reported by Essex and Graña52 in the newly formed regenerated blood vessels in transparent chambers inserted in the ears of rabbits.

Experiments are in progress to determine nontoxic substances which will prevent the formation of platelet and leukocytic thrombi. The anticoagulants heparin and dicumarol do not appear to be promising therapeutic substances in this respect, because, per se, they cause leukocytes to adhere to the vascular endothe-
leukocytes, and in addition dicumarol produces leukocytic emboli.

**Platelet and Leukocytic Coating on the Vascular Endothelium, and Embolism During Infection**

The endothelial lining of venules, capillaries and many small arterioles in the cheek pouch was coated with a continuous layer of platelets and leukocytes varying in thickness. This was observed from 24 to 120 hours after intraperitoneal inoculation with a suspension of *Staphylococcus aureus*. In the smallest vessels the leukocytes adhered to the endothelium and did not roll along the vessel wall in the usual way. Frequently the blood stream in a vessel 20 to 30 microns in diameter was confined to a tortuous channel sufficient only for the passage of erythrocytes in single file. The leukocytic coating was greatest 24 to 48 hours after inoculation, but persisted during the course of the infection. Septicemia was demonstrated by the preparation of positive cultures of the original organism from cardiac blood obtained 24, 48, 96 and 120 hours after injection. Phagocytized bacteria were found within leukocytes in blood smears made from infected hamsters. Several hamsters died within 96 hours after inoculation, but the majority recovered. The intimal endothelium of the blood vessels in the cheek pouches of hamsters which recovered was normal.

Numerous venules were lined by a white adhesive coating (fig. 3A and B). Commonly white emboli were seen (fig. 3B). Smear preparations of the coatings were made and blood platelets, leukocytes and erythrocytes were demonstrated, but no fibrin was seen. The erythrocytes were trapped by platelet aggregations. Mixed thrombi were seen also at venous junctions, completely blocking the circulation in one venule (fig. 2F).

In staphylococcus infected hamsters, large white emboli composed predominantly of clumped leukocytes were seen in the blood vessels of the cheek pouch. We have observed the formation of leukocytic emboli from the coating on the vascular endothelium. Occasionally we have followed successfully the course traversed by a white cell embolus and have observed it entering a precapillary arteriole by stretching longitudinally and “worming” through the lumen to lodge at a capillary junction and form a thrombus unresolved during the period of observation.

The appearance of mixed platelet-leukocytic thrombi and emboli in animals with *Staph. aureus* infection is accompanied by a thrombopenia and leukocytosis. The platelet count in cardiac blood decreased in all cases and dropped as low as 27,000 and 30,000 at 48 and 72 hours respectively. The differential white cell count varied between the following limits during the course of the infection: 60 to 86 per cent polymorphonuclear leukocytes, 6 to 32 per cent lymphocytes, and 6 to 19 per cent monocytes. This is in sharp contrast with normal values, as reported by Stewart and co-workers and confirmed by us, of 32 to 35 per cent polymorphonuclear leukocytes, 63 to 66 per cent lymphocytes and 1 to 2 per cent monocytes.

The characteristics of blood flow were investigated also in the retrolingual membranes and the mesenteries of many frogs with the infectious disease known as “red leg.” The preparations were characterized by an increase in leukocytes in the small blood vessels. However, emboli and the extensive leukocytic coating comparable to that found in the hamster were not observed in the frog. The vascular pattern in the retrolingual membranes of frogs with “red leg” appeared to be in a state of flux. Vasodilation predominated and considerable stasis was found. Some capillaries appeared to be degenerating, and in other parts of the field capillary endothelial sprouts were seen. Portions of the capillary network consisted of unusually small and atypical complex anastomoses.

The discovery of platelet and leukocytic aggregates in the vascular system of hamsters during infection may be highly significant. The mechanism involved in the formation of the aggregations has not been determined. Considerable controversy exists concerning the possible role of blood platelets in the removal of bacteria from circulating blood. Houlihan and Copley have demonstrated in vitro an increased adhesiveness of platelets and a marked clumping when rabbit blood was
mixed with *Staph. aureus* and other bacteria. The possibility exists that platelet and leukocytic masses may line the small blood vessels in various vital organs, including the brain, heart and lungs during severe infections. Such coatings on the endothelium might conceivably decrease the effective oxygen, carbon dioxide, and nutrient exchange between blood and...
tissue fluids. The effect of larger thrombi incompletely or completely blocking the small blood vessels would be severe. Aggregates of this type might well explain the central nervous system manifestations and mental confusions which frequently accompany severe infection. The symptoms associated with many infectious diseases are often more severe than would be expected in consideration of the known characteristics of the infecting agent. Furthermore, an analysis of the case histories of the twenty or more sudden deaths characterized by autopsy findings of extensive generalized platelet thrombosis of arterioles, capillaries and venules indicates a correlation between infection and platelet thrombosis.

White Thrombosis, Embolism and Leukocytosis Associated with Malignant Neoplasia

Large circulating white emboli were seen routinely by reflected light (X 15) in the cheek pouch vessels of nearly all hamsters (150) with advanced sarcomas induced by methylcholanthrene, and with large advanced transplants (2 to 3 cc.) of methylcholanthrene-induced sarcoma in the opposite cheek pouch. When the blood flow in 14 of these hamsters was examined by transillumination at X 260 and X 520, the walls of venules, capillaries and many arterioles were seen to be lined with immobilized leukocytes and platelets (fig. 3D). In many areas a coating of these elements nearly occluded the lumen for a considerable length (fig. 3C). At certain points thrombi were alternately forming and breaking away, producing emboli of various sizes (fig. 3E, F). Microscopic examination of blood smears showed many of these emboli to be composed of blood platelets. Leukocytic emboli were also found, in some cases large enough completely to plug small vessels.

The tumor bearing hamsters were asthenic and anemic, as indicated by pallor of the nose and extremities. The blood stream was translucent, further suggesting a severe anemia. Red cell counts were made on 12 hamsters with cheek pouch tumor transplants 60 to 108 days old. One additional hamster was used with a transplant 22 days old. The red cell counts varied from 4.6 to 10.8 million. The extremes were represented by one case each. The other counts fell within the accepted normal values for this age group. The anemic appearance was therefore not due to erythrocyte deficiency, but presumably to lowered hemoglobin. In all cases except one there was a definite leukocytosis, and in 8 cases the normal ratio of polymorphonuclear leukocytes to lymphocytes was reversed, as in hamsters infected with Staph. aureus. In 6 cases with extreme leukocytosis (23,200 to 35,500 per cu. mm.) the ratio varied from 63 per cent polymorphonuclear leukocytes and 22 per cent lymphocytes to 93 per cent and 3 per cent, respectively. In all cases there was an increase in monocytes. In 11 cases the monocytes constituted 5 per cent to 21 per cent. The blood platelet count varied from a low of 97,000 to a high of 1,255,000 per cu. mm. Two cases were below and 5 above the normal range, which is between four and five hundred thousand. The variability in the white elements of the blood may be due to the heterogeneity of the group so far as the ages of the transplants were concerned. In some cases ulceration of the tumor and secondary infection were apparent. In other cases very extensive white coatings and numerous white emboli and plugs were observed. Further work is in progress in this laboratory on the properties of the blood in hamsters with advanced tumors of carcinogenic origin.

Absence of Sludged Blood in Trauma, Infection and Malignant Neoplasia

We have studied the characteristics of blood flow in transilluminated membranes under high magnification for the possible formation of sludged blood under pathologic and traumatic conditions. Sludged blood was reported originally by Knisely in monkeys in the terminal stages of malaria and described as an aggregation of erythrocytes forming circulating "rafts" held together by means of a glassy precipitate as determined by microprobing in vitro. These erythrocyte emboli were seen lodging at the capillary junctions to block the blood flow. The presence of sludged blood, reported by Knisely and co-workers, was de-
determined in man under conditions which did not permit clear visualization and adequate testing for the presence of a sticky coat binding erythrocytes together. In hamsters we have simulated experimentally the general conditions in which sludged blood has been reported in man. We find that erythrocytes have a tendency to circulate at times in groups separated by plasma, platelets and leukocytes, due to vasomotor rhythmicity and consequent plasma skimming. This is undoubtedly the phenomenon described by Fowler after stimulation of vasoconstrictor nerves and intravascular injection of vasoconstrictor substances and reported as sludged blood. We think that Knisely has extended his concept, without crucial evidence, to include blood flow characteristics which differ from those of the sludged blood he originally defined.

In several clinical laboratories, the classic rouleau formation of Fahraeus has been confused with the concept of sludged blood. Hirschboeck and Woo and Robertson, Wolf, and Wolff report that the conjunctival intravascular phenomena referred to as sludged blood occur in both normal and diseased states and are of no great clinical significance. The term “sludged blood” is obviously a misnomer in the majority of circumstances in which it has been used. We have microprobed, in vivo, erythrocytes circulating in groups, and find no evidence for sludge in the original sense of the term. Furthermore, erythrocytes in stasis for long periods (one to five days) remained unagglutinated and free to move individually when the vessel wall was compressed with a microneedle.

Although Knisely has reported sludged blood in frogs, we have not seen it in the retrolingual membrane, web, lungs, bladder, mesentery or vocal sac, even in conditions of severe trauma produced by burning or physical injury. We find no sludged blood in the blood vessels of the cheek pouches of hamsters with staphylococcus infection, malignant neoplasia, and trauma including massive limb crushing, visceral manipulation, a combination of both procedures, and production of small burns in the pouch.

**Summary**

1. Platelet thrombosis and embolism were produced in the distal blood vessels of the transilluminated cheek pouch of the hamster by trauma of the endothelium resulting from vascular occlusion, for 20 to 45 minutes, at the attached end of the pouch. Cinephotomicrographs were taken demonstrating the formation of platelet thrombi and emboli, especially at venous junctions, both in the same pouch and in the pouch opposite to that occluded. Thrombus fragmentation produced circulating platelet emboli, which were recorded in venules and arterioles.

2. DICumarol prevented the formation of platelet thrombi and platelet emboli after vascular occlusion. However, without vascular occlusion, dicumarol produced both leukocytic thrombosis and embolism.

3. Heparin produced platelet emboli, per se, but no platelet thrombi were found. However, a leukocytic coating (thrombosis) was produced by heparin, but no leukocytic emboli were seen.

4. Infection with *Staph. aureus* produced mixed platelet and leukocytic coatings and emboli of similar nature, accompanied by thrombopenia and leukocytosis. The correlation between infection in man and white thromboembolism is considered.

5. In malignant neoplasia (cheek pouch transplants of methylcholanthrene-induced sarcoma) white emboli composed largely of platelets were found. Mixed platelet and leukocytic coatings, as well as leukocytosis were characteristic.

6. Sludged blood was not produced by trauma, infection, and malignant neoplasia.

**REFERENCES**


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