Physical Effects in Red Blood Cell Trauma

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Red blood cell damage and destruction are important problems in the use of artificial valves, heart-lung machines, and other devices which pump or process blood. An experimental study has been made on the mechanism of cell damage. Damage was defined by three types of observations on blood which had been subjected to trauma: (a) release of hemoglobin from cells (hemolysis), (b) morphological changes observed microscopically, and (c) red cell life span studies in rabbits using a Cr^{51} tagging technique.

Three types of physical forces which might be injurious to red cells were studied; shearing stress (of known, constant magnitudes from a concentric cylinder viscometer), pressure variations (from studies in a static pressure cell), and direct impact of solid surfaces (from studies in a device which simulates the seating action of artificial heart valves).

The study shows that high shearing stress may be primarily responsible for mechanical cell damage under certain important circumstances. There is a critical shearing stress above which cell damage increases markedly. Much of the cell damage does not appear as an immediate release of hemoglobin. Many cells undergo morphological changes and exhibit shortened average life span *in vivo*. The morphological changes due to shearing stress are very similar to the changes observed in patients who have hemolytic anemia associated with artificial valves.

For many years it has been apparent that certain circulatory protheses can cause hemolysis. Well-known examples are artificial heart valves, heart-lung machines, and artificial kidneys. There have been frequent clinical reports of anemia resulting from hemolysis caused by such devices.

A number of studies have appeared on rates of hemolysis in prosthetic devices. The literature of most interest has been reviewed recently (*2, 6*). In most cases it has been difficult or impossible to establish the mechanism of red cell damage because of the complexity of the system. In addition, in many of the prior studies the free hemoglobin released in the plasma was used as the only index of red cell damage. As will be discussed in detail below, there are cases in which this measurement alone does not adequately reflect the extent of the damage.

The purpose of the work reported here was to study physical factors one at a time in an isolated way with as nearly as possible a complete analysis of the resulting red blood cell trauma. To assess the effects of trauma, three types of observations were made:

1. Free hemoglobin in the plasma is the most direct measurement of red cell trauma since the release of hemoglobin signifies a cell has somehow lost its integrity, for example, by rupture of the membrane in such a way that the contents of the cell are released into the plasma.
2. Morphological changes in red blood cells (changes in shape and size) were observed by direct microscopic examination.
3. The life span of cells *in vivo* following trauma was determined by a tagging technique. These *in vivo* measurements are of considerable importance since some cells may be damaged in a way which reduces their life span yet does not result in complete loss of hemoglobin.

The three mechanisms considered were pressure fluctuations, direct impact or crushing due to solid surfaces as in valve seating, and shearing stress. Each of the above listed mechanisms was investigated with apparatus that was specifically designed to isolate the particular mechanism and minimize all other possible causes of damage.

Surprisingly enough, there have been very few prior studies in which an attempt was made to isolate the mechanism of damage, and apparently this is the first work of this type that attempted to isolate mechanical factors and used a careful definition of trauma including *in vivo* life span determinations.

Blackshear and co-workers have studied the effect of both pressure fluctuations and shearing stress on the release of free hemoglobin (*2, 3, 4*). Although they did not report the rate of change of pressure, they reported that in their preliminary results they found that pressure variations did not seem to have an important effect. Apparently, Andres and co-workers (*1*) were the first to suggest that high shearing stress in jets (from rapid injections) causes hemolysis although previous workers had noted that hemolysis occurred in jets of sufficiently high velocity. Blackshear and co-workers (*2, 4*) analyzed the jet data of several workers and concluded that the critical shearing stress for hemolysis was in the range of 10^4 to 10^5 dynes/sq. cm. As will be outlined below, the results of the present work are consistent with these conclusions although substantial cell damage in the form of reduced *in vivo* life span occurs at somewhat lower shearing stresses.

There is a considerable literature on clinical studies and on effect of larger scale systems, especially pumps, which has been reviewed elsewhere (*6*). Bernstein, Blackshear and Keller (*2*) have also reviewed some of this work. The discussion to follow will cover the aspects of the work which seem to be of most interest to engineers with a general interest in application related to medicine. Spe-

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cific details on procedures and results more closely related to hematology are reported elsewhere (*6, 12*).

EXPERIMENTAL WORK

Pressure Cycling Device

A pressure cell which allows the application of up to 10 atm. of pressure at cyclic rates of up to 30 cycles/sec. was constructed to permit the investigation of rapid static pressure changes without the shearing stresses which accompany flow. The system is a heavy walled acrylic chamber into which a sample plug may be fitted and to which are attached a stainless steel cylinder and a pressure transducer. A silastic bag which contains the blood sample is fitted over the end of the sample plug. The sample plug is equipped with parts for filling with blood and purging of air. A piston fitted with two O-rings is placed into the cylinder. A driver cylinder fits over the cylinder attached to the chamber and is loosely connected to the piston through a coil spring. Moving the driver cylinder toward the chamber compresses the spring and the force is transmitted through the piston to the fluid in the chamber.

A displacement is applied through an eccentric cam on a d.c. motor which is equipped with speed controls. The pressure wave which is produced is approximately sinusoidal.

Simulation of Valve Seating

An experimental system was developed to simulate the seating of artificial heart valves. The system consists of an electronic oscillator, an electronic amplifier, a force transducer, and the driver portion of the system which will be called the vibrator. The vibrator is similar to a speaker, without a cone, with a powerful diaphragm. The only purpose of the vibrator is to produce a periodic reciprocating motion of a shaft. The motion is used to cause an object fixed on the shaft to strike a fixed substrate in the presence of blood.

Two valve simulation systems were used. In the first system an annular ring of approximately the same surface area for seating as an artificial valve was attached to the shaft (the shaft was aligned with the axis of the concentric cylindrical surfaces of the ring). The ring was moved up and down striking a fixed silastic substrate. Both the ring and the substrate were immersed in blood. In the second system a Starr-Edwards 9A aortic artificial valve was used. The Starr-Edwards valve is a ball check valve with a silastic ball and a metal seating ring. The ball was attached to the shaft and the seating ring was the fixed substrate.

A device to measure the force with which the reciprocating member strikes the fixed substrate was developed. A force transducer was devised using four strain gauges placed on the aluminium member which supports the substrate. The transducer was calibrated directly. It was possible to show that momentum effects are small and consequently the force measured closely agrees with the actual force of closure.

Concentric Cylinder Viscometer

A Fann Model 38A viscometer was modified to suit the purposes of this work. The usual rotor-bob setup was closed and the bottom of the bob was machined to a cone designed to yield uniform shearing stress throughout the gap. The rotor was made of acrylic plastic to allow visual observation for elimination of air bubbles in the test section. There is a valve which permits sample removal from the bottom of the rotor. At the highest shearing stresses studied the top speed of the rotor (cup) had to be increased. The modified instrument produces a maximum shear rate of approximately 131,000 $sec.^{-1}$ (at 29.83 $sec.^{-1}$/rev./min. The smallest clearance used was 0.0025 in. and the maximum speed was 4,400 rev./min. In the highest shearing stress runs, the blood sample in the gap was only 0.5 cc. However, this sample was sufficient in nearly all cases. In the instances where larger samples were desired, repeated runs were required.

Experimental Method

Bovine, rabbit, and human bloods were used in this work. The usual procedure was to anticoagulate the blood with a standard ACD (acid citrate dextrose) solution (*9*). The effect of anticoagulant was studied by using heparin instead of ACD and by using difibrinated blood (blood in which fibrinogen is removed by contact with glass spheres so that no anticoagulant is required). The results were surprisingly uniform, showing little or no effect of anticoagulant in the ranges of concentrations studied.

Red cell hemolysis was evaluated by measuring the plasma hemoglobin concentration before and after trauma. Blood films were prepared on microscope slides using Wright's stain. The magnitude of morphological change was estimated by counting 1,000 cells and enumerating the abnormally sized or shaped cells.

Studies were made to determine that the results were free from undesired side effects due to blood-air interaction, blood-solid interface interaction and viscous heating. Control samples were used in all cases. The control samples were subjected to the same handling and to the same air and solid interface contact as the working samples. The effect of temperature had to be studied in detail to establish that effects of viscous heating were negligible. The temperature rise at various shear rates was studied experimentally with use of the elementary theory. The maximum temperature in any experiment was controlled to avoid cell damage.

The usual rate of closure for simulated valve seating was 15 cycles/sec. and the usual duration was 200 sec. This number of cycles corresponds to about 50 min. of operation in the human range of frequencies. The usual duration of trauma in the viscometer was 2 min. This interval was selected after study of the effect of viscous heating and the effect of time on hemolysis. It was found that duration of the run in the viscometer had little effect on results in the range of 0.5 to 7 min.

In addition to cell hemolysis as measured by free plasma hemoglobin, there may be extensive damage of a more subtle type. The spleen and liver tend to remove, from the circulation, red cells which are abnormal in shape and size. Such cells of course may contain substantial amounts of hemoglobin; hence, the need to determine whether physical trauma had an effect on red cell life span.

Rabbits were selected as convenient laboratory animals having red cells remarkably similar to those of humans (*10*). The survival of rabbit red cells was determined by using the well established radioactive sodium chromate tagging technique (*5, 7*). Ascorbic acid was added to the blood to reduce any chromium not in the red cells and thus prevent labeling of circulating cells following injection into the donor animal. The reduced chromium complex cannot penetrate the cell membrane and hence will not label the red cell. An unexpected finding was that it is important that the ascorbate be added after the blood sample was traumatized. Apparently small concentrations of ascorbate have a marked effect on the resistance of red blood cells to shearing stress.

Traumatized and labeled blood was injected into the ear vein of the donor animal. Blood was withdrawn from the opposite ear at 30 min., one day, two days, and at one or 2 day intervals for about two weeks. In some cases, more frequent samples were taken in the first few hours. The activity was measured in an automatic gamma scintillation counter and the data expressed as counts/min./ml. of packed red blood cells. The approximately exponential survival curve was plotted on semilog paper and the half-life of the Cr^{51} was recorded in addition to observations of the shape of the curve of activity vs. time.

The curve as reported here is simply the radioactivity in a sample of a given size as a percent of the initial value. There are well established techniques (*7*) for correcting the results for chromium decay and elution. These corrections have negligible effect on the conclusions in this work which we based on comparison of life span for control with traumatized samples. The example results to be presented here (Figure 4) are uncorrected values of radioactivity.

RESULTS

Pressure Variation

Several experiments were carried out in the static cell at rates of change of pressure of up to 1.65×10^5 mm. Hg./sec. for periods of 1 hr. These studies revealed no

significant hemolysis. Furthermore, there was no change in morphology of these cells and the cells which survived the test had a normal life span. Hence, it can be concluded that pressure variations of the magnitude studied seem to have little effect on red blood cells. It should be mentioned again that this finding is consistent with the prior work of Blackshear and co-workers (3) although the magnitude of the pressure variation studied in their work was not reported.

Valve Seating

The results of a series of typical experiments to determine the hemolysis resulting from the seating of the Starr-Edwards valve are shown in Figure 1. All these results are for runs of 200 seconds duration at 15 cycles/sec. It can be seen that the amount of hemoglobin released in the test period increased with the increasing force monitored in the force transducer. The increase was relatively rapid at low force levels but the hemolysis appeared to level off at about 3 force pounds. This phenomenon may be explained by an increase in seating area as the silastic ball increasingly conforms to the seat shape with increasing force. The asymptotic levels of hemoglobin release in these experiments and those of an experiment using the flat ring simulation (silastic substrate) are plotted vs. red cell concentration (hematocrit) in Figure 2. The data falls along straight lines which might be expected since as the concentration of cells present is increased, the probability of cells being trapped between the two surfaces increases. It is interesting to note that the ratio of damage in the case of the flat ring simulation to that of the Starr-Edwards valve is about 1.8:1. This ratio is close to the ratio of the areas of contact (0.12 to 0.07 sq. in.) and thus confirms that the amount of hemolysis is proportional to the area of contact. This conclusion is further substantiated by the fact that there was little difference in he asymptotic hemolysis rate in experiments in which the relatively soft silastic substrate was replaced with a relatively hard acrylic substrate. Blackshear and co-workers (3) found a similar effect of area in work on a roller pump although the levels of hemolysis were much lower. The only abnormal morphology observed was the presence of ghost cells (that is, cells in which the hemoglobin has been removed

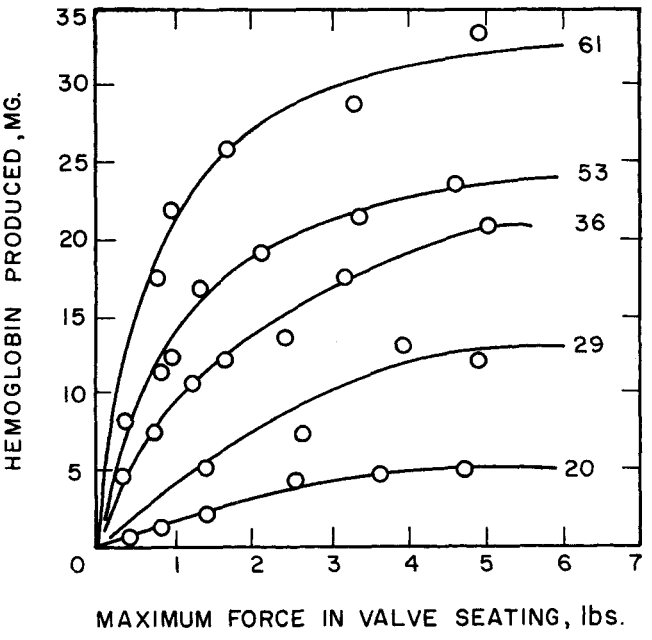

Fig. 1. Hemolysis caused by seating of Starr-Edwards valve. The parameter is concentration of red blood cells in volume percent (bovine blood).

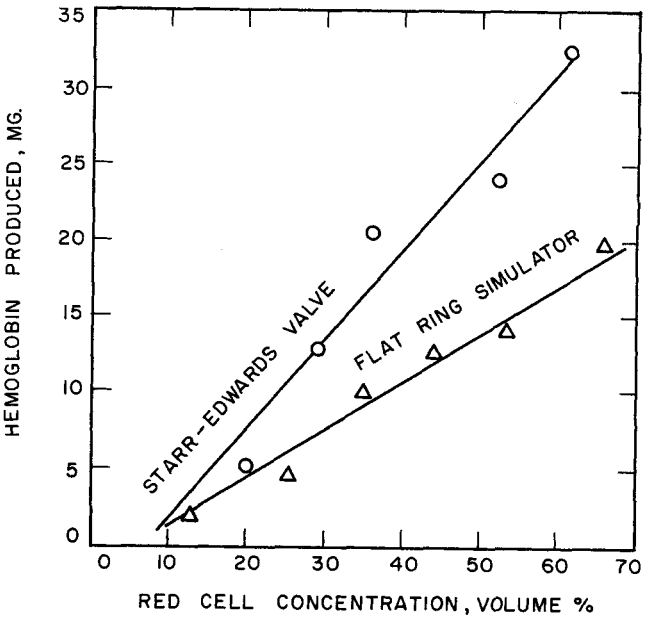

Fig. 2. Comparison of hemolysis for the two valve seating devices (bovine blood).

but the membrane remains relatively intact). The *in vivo* life span studies showed that the Cr^{51} half-life was reduced from 11.7 days for control samples to 8.9 days for samples subjected to the valve seating simulator. This change might appear to be important. However, it will be shown later that it is negligible when viewed in consideration of the effect on a human subject.

Shearing Stress

Typical results on hemolysis reulting from runs of 2 min. duration in the concentric cylinder viscometer are displayed in Figure 3. It can be seen that relatively little hemolysis occurs at low shearing stresses. However, there is a marked increase in hemolysis with increasing stresses above 3,000 dynes/sq. cm. These results are for human red blood cells. Similar results were obtained with both rabbit and bovine blood except that the transition occurs at somewhat higher stresses for bovine blood and slightly lower stresses for rabbit blood. The figure also shows the results of a study on the effect of viscosity of the suspending medium. The albumin content of the plasma was varied over the range 3.5 to 14% which results in a two-fold change in viscosity. At a particular shearing stress the shearing rate is different for different viscosities.

It can be seen that the hemolysis appears to be independent of the viscosity of the blood. In other words, shearing stress has an important effect but shearing rate does not, at least over the range studied in this work.

This work showing no effect of shearing rate is important since it lends additional support to the reliability of results. Shearing rate presumably would have a measurable effect if blood-solid material or blood-air interactions were having an important influence. Of course the primary evidence of freedom from solid or air interaction effects is from control samples subjected to the same conditions as the working samples. Changing the hematocrit (the red blood cell concentration in volume percent) also changes the apparent viscosity of the suspension. Similar studies over a wide range of hematocrits confirms that there is no important effect of hematocrit.

Some of the most interesting results from this work are on the effect of shearing stress on morphology and cell survival. A typical cell survival curve for blood subjected to high shearing stress is shown in Figure 4. The curve has been artificially fitted with two straight lines to emphasize two important points. First, there is a very rapid decrease

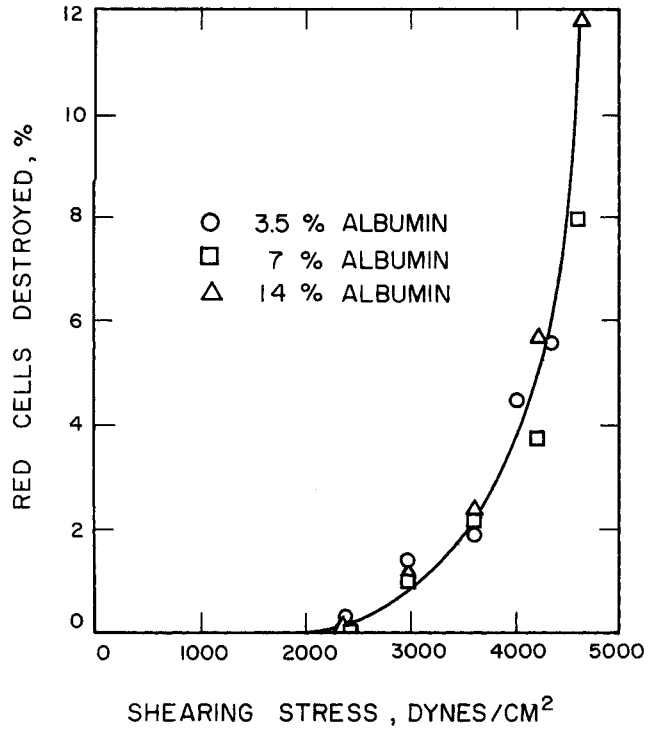

Fig. 3. Cr^{51} survival curve of rabbit red blood cells subjected to 5,100 dynes/sq. cm. shearing stress.

in the number of tagged cells within the first day. Specifically in Figure 4 about 30% of the traumatized cells disappear in one day, whereas for control samples, only about 6% disappear. It was possible to quantitatively account for most of the cells which disappear from the circulation by analysis of the spleen and liver of subject animals. Secondly, the cells which survive beyond one day have a normal life span. The second straight line in the figure is drawn with a slope corresponding to the normal Cr^{51} half-life of 11 days. It should be emphasized that the magnitude of cell damage by shortened *in vivo* life span is far in excess of that which is revealed as free hemoglobin. This result is in contrast to the results on valve seating where nearly all damage was by direct hemolysis.

The studies on morphology of cells subjected to shearing stress were related directly to the life span studies. Blood smears from thirty-six runs at various shearing stresses were examined and classified in detail. The abnormal cells include cell fragments, in which the cell membrane reseals and retains some hemoglobin, as well as cells of apparently original size but of altered shape. A well defined linear correlation of abnormal cell fraction with shearing stress was developed. The abnormal cell fraction increases from zero at 1,500 dynes/sq. cm. (the threshold value for cell damage) to 40% at 4,000 dynes/sq. cm. The fraction abnormal cells seems to agree almost quantitatively with the cell disappearance during the first 24 hr. *in vivo*. Evidently the morphologically altered cells are rapidly removed from the circulation, principally by the spleen and liver. In particular, the *in vivo* cell survival beyond 24 hr. for nine control samples was 93.4 ± 4.6% (the last figure denotes the standard deviation). For six samples subjected to 4,200 dynes/sq. cm. the corresponding survival figure was 78.1 ± 7.7%. Morphological examination of seven samples subjected to the same shearing stress revealed 65 ± 6% normal cells. The results of examinations of the spleen and liver are also expressed in terms of percent of the initial radiation. For three control samples the total radioactivity in the spleen and liver averaged 3.9% whereas for three samples subjected to 4,200 dynes/sq. cm. the corresponding figure was 15.9%.

DISCUSSION OF APPLICATIONS

Pressure Variations

To illustrate the magnitude of the rate of change of pressure which may occur as the result of the failure of a prosthesis, consider the case of back flow due to improper seating of a valve or leakage around a valve. The cause of the backflow could be an insufficient natural valve or an artificial valve which has been slightly dislocated from its sutures. A similar flow could arise from the failure of a septal patch resulting in leakage between the ventricles. A pressure drop of the order of 100 mm. Hg. in distances of the order of 1 cm. would develop. If the flow is assumed to be that of an ideal nozzle, the velocity may be estimated in the usual way to be 500 cm./sec. This means that a red cell would undergo the 100 mm. Hg. pressure drop in 0.002 sec. yielding a rate of change of pressure of 5.0×10^4 mm. Hg./sec. Rates of change of pressure almost an order of magnitude higher than this were studied in this work and found to have negligible effect.

Valve Seating

Consider an artificial heart valve in an average 150 lb. man. The blood volume of such a subject would be about 5.2 liters and the cardiac output would be about 6 liters/min. At 72 beats/min., the valve will open and close 103,680 times per day. Reference to the data given above shows that the damage due to closure by a Starr-Edwards valve at a normal cell concentration of 45 vol. % is about 0.006 mg. hemoglobin liberated per stroke or 600 mg. of hemoglobin per day. There would be 780 g. of hemoglobin in the circulatory system. Hence, the hemolysis rate is less than 0.1% per day. The body regenerates approximately 0.8% (normal red cell life span is 120 days) of the blood volume each day and has the ability to increase this several fold (*8*). Therefore, the damage done by the seating of even several valves is apparently not important.

To complete our consideration of valve seating, the results of the *in vivo* experimentation will be reviewed. The Cr^{51} half-life of the traumatized cells appeared to be shortened by about 20%. It must be remembered that the sample of blood under consideration was taken from a traumatized sample of a volume of 10 cc. This sample, however, was subjected to about 162,000 valve closures. This amounts to 1.5 days of a valve's normal cycles imposed on only 0.2% (10 cc.) of the total blood volume. Therefore, the effect would be 500 times smaller in a

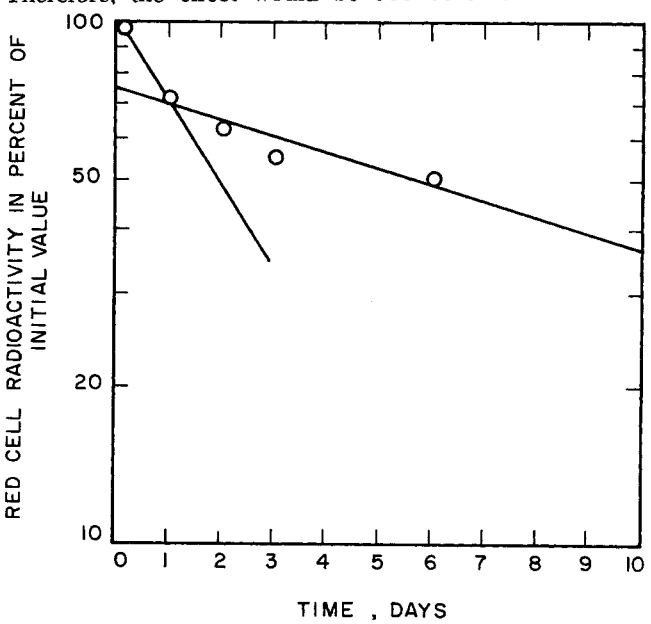

Fig. 4. Comparison of red blood cells from viscometer with those from patient.

human subject and, hence, presumably would be entirely negligible in applications.

Shearing Stress

When stresses in excess of about 1,500 dynes/sq. cm. are applied to human blood morphologically abnormal cells are produced. When stresses in excess of about 3,000 dynes/sq. cm. are applied significant hemolysis results. These stresses seem to be well in excess of those incurred in normal circulation (approximately 100 dynes/sq. cm.). Consider again the case where an aortic valve becomes dislocated from its sutures and the valve is pulled slightly away from the wall of the vessel. During systole (the valve is open) the pressure in the aorta is increased to above 100 mm. Hg. by the contracting ventricle. When the ventricle relaxes (diastole) the pressure in the ventricle falls rapidly to about atmospheric pressure, the pressure in the aorta remains near 100 mm. Hg., and the valve should close and prevent back flow. If the previously mentioned defect is present there will be a pressure drop of approximately 100 mm. Hg. over a small orifice and a jet of blood will bypass the valve and flow back into the ventricle. The velocity of the jet and the maximum shearing stress developed can be estimated from prior work in fluid mechanics (*11*). The resulting estimated shearing stress is about 5,000 dynes/sq. cm. which is in excess of the critical shearing stress for extensive red cell damage.

A Clinical Example

Relatively little serious hemolysis occurs in most patients with prosthetic valves. Some patients, however, have been seen to exhibit extraordinary anemia. The regurgitant flow (back flow) described in the previous paragraph frequently accompanies this anemia. The case of a patient (Patient C) is representative. Several months after replacement of an aortic valve, Patient C developed severe anemia. Tests showed evidence of regurgitant flow around the aortic valve. Upon reoperation of the patient, some of the sutures to the valve were found to be pulled loose. These were repaired and the patient recovered. The most interesting observation of this case related to the present work was the morphology of the red cells during the period of anemia. Figure 5 shows a view of normal red blood cells, of the cells of Patient C, and of cells subjected to a shearing stress of 2,600 dynes/sq. cm. in the concentric cylinder viscometer. The similarity of the cell abnormalities lends support to the tentative conclusion that shearing stress in the backflow due to defective sutures caused the anemia.

CONCLUSIONS

The study suggests answers to certain questions about how blood is damaged when circulating through prosthetic devices as reviewed below.

1. When red blood cells are subjected to high shearing stresses (in the range of 3,000 dynes/sq. cm.) hemolysis occurs, morphological changes are observed, and the *in vivo* survival times of these cells is shortened.
2. Morphological changes occur at a somewhat lower critical shearing stress than hemolysis (approximately 1,500 dynes/sq. cm. for human blood). The morphologically changed cells are rapidly removed from the circulation by the spleen and liver. Damage of this type can be more important than the damage revealed as direct hemolysis.
3. The shearing stress which develops in a jet of blood may exceed the critical value necessary to injure red blood cells and shearing stresses of this magnitude can occur in regurgitant flow about an artificial aortic valve.
4. The morphological changes induced by shearing stress are similar to those observed in patients with hemolytic anemia associated with artificial valves.
5. Pressure variations of the rate of 1.65×10^5 mm. Hg./sec. or less produce little or no red cell trauma.
6. Crushing of red blood cells in seating of the Starr-Edwards valve does not appear to contribute significant hemolysis, morphological changes, or shortened cell survival.

It should be emphasized that some of the conclusions reviewed above are not entirely new. In particular, it has been known previously that shearing stress could hemolyze red blood cells. However, there have been no previous direct quantitative studies on the important effect on life span *in vivo*. Conclusion 3 is also new only in the sense that we have applied the very simple calculation to the cardiovascular system and related the results to clinical observations. Conclusion 5 is new only in that new quantitative figures are reported here, in addition to the new information on life span *in vivo*.

It should also be emphasized that this work has been concerned only with certain physical factors. It is known of course that under some circumstances chemical changes can be of most importance.

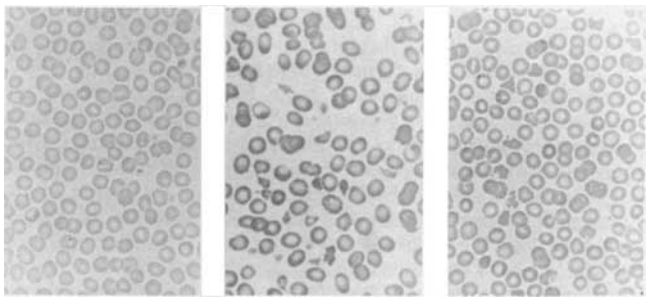

Fig. 5.

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