STUDIES ON PLATELETS

V. The Effect of Platelets on the Surface Tension of Saline Solutions and Heparinized Plasma

By Alfred L. Copley, M.D., and Daniel F. Glaser, M.D.

IN THE literature on blood platelets there is no account of any work pertaining to their surface tension.[1] It has been the purpose of this study to determine the effect of the platelets on the surface tension of physiologic saline solutions and how it varies in different platelet concentrations suspended in saline. It was of further interest to ascertain whether heparinized plasma, in which platelets are suspended, would exhibit surface tension phenomena similar to those exhibited by platelets suspended in saline.

These experiments have been made on isolated platelets from the blood of 6 dogs. The method of platelet isolation as employed here differs somewhat from a standard procedure developed later and described elsewhere by Copley and Houlihan.[2] The Cenco-du Nouy Tensiometer Precision Form, no. 70530, was used. The ring was of platinum-iridium with a mean circumference of 4 cm.

PROCEDURES

I. Obtaining the Blood

A clean, dry, sharp 18 gauge needle is affixed to one of two 30 cc. clean, dry syringes, and blood is drawn from the jugular vein of normal dogs. Previous to making the venipuncture, 0.5 cc. of 1 per cent sodium heparin solution (Roche-Organon*) is placed into each of two 50 cc. Lusteroid tubes. The first 30 cc. of blood drawn from the dog are placed immediately in one of the tubes, leaving the needle in the vein. The other 30 cc. syringe is fixed to the needle and 30 cc. more of blood is drawn. The needle and syringe are removed, and the blood is placed immediately into the second tube. Both tubes are inverted several times to mix the heparin with the blood thoroughly, care being taken to avoid bubble formation. In the blood sample from dog 923, 2 cc. of 1 per cent heparin solution was used for 80 cc. of blood, or 0.25 mg. per cc. blood. The blood samples from the other dogs contained 0.17 mg. per cc.

II. The Preparation of Platelet Suspensions

The heparinized blood obtained in Lusteroid tubes is placed in a centrifuge (International SB-1) and rotated for 30 minutes at 2900 to 3100 rpm. The tubes are removed and the plasma removed from the top by means of a capillary pipet.

From the Laboratory of Cellular Physiology, Department of Biology, New York University, New York City.

The experiments on which this paper is based were done in the Department of Surgery and Gynecology, School of Medicine, University of Virginia, Charlottesville, Virginia.

*Kindly supplied by Dr. Leo A. Pirk of Roche-Organon, Inc., Nutley, New Jersey.

Saline (chilled in ice water) is then placed over the buffy coat. The buffy coat is then removed together with a small portion of the red cell layer. This is placed in a special tube which is drawn to a point. Saline is added to within about 0.5 cm. of the top of the tube and the suspension is thoroughly agitated. The tube is then centrifuged at 2500 rpm for 30 minutes. The buffy coat is again removed after removal of the supernatant and additions of chilled saline. The buffy coat is placed again in one of the special tubes and the tube is filled with saline and agitated as before. The tube is centrifuged for 20 minutes, at 2500 rpm. The process of removal of the buffy coat and addition of saline continues until 4 more washings are all centrifuged at 2500 rpm for 20 minutes. As the washings progress, the buffy coat will be seen to become sharply differentiated into a very white layer and a pink layer. At the fifth washing only the white layer is taken off. These are the platelets. After the sixth washing the supernatant fluid is withdrawn and the platelets are overlaid with a layer of chilled saline just large enough to permit the removal of the platelets. These are then transferred to a centrifuge tube and diluted to 5 cc. The count is then made. In dog 923 all centrifugations subsequent to the first one were made at a speed between 1900 and 2100 rpm instead of 2500 rpm.

III. The Tensiometer Readings

A. *Preparation of Glassware.* All glassware is thoroughly cleaned with soap and water, and placed in hot boiling cleaning solution (potassium bichromate, 20 Gm., concentrated sulfuric acid, 1000 cc.) for 1 hour. It is then washed in distilled water 4 or 5 times and placed in a drying oven. When thoroughly dry, it is used immediately after allowing it to cool to room temperature. Fifteen to twenty minutes before use, it is run through the flame of a microburner. The surface of the watch crystals to be in contact with the suspensions are run through the flame of a microburner 15 to 20 minutes before use.

B. The tensiometer is standardized.

C. After standardization, the stirrup that stops the arm carrying the loop is adjusted so that the lever arm can only go 1 or 2 mm. beyond the zero reading. This is done so that the loop will not pull completely away from the suspension each time a reading is made. However, the point at which the loop breaks away from the suspension is easily determined if that point is approached slowly. This saves time and does not agitate the suspension as much as when the loop breaks away from the suspension with each reading. The various suspensions and solutions are each introduced in turn. A reading is made as soon as possible after placing the suspension in the watch crystal. Readings are then taken at intervals of 1, 2, 3, 4, 6, 9, 14, and 19 minutes. Readings are begun 15 seconds before each minute at which the reading is recorded. The temperature is recorded to the nearest 0.1° C. at each reading.

RESULTS

In table 1, 8 samples of saline are shown with their corresponding surface tensions in dynes per cm. Eight to nine determinations were made within 19 minutes in

7 cases and within 24 minutes in 1 case. The variation of temperature in each series of tests was never more than 0.8° C. Five series were run within 24.8 to 25.8° C. and the surface tensions ranged from 72.2 to 74.2 within these temperatures.In

TABLE 1.—*The Surface Tension of Different Samples of 0.9 Per Cent Sodium Chloride Solution*

Sample	Determinations		Range of Temp. degrees C.	Surface Tension Dynes per cm.	
	Number	Time Interval		Range	Average
		min.			
1	9	19	24.8–24.9	72.5–72.9	72.8
2	8	19	25.0–25.8	72.2–72.5	72.4
3	9	24	25.0–25.8	72.8–73.0	72.8
4	9	19	25.2–25.4	73.8–74.0	74.0
5	9	19	25.2–25.4	73.7–74.2	74.0
6	9	19	28.5–28.6	73.4–73.8	73.6
7	9	19	28.5–28.6	74.1–74.6	74.3
8	9	19	28.8–28.9	73.0–73.4	73.2

TABLE 2.—*Surface Tension Values of Platelet Suspensions in Saline in Comparison with Values Obtained on the Supernatant Liquids of Same Suspensions Following Centrifugation*

Dog Number	Added Platelets × 10^3 per mm.^{3}	Surface Tension of Nine Determinations within Nineteen Minutes							
		Platelets Plus Saline				Supernatant of Platelet Saline			
		Temp. degrees C.	Dynes/cm.			Temp. degrees C.	Dynes/cm.		
			Range	Average	Time Drop		Range	Average	Time Drop
871	1100	25.0–25.8	58.0–52.8	55.2	5.2				
	275	25.5–25.8	66.1–58.7	61.4	7.4	25.0–25.8	66.3–59.3	61.2	7.0
	55	25.0–25.8	66.0–60.4	63.7	5.6	25.0–25.8	73.3–68.5	71.2	4.8
914	1.384	28.4–28.8	59.2–52.8	55.5	6.4	28.8–29.0	63.8–57.0	59.5	6.8
	69	28.8–29.1	66.6–57.3	61.1	9.3	28.8–29.0	74.4–71.9	73.8	2.5
911	660	25.6–26.2	59.1–50.5	53.7	8.6	24.6–24.8	60.2–52.8	55.6	8.4
	76	25.5–25.6	71.6–63.0	67.6	8.6	24.7–24.9	72.2–72.9	72 6	0
879	2290	29.0–29.2	57.3–50.8	53.8	6.5	29.6	60.6–55.8	57.4	4.8
	115	29.3–29.4	65.5–59.0	61.2	6.5	29.3–29.7	74.2–68.8	71.3	5.4
872	230	25.0–25.2	64.9–56.4	59.4	8.5	25.7–25.9	65.4–56.4	59.5	9.0
	23	25.3–25.5	71.5–60.4	65.2	11.1	25.7–25.9	73.9–67.0	71.2	6.9
923	1148	26.4–26.6	59.1–54.6	56.2	4.5	26.4–26.8	63.5–57.7	59.6	5.8
	115	26.4–26.7	61.4–57.4	58.7	4.0	27.1–27.4	74.0–68.7	71.2	5.3

a group of 3 series of tests the temperatures ranged from 28.6 to 28.9° C., and the surface tension at this temperature from 73.0 to 74.6. In each of the 8 series the surface tension differs only by a value of 0.2 to 0.5 dynes per cm.

Surface tension determinations for various concentrations of platelets suspended in saline and obtained from heparinized blood of 6 dogs are presented in table 2.

Surface tension values of the supernatant liquid following centrifugation of the same suspensions are given for comparison. The number of platelets presented in the lower concentrations represent values obtained by diluting the original platelet suspension. No actual counts were made on these latter dilutions and the count is therefore only an approximation. Original counts were made in 24 fields of the Neubauer chamber. It has been shown by Copley and Houlihan[2] that as a rule such platelet suspensions, when diluted, give counts approximating the calculated values.

Platelet suspensions in higher concentrations uniformly showed greater decreases in surface tension than suspensions of lower concentration. It is of interest that even such a minute number of platelets as 7000 per cmm. showed decrease in surface tension as compared with the saline controls as given in table 1. These determinations have been made on 9 successive tests over a period of 19 minutes. The few exceptions from this rule are noted in the table. In all platelet suspensions in saline there is a range of surface tension values, differing within the range of 4.0 to 11.1 dynes per cm. The surface tension usually decreases successively with each subsequent determination within the 19 minute time interval. No determinations have been made beyond this time limit. The average of each of the 9 determinations is given and serves as a figure for comparison with other determinations. Following centrifugation of the given platelet suspensions, the supernatant liquid tested usually showed variations in surface tension which likewise were further lowered upon successive determinations. The platelet-free supernatant did not exhibit any appreciable decrease in surface tension as compared with the saline control given in table 1 when prepared from the lowest platelet concentrations. These lowest concentrations ranged from approximately 7000 to 115,000 per cmm. In case the range exhibits in these lower concentrations up to 6.9 dynes per cm. difference, the drop in surface tension was not usually observed before the 9 minute reading. In the saline supernatant from centrifugation of suspensions with higher platelet concentration there was always a decrease in surface tension as compared to that of the saline controls of table 1. Supernatant samples of dogs 871 and 872 with higher platelet concentration did not show any difference in surface tension when compared to the corresponding platelet suspension. In samples from the other 4 dogs, the surface tension was from 1.9 to 4.0 dynes per cm. higher in the supernatants than in the samples of platelet suspensions. All these comparisons are average values.

The so-called "time drop" expresses the difference between the surface tension values in dynes obtained in certain time intervals.[3] The time drop is about the same in platelets in saline suspensions and in the supernatant of this system when high platelet concentrations are considered. However, in lower platelet concentrations there is a decrease in time drop in the materials from dogs 914 and 872. In dog 911 the values in the saline supernatant of low platelet concentration are similar to those of the saline control.

Table 3 demonstrates surface tension values obtained in plasma of 4 dogs with and without the addition of platelets. Results following the use of two concentrations of platelets in plasma are given. The heparinized plasma is from the same

blood used as a source of platelets. The heparin concentration of the plasma of dog 923 was about 50 per cent higher than in the plasma from the other dogs. There was always a successive decrease in surface tension within the 19 minute test period, the differences ranging from 3.1 to 7.5 dynes per cm. in platelet suspensions in plasma. This decrease was of about the same order in both the supernatant of the platelet plasma and of the plasma itself. In comparing the average values, each obtained from 9 determinations within 19 minute test periods showed the following: There was no change in surface tension between the higher and lower

TABLE 3.—*Comparison in Surface Tension of Platelets Suspended in Heparin Plasma, the Supernatant Plasma Thereof, and of Heparin Plasma*

Dog Number	Added Platelets $\times 10^3$ per mm.^{3}	Surface Tension of Nine Determinations Within Nineteen Minutes											
		Platelets Plus Plasma				Supernatant of Platelet Plasma				Plasma			
		Temp. degrees C.	Dynes/cm.			Temp. degrees C.	Dynes/cm.			Temp. degrees C.	Dynes/cm.		
			Range	Average	Time Drop		Range	Average	Time Drop		Range	Average	Time Drop
914	1384	29.1–29.5	57.0–52.8	54.2	4.2					28.7–29.1	59.0–53.4	55.5	5.6
	69	29.1–29.2	56.3–53.2	54.5	3.1								
897	2290	29.6–29.5	56.4–52.9	54.1	3.5	29.7–29.8	50.5–49.5	49.9	1.0				
	115	29.7–29.8	57.0–52.3	53.8	4.7	29.7–29.8	54.4–52.1	53.1	2.3				
872	230	25.6–25.8	64.0–59.7	61.2	4.3	26.1	61.0–54.6	58.2	6.4	25.5–25.9	61.7–54.6	57.9	7.1
	23	25.4–25.8	59.5–54.5	57.4	5.0	26.1–26.2	60.6–54.8	58.0	5.8				
923	1148	26.9–26.4	63.7–56.2	60.2	7.5	27.2–27.4	54.7–51.4	52.3	3.3				
	115	26.9–27.3	62.3–57.3	59.7	5.0	27.3–27.5	54.5–51.7	52.5	2.8	25.0–25.2	55.4–52.7	53.4	2.7

concentrations of platelet suspensions in 3 dogs, whereas in dog 872 there is an increase of 3.7 dynes per cm. in the higher concentration. In comparing the supernatant platelet plasma or the plasma from dogs 872 and 923, there is no marked change in surface tension in either concentration from dog 914 and in the lower concentration from dogs 879 and 872. However, there is an increase in the higher platelet concentration of sample 987 and 872 and in both concentrations from dog 923. This increase of surface tension in these platelet suspensions in plasma ranges between 3.0 to 7.2 dynes per cm. In comparing the surface tension of the supernatants from the 2 concentrations of platelets, there is no change in values when comparing dogs 872 and 923. However, there is a decrease of 3.2 dynes per cm. in the surface tension values of the supernatant of the highest concentration of platelets used.

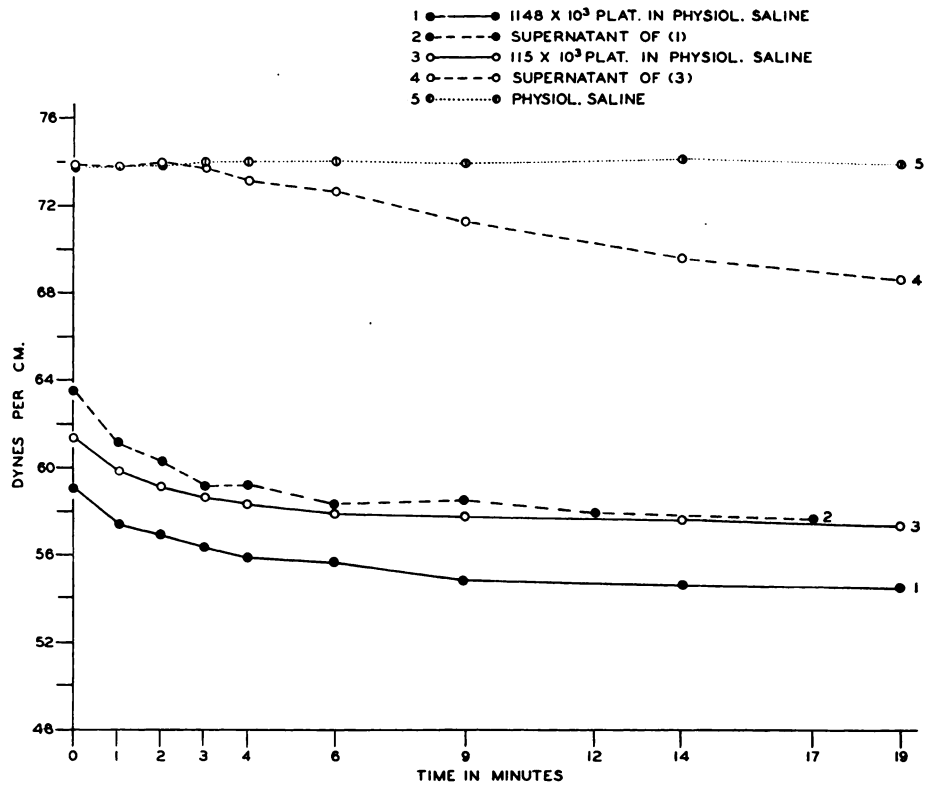

FIG. 1. THE EFFECT OF PLATELETS ON THE SURFACE TENSION OF SALINE SOLUTIONS

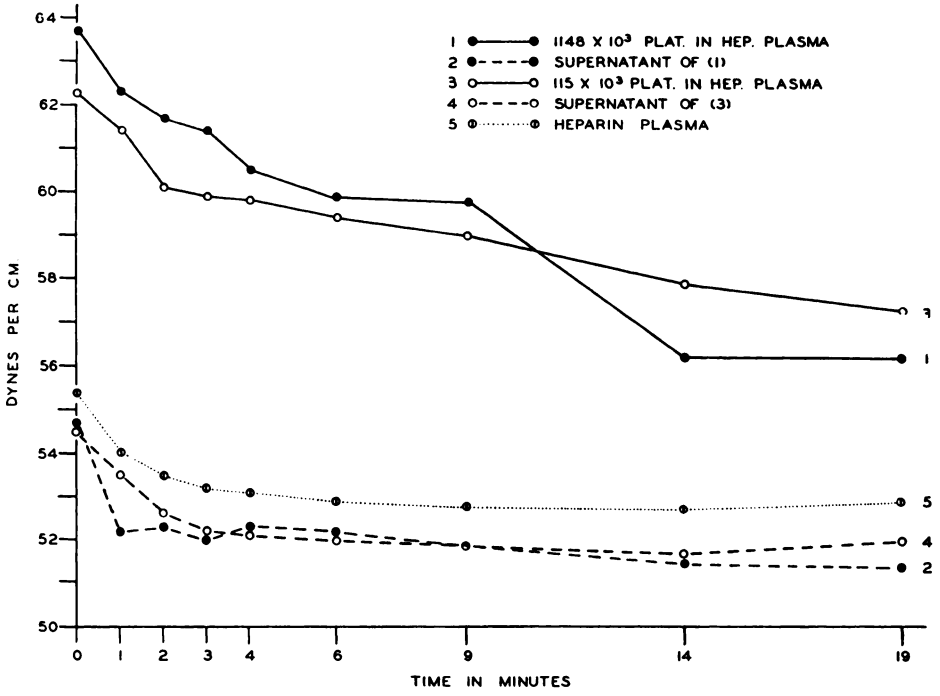

FIG. 2. THE EFFECT OF PLATELETS ON THE SURFACE TENSION OF HEPARINIZED PLASMA

The time drop is either slightly lower in the platelet plasma systems of dog 914 and dog 872 as compared to their supernatants or plasma controls. In the systems from dog 879 and 923 the reverse occurred, the platelet suspensions in plasma showing a higher time drop than their supernatants or the plasma control.

Figures 1 and 2 present the time drop within a 19 minute test period in two platelet concentrations suspended in saline and plasma respectively, their supernatants, saline or plasma controls. It can be seen that the largest time drop varies with different systems tested.

COMMENT

The decrease in surface tension (T) seen in the higher platelet concentrations in saline cannot be found with the corresponding suspensions in plasma. There is rather a trend toward an increase in surface tension of platelets suspended in plasma, especially with higher platelet concentration. Whether this phenomenon is only true in heparin plasma or is general in plasma as obtained with any other anticoagulant, further studies will have to establish. The positive increment of surface tension (+ Δ T) of the platelet-plasma system suggests pellicle formation in which measurements represent T plus yield value.

It is of interest to note that decrease in surface tension has been generally observed within the 19 minute test periods employed. This phenomenon was not present when saline alone was used. However, saline which previously served as the liquid phase for the suspension of platelets, when made platelet-free, still showed decrease in surface tension as a rule in samples from higher platelet concentrations and in some samples of the lower platelet concentrations. From these observations it is evident that the platelets by their presence in saline have altered this liquid. It cannot be stated which factor or factors cause this change in saline. There are two possibilities: one that some substance has been released from the platelets by diffusion, and the other that the platelets may have disintegrated and thus liberated certain substances. It may be argued that the latter possibility is unlikely, since such platelet suspensions prepared from heparinized blood may be kept for several weeks without significant change in the platelet count.[2] Even centrifugation at high speed to separate the platelets from the liquid phase should not destroy the platelets and cause lysis of their particles. It has been shown that these platelets are rather stable and that only repeated centrifugations following suspension of platelets in distilled water may bring about any degree of lysis.

The argument presented does not eliminate the possibility that breakdown of platelets is responsible for $-\Delta$ T. It only requires a monolayer of T active molecules to reduce T. The methods available for determining the number of platelets are probably insensitive to measure minute changes in platelet count. The fact that salt-platelet systems give greater $-\Delta$ T/time suggests that platelet breakdown is more extensive than in platelet-plasma systems. The progressive $-\Delta$ T/time indicates accumulation of surface active molecules at surface. The relatively slow $-\Delta$ T/time suggests that rather large molecules are probably involved. Disruption of surface usually produces $+\Delta$ T, until new surface active molecules are restored at the surface.

Du Nouy[3] pointed out that in studying the surface tension of any colloidal solution with his technic, the value of the tension decreases regularly. He showed that stirring causes a return to initial surface tension. He differentiates between dynamic and static values, the latter being obtained when the system has attained a state of equilibrium. He found that when solutions of different substances contain a small quantity (1 per cent) of sodium chloride, the drop of the surface tension becomes greater in two hours. The time drop after immunization with certain antigens was greater than before immunization.

In our experiments the differences in time drop between the platelet suspensions in saline or plasma and their substances or the plasma controls are of a small order and do not exhibit any clear-cut evidence. Perhaps if longer time intervals—as, for instance, the two hour interval used by du Nouy—were employed in a larger series of tests, differences in the time drop might become more evident.

Neurath and Bull[4] reviewed the literature on the surface tension of protein solutions and discussed the limitations of the technic so far employed. The insoluble film which is formed at a protein solution surface is, according to Astbury, Bell, Gorter, and Van Ormondt,[5] probably highly organized with the protein molecules oriented. The organization of this film is disrupted during each T measurement and thus the measured T may be different from that in its quiescent condition. On this basis Neurath and Bull believe that the du Nouy tensiometer induces inaccuracies in T measurements. These authors assert, since it is extremely difficult to measure accurately T of protein solutions, that "it may be fairly stated that the equilibrium surface tension of such solutions has never been determined."[4]

It cannot be stated that a suspension of 0.9 per cent sodium chloride solution or a so-called physiologic saline would necessarily be physiologic for such isolated platelets. Our experiments may be suggestive that this is not the case since the platelets, possibly by diffusion, have liberated some of their inert substances to the surrounding medium of saline. It is of interest that the surface of the blood platelet was found by Enders and Hergert[6] to be more transmissible for ions than the surface of the red blood cell.

The observations on plasma should be considered only preliminary since they represent a small number of cases which are reported mainly because of findings which should stimulate further studies. $+ \Delta T/\text{time}$, as mentioned above, may be the result of a pellicle formation—possibly an intermolecular complex between plasma proteins and products of platelets. With protein solutions, one is frequently concerned with pellicle formation. Such phenomena invariably cause a $+ \Delta T$, but such changes are apparent for ΔT. The yield value of the pellicle must be considered, preferably according to the treatment made by Kopac,[7] viz.,

$$\text{Apparent } T_a = T + y, \text{ where y equals yield value in dynes/cm.}$$

Attempts are being made by Kopac to measure this yield value.[7]

SUMMARY

1. Washed isolated platelets added to physiologic saline produce fall in surface tension.

2. Higher concentrations of platelets in saline cause a greater fall in surface tension than lower concentrations.

3. After centrifugation of platelets suspended in saline, the supernatant may show a fall in surface tension as compared to saline in which no platelets have been suspended. In the higher concentrations of platelets, the fall is much greater than in the lower concentrations.

4. When platelets are suspended in heparinized plasma, they exhibit either no change or a higher surface tension than do either their supernatants following centrifugation or the heparinized plasma alone.

5. No full explanation for the observed phenomena can be given.

The authors are indebted to Dr. M. J. Kopac for his valuable suggestions.

REFERENCES

[1] Tocantins, L. M.: The mammalian blood platelet in health and disease. Medicine *17:* 155, 1938.

[2] Copley, A. L., and Houlihan, R. B.: Studies on platelets. VI The isolation of platelets from human and dog blood. Blood (Supp) *1:* 170, 1947.

[3] Du Nouy, P. L.: Surface Equilibria of Biological and Organic Colloids. New York, The Chemical Catalog Company, 1926.

[4] Neurath, H., and Bull, H. B.: The surface activity of proteins. Chem. Rev. *23:* 391, 1938.

[5] Astbury, W. T., Bell, F. O., Gorter, E., and Van Ormondt, J.: Optical and x-ray examination and direct measurement of built-up protein films. Nature *142:* 33, 1938.

[6] Enders, G., and Hergert, L.: Ztschr. f. Biol. *98:* 136, 1937.
(Cited by Wolpers, C., and Ruska, H.: Strukturuntersuchungen zur Blutgerinnung. Klin. Wchnschr. *18:* 1077, 1111, 1939.)

[7] Kopac, M. J., *in:* Reyniers, J. A.: Micrurgical and Germ-Free Methods. Springfield, Ill., Charles C. Thomas, 1943, p. 26.