total oxygen flux inward across the cornea. That fraction of the oxygen flux resulting from the gradient in oxygen tension from the lens reservoir to the anterior chamber could account for 25 percent of the total observed. This would be the flux calculated from Fick's first law of diffusion for an inert cornea in which the oxygen diffusion coefficient and oxygen solubility are taken as equal to that in a 25-percent protein solution. The metabolic use of oxygen by the cornea will reduce the tendency of oxygen to move from the outer surface of the cornea to the anterior chamber. This movement of oxygen is probably much below the 25 percent of the observed flux as estimated herein for the limiting condition of an inert cornea. In view of the limited precision of our data we have chosen not to attempt a correction for this flux.

The best estimate for the initial rate (at an oxygen tension of 155 mm-Hg), taken from the data in Fig. 2, is 6.3 μl/hour. On a per unit area basis, taking 1.3 cm^2 as the area of the human cornea, this is 4.8 μl/cm^2 per hour. At an oxygen tension of 100 mm-Hg the rate is 3.1 μl/cm^2 per hour; at 50 mm-Hg it is 1.5 μl/cm^2 per hour. The extremes of the initial rate caused by the scatter of the data in Fig. 2 are 3.2 μl/cm^2 per hour and 7.2 μl/cm^2 per hour.

Table 1 shows representative oxygen uptake rates given in the literature for several animal corneas as measured by the Warburg respirometer. They have been recalculated here to give an oxygen flux across the cornea surface based on the assumption that all of the cornea's oxygen demand is met from the atmosphere. Our results for the human cornea compare favorably with those for rabbit and bovine corneas (*5, 7*).

At the rates we have observed it is evident that the cornea would, in the absence of tear circulation, very quickly exhaust the limited supply of oxygen, as for example, under a scleral contact lens having a minimum reservoir volume of only a few microliters. Without replenishment through tear circulation, the cornea would be forced to draw on other sources of oxygen or undergo loss of transparency through slowing of normal metabolic processes. Smelser (*11*) has reported loss of cornea transparency often as early as two hours after insertion of such contact lenses. We have confirmed, by means of our recordings of oxygen tension versus time with minimum-clearance lenses, what has long been known clinically, that movements of the eye, when allowed, can continuously replenish the oxygen supply under such a lens (*12*).

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8. The platinum reducing surface was 25 μ in diameter; both this and the silver–silver chloride reference electrode were in a borate buffer solution of *p*H 9 and 0.1 molar KCl. The polyethylene membrane was 12 μ in thickness. The time constant for the entire system averaged 20 seconds.
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Heparin Bonding on Colloidal Graphite Surfaces

Abstract. *Experiments on clotting, both in vitro and in vivo, showed that a colloidal graphite surface, when rinsed with a cationic, surface-active agent, was capable of bonding heparin. The resistance of this graphite-heparin surface to the formation of clots was far greater than plastic or silicone surfaces in comparable studies.*

Artificial valves in animal hearts have not been very successful. More than 95 percent of the animals have succumbed within 1 month after valve replacement, and the overwhelming cause of failure has been the formation of thrombi on the valves, with subsequent disruption of function. Because of the severity of this problem, especially in the canine heart, long-term experimental evaluation of artificial valves for human use has been virtually impossible. Fortunately, thrombus formation on prosthetic valves placed in the human has not been as serious a problem, but it is still a very significant complicating factor and frequently necessitates prolonged administration of anticoagulants.

In an attempt to reduce clot formation on artificial valves, a number of plastic materials and coatings were previously evaluated in this laboratory for their relative abilities to resist the formation of clots (*1*). Of all the materials tested in this earlier study, a new type of intravascular coating substance, colloidal graphite, appeared to give the best results. It was thought that this property of graphite was related to several factors. First, as demonstrated by microscopic studies, the graphite coating provided an extremely smooth surface, filling in small defects on a polished plastic surface. Secondly, the inertness of the carbon in colloidal graphite was considered to be of importance in inhibiting clot formation. Additional properties of graphite, considered at that time to be of questionable importance in preventing thrombus formation, included nonwettability, good lubricity and conductivity, and a negative Zeta potential. More recent data from this laboratory suggest that the most important anticoagulant property of graphite is its apparent ability to bond heparin to its surface. This factor was active in the previous study but was not appreciated by us.

The coagulation of canine blood was tested in vitro and in vivo. Test tubes measuring 9 mm in diameter were used, 1 ml of blood being placed in each one. Glass, polycarbonate (Lexan), silicone-coated, and graphite-coated tubes (*2*) were used. Three tubes of each type were prepared for each study. The first was untreated, the second was filled with heparin and then thoroughly rinsed (*3*), and the third tube was filled with a cationic, surface-active agent (*4*), then heparin, and then thoroughly rinsed (*3*). The time taken for the blood to coagulate in each tube was noted, and some of the results are shown in Table 1.

The coagulation of blood in vivo

Table 1. Results of experiments on the coagulation of blood in vivo and in vitro. Mean values are given for the results in vitro, together with the standard error of the mean.

Material	Time taken for coagulation to occur in vitro (min)			Degree of coagulation in vivo		
	Non-treated	Heparin and rinse*	Zephiran, heparin, and rinse*	1 hour	2 hours	14 days
Glass	6.6 ±.4	8.2 ±.8	17.2 ±.7			
Lexan	14.8 ±1.5	20.4 ±1.0	26.2 ±.4	●●●●	●	
Silicone	17.4 ±1.6	19.0 ±1.7	20.6 ±1.9		●○○○○	
Graphite	21.2 ±.8	52.6 ±3.0	>600†			○○○○○

* All tubes were filled ten times with 0.9 percent saline after heparin had been applied. † The blood did not clot in any of these tubes during the 10-hour time limit of this study.

was studied by placing plastic rings (measuring 7 mm, inner diameter; 8 mm, outer diameter; 9 mm in length) in the thoracic inferior vena cava of dogs weighing approximately 10 kg. This is a very severe test because of the low velocity of the blood in this venous segment. All the plastic rings were placed in the cationic, surface-active agent (Zephiran) for 24 hours, rinsed in saline, and then placed in a dilute heparin solution (0.6 mg/ml) for 1 hour.

Silicone coatings have generally been considered to be the most resistant to clotting, and the advantage of coating a glass surface with silicone is shown in Table 1. It also appears that graphite will absorb heparin directly, since the time taken for coagulation to occur in the graphite-coated tubes, which were filled with heparin and rinsed with saline, was 2.5 times longer than in the tubes with an untreated graphite surface.

Of considerable significance is the fact that blood placed in graphite-coated tubes, prepared with the cationic, surface-active agent, then heparin, and finally thoroughly rinsed with saline, did not clot in the 10-hour time limit of this study. In a further study with these graphite coated tubes, prepared with the surface-active agent, heparin, and saline, the blood was transferred to glass test tubes after 10 hours. Coagulation occurred only after the addition of a small amount of protamine. This indicated that a small amount of heparin had passed from the graphite surface into the blood during the 10-hour period. However, the continued presence of heparin on the graphite surface was suggested by the fact that if these same tubes were then refilled with fresh blood after a saline rinse, clotting was again not observed during an additional 10-hour period. A second series of graphite-coated tubes was similarly prepared with Zephiran, and heparin, but was rinsed 100 times with 0.9-percent saline. In spite of this extreme effort to wash the heparin from the graphite surface, blood placed in these tubes did not clot in 10 hours.

In the experiments in vivo, the fact that all five of the graphite-heparin coated rings were free of clots at 14 days is of considerable significance (Table 1). When untreated graphite rings (not included in Table 1) were placed in the vena cava, severe clotting usually resulted within 2 hours.

At present we do not know how long heparin remains on the surface of graphite-coated prostheses placed in the blood stream. Under ordinary conditions, free heparin in the blood stream is cleared fairly rapidly on passage through the kidneys, and additional heparin is inactivated in the liver and reticulo-endothelial system. Heparin bonded to a fixed prosthesis, however, might remain active for a considerably longer period than free heparin in the blood stream. The presence of the cationic, surface-active agent is thought to be an essential factor in the graphite-heparin bond. The affinity of this cationic agent for the oleophilic surface of graphite, in combination with the adsorptive properties of graphite, might enable a very firm bond to be formed. Once the cationic, surface-active agent was adsorbed on the graphite surface, the negatively charged heparin molecule could, in turn, be bonded by the cationic agent. The heparin molecule has a molecular weight of approximately 16,000 and consists of tetrasaccharide units with at least one sulfate or sulfonate radical for each saccharide. Heparin has the highest negative charge of any organic substance in the body and some investigators have suggested that its anticoagulant properties are related to this high negative charge.

Our results show that at least some of the heparin bonded to a graphite surface gradually moves into the blood within the tube. This suggests that when heparin-coated graphite rings are placed in the blood stream, the heparin might be depleted from the surface of the rings within several hours or days. The experiments in vivo indicate, however, that the rings remained free of clots for at least 14 days, but when graphite rings not coated with heparin were used, clots formed within 2 hours. It is possible, however, that heparin is removed from the graphite surface by the flowing blood, but is replenished by endogenous heparin, or by other anticoagulant substances, which might be adsorbed by the graphite from the blood stream. A number of graphite-coated plastic valves, prepared with Zephiran and heparin and placed in the canine heart, have been examined after 1 year and have revealed an intact graphite surface with little or no clot present (*5*).

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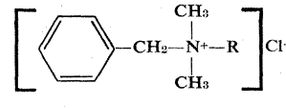
2. The graphite is available from the Acheson Colloid Company, Port Huron, Michigan.
3. The tubes were rinsed ten times with 0.9 percent saline.
4. The cationic, surface-active agent used was Zephiran chloride. This is a quaternary ammonium germicidal agent manufactured by Winthrop, Inc., New York, and has the formula



in which the R is an alkyl group ranging from C_8H_{17} to $C_{18}H_{37}$. A concentration of 1 to 1000 was used in these studies.
5. This work was supported by U.S. Public Health Service grant H-4162 and by the Wisconsin Alumni Research Foundation.

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