Methylcellulose as a Wetting Agent in Blood Clot: In Vivo Studies

By Morris Rosenfeld

THE physical properties of colloidal fluids and gels can often be modified by additives of very mild chemical reactivity. In a previous study[1] certain characteristics of plasma were found to be strongly influenced by the incorporation, in vitro, of the polysaccharide, methylcellulose. Two interrelated effects were observed; fluid plasma showed a pronounced change in surface tension, clotted plasma took on hydrophilic surface properties as opposed to its normal hydrophobic character. Methylcellulose stood out distinctively from other colloids in this surface behavior. It is a water soluble, gum-like derivative of cellulose that is widely used to adjust the characteristics of colloid products. Among its uses are the regulation of viscosity, stabilization of emulsions, enhancement of adhesiveness, promotion of strength and flexibility of plastic films. Owing to its chemical inertness, methylcellulose can be introduced into the blood stream for the study of possible effects in circulating plasma.

Methods and Materials

Plasma Concentration. Methylcellulose, 10 centipoises viscosity, was injected intravenously as a 2 per cent solution in saline. Two unanesthetized dogs were used, each receiving 35 to 50 mg. per Kg. within a 5 minute period.

Analytic measurements were made by an adaptation of the anthrone reaction. The general procedures and optimal conditions for applying this color reaction to methylcellulose and other polysaccharides have been worked out by Samsel and DeLap[2] and by Scott and Melvin.[3] In extending the analysis to blood plasma, two additional problems were met; separation of blood glucose which also reacts with anthrone, and removal of plasma proteins without loss of methylcellulose.

Glucose was removed with baker's yeast (Fleischmann) washed six times with several volumes of water; 2.0 ml. of a 50 per cent suspension of the washed yeast was centrifuged in a small test tube and the supernatant water layer was removed with a fine-tipped pipet; 2.0 ml. of oxalated plasma was added, the yeast was stirred to a smooth suspension and the tube was repeatedly agitated during a 15 minute period of contact at room temperature. The yeast was removed by centrifugation for 10 minutes at 2000 rpm. and the supernatant was again centrifuged to insure complete removal.

For protein precipitation, dialyzed iron was found to give complete recovery of added methylcellulose, whereas poor recoveries were obtained with sodium tungstate and with trichloroacetic acid. The deproteinization was essentially that of Jackson, Kuhl and Irvin.[4] To 3.0 ml. of distilled water was added 1.0 ml. of glucose free plasma and the tube was placed in a boiling water bath for 3 minutes; 0.6 ml. of dialyzed iron (Merck, 5 per cent Fe_2O_3) was added, the mixture was shaken vigorously, and then was replaced in the boiling bath for another 2 minutes. The tube was then cooled in ice-water with repeated shaking to insure complete solution of methylcellulose, which has the property of being more soluble

From the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore.

This investigation was supported by a research grant, C-1841, from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

The author gratefully acknowledges the technical assistance of Miss Dorothy Ann Wood.

Submitted July 13, 1956, accepted for publication Sept. 20, 1956.

at low temperature. After addition of 0.4 ml. of 22 per cent sodium sulfate to precipitate excess colloidal iron, the tubes were centrifuged for 10 minutes at 2000 rpm. at 1–4 C. The supernatant was recentrifuged to remove all precipitated iron.

The anthrone reaction was applied in the following manner: 50 mg. of anthrone (Matheson Coleman and Bell) was dissolved in 100 ml. concentrated sulfuric acid. The solution was allowed to age 4 hours and was used within 48 hours; 4.0 ml. quantities were distributed in test tubes immersed in cold tap water; 2.0 ml. of plasma filtrate was stratified over the acid. The two layers were first gently, then more vigorously mixed with individual glass stirring rods. Only a faint color developed during this mixing at low temperature. The tubes were placed in a boiling water bath for 5 minutes to develop color and then were returned to the cold bath. Optical density readings were made at room temperature in a Beckman, model DU spectrophotometer at the wave length, 625 mμ.

Standards were prepared both in water and in blood plasma taken prior to injection of drug. Glucose-free control plasma contained anthrone reacting material equivalent to about

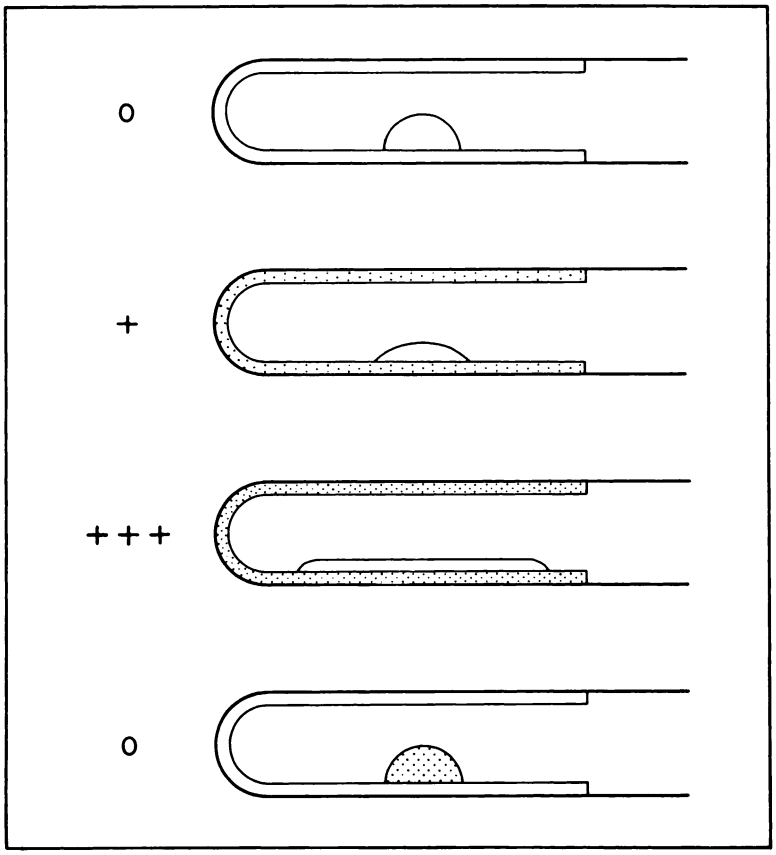

FIG. 1.—Diagrammatic sketch of clot-wetting test. O denotes absence of wetting action. Normal plasma, deposited on surface of drug-free clot, remains associated as a lens-like droplet with a contact angle of approximately 90°. + denotes slight wetting action. Normal plasma, on surface of clot containing a low concentration of methylcellulose, forms a lens with a contact angle of approximately 45°. + + + denotes positive wetting action. Normal plasma, on surface of clot containing a high concentration of methylcellulose, exhibits extensive spread, with a contact angle of approximately 0°. The lowest diagram shows absence of wetting action when fluid plasma, containing methylcellulose, is deposited on surface of drug-free clot. In all cases, stippling indicates presence of methylcellulose.

10 mg. of methylcelluose per 100 ml. A standard glucose solution (100 mg. per 100 ml.) was carried through the procedure to check the effectiveness of the yeast suspension and a water blank was similarly treated to insure that no anthrone reacting material was contributed by the yeast or other reagents.

Clot-wetting action, surface tension effects and *coagulation time.* Five dogs anesthetized with sodium phenobarbital, 140 mg. per Kg., given intraperitoneally, were used; three for methylcellulose studies, two for comparative tests on other colloids. Drug injections were made into the radial vein of the foreleg, the femoral veins were exposed for withdrawal of blood samples.

For test of clot-wetting action, the sample was taken in a siliconed syringe containing heparin in salt solution, 2 μg. per ml. of blood. It was centrifuged for ½ hour at 2000 rpm. at 0–4 C. Plasma was separated and stored in siliconed tubes immersed in cracked ice. About 10 drops of plasma were flowed over the interior lower half of a culture tube and the excess was drained to the bottom. One drop of thromboplastin extract (Difco, from rabbit brain) was added, the mixture was again flowed over the previously moistened surface, and the excess was withdrawn by pipet. The tube was held vertically a minute or two to permit formation of a uniform film during coagulation. Then with the tube placed horizontally, a drop of normal fluid plasma was gently deposited on the surface of the clot with a bent-tip pipet and the extent of spread was noted (fig. 1).

The sample for surface tension measurement was drawn in an unsiliconed syringe moistened with 38 per cent sodium citrate, 0.015 ml. per ml. of blood. Plasma was separated as above except that unsiliconed glassware was used. Measurements were made within a few hours after removal of the sample from the dog. The tube of citrated plasma was held in a water bath 10 or 15 minutes to bring it to room temperature and a 5.0 ml. aliquot was transferred by pipet to a watch glass for measurement in the Cenco-du Noüy tensiometer. Readings were made at 1 or 2 minutes and at 5 or 10 minute intervals thereafter. Between measurements, the watch glass was kept in a moist chamber consisting of a petri dish provided with a wet cotton pledget.

In measuring coagulation time, the precautions emphasized in Tocantins'[5] manual were observed. A 15 ml. sample was drawn in a siliconed syringe; 2 ml. was discarded, then 1 ml. quantities were distributed to two sets of small tubes, 5 siliconed and 5 untreated, all par-

TABLE 1.—*Wettability of Plasma Clot Following Intravenous Administration of Methylcellulose and Other Colloids*

Colloid	Dog No.	Dose mg./Kg.	Before drug	Hour after drug: Degree of wetting		
Methylcellulose	4-4	100		½	7	19
			0	+++	+++	+
Methylcellulose	4-11	100		½	6	
			0	+++	+++	
Methylcellulose	4-24	100		2	11	24
			0	+++	++	+
Carboxymethylcellulose	4-27	100		½		
			0	0		
Dextran	5-2	300		½		
			0	0		
Polyvinylpyrrolidone	5-2	175		½		
			0	0		

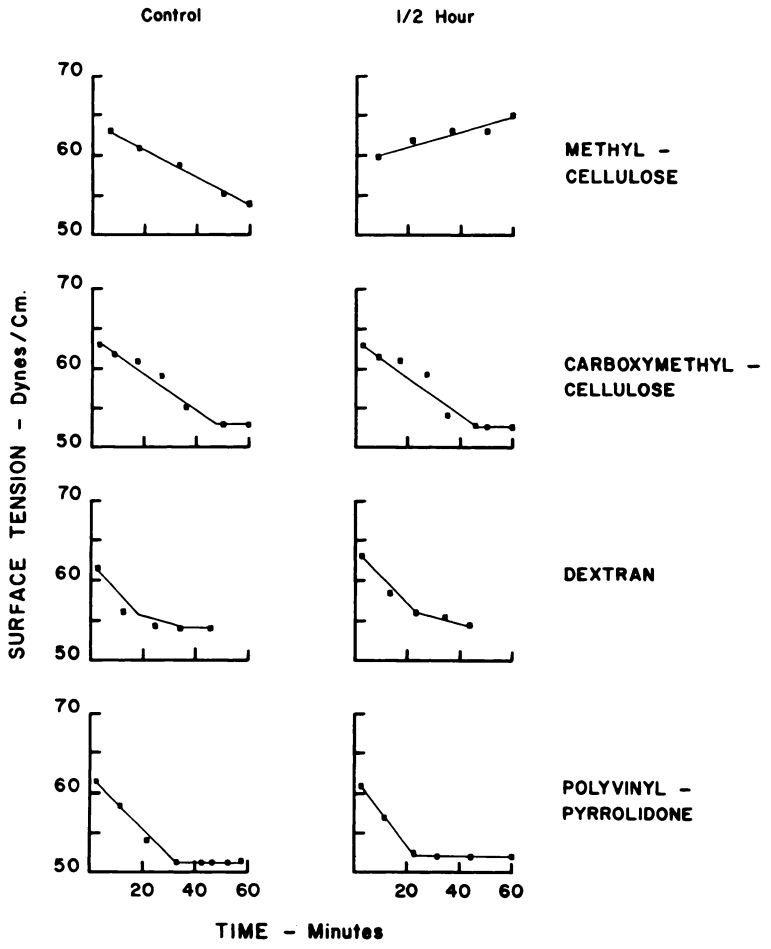

FIG. 2.—Surface tension-time curves on plasma taken before and after intravenous injection of methylcellulose and other colloids.

tially immersed in a water bath at 37 C. Starting near the expected coagulation time, the tubes were examined at 1 to 3 minute intervals, seriatim, after coagulation of the preceding tube. The median time for coagulation in the last four tubes was taken as the end point.*

RESULTS

Increased wettability of the clot surface was readily demonstrated in samples taken after injection of methylcellulose (table 1). Plasma films prepared from the pre-drug and post-drug specimens showed a striking difference. A droplet of normal fluid plasma deposited on a clotted film of control plasma remained aggregated as a lens-like globule; the same normal plasma, deposited on a clotted film

* Methylcellulose was generously supplied as Methocel, National Formulary grade, 10 centipoises type, by the Dow Chemical Company, Midland, Michigan. The Hercules Powder Company, Wilmington, Delaware, very kindly furnished the carboxymethylcellulose as Cellulose Gum, premium grade, CMC-70, extra low viscosity. Dextran and polyvinylpyrrolidone were obtained commercially as clinical solutions for intravenous use as plasma volume extenders.

from a methylcellulose treated animal, spread at once. Wettability was pronounced in the ½, 2, 6, 7, and 11 hour samples. The 19 and 24 hour samples showed definite hydrophilic activity, but of a reduced degree. In these late samples the lens displayed a contact angle of about 45° compared to 0° in the earlier specimens.

Injection of the other colloids, carboxymethylcellulose, dextran, and polyvinylpyrrolidone did not alter the wettability of the clot surface. These inactive materials were tested in two or three times the methylcellulose dosage. The clot surface remained hydrophobic with contact angles grossly the same as before drug injection.

The time course of surface tension measurements on control specimens showed a characteristic pattern (fig. 2). The initial readings, which may be regarded as dynamic values, lay between 60 and 65 dynes per cm. With the plasma left in the watch glass, undisturbed except for the measurements, the surface tension slowly fell. The tension-time curve exhibited a negative slope, usually leveling off to a stationary value at about one hour. This static reading lay between 50 and 55 dynes per cm.

Following methylcellulose injection, the initial reading was depressed about 2 to 5 units. Instead of a pattern of diminishing values, the readings increased with time, usually reaching a plateau in one-half to one hour. The other colloids, on the other hand, did not alter the normal tension-time curve. The dynamic readings and the slope characteristics remained unchanged from the controls.

In figure 3 are recorded the tension-time curves on plasma samples taken at different intervals after methylcellulose administration. A reduced dynamic level

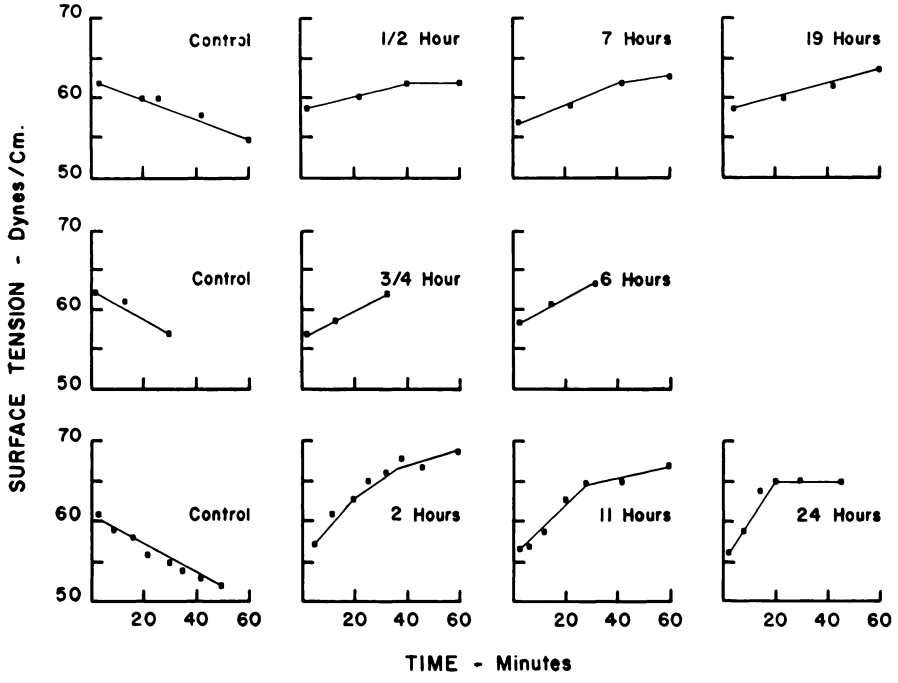

FIG. 3.—Surface tension-time curves on plasma taken at different time intervals after intravenous administration of methylcellulose.

and a positive slope characterized all the post-injection curves up to and including that of the 24 hour sample. The static level lay about 5 to 10 units above the initial dynamic value. The effect of the drug was demonstrable over the whole 24 hour period.

Whole-blood coagulation time, measured in glass tubes, showed no change following methylcellulose injection. The results are shown in table 2. No effect was discernible with the other colloids. Clotting time in siliconed tubes was prolonged after methylcellulose injection to the extent of 20 to 40 per cent above pre-drug levels. This effect was not peculiar to methylcellulose, but was encountered with the other colloids, though possibly to a lesser degree. A trend toward

TABLE 2.—*Coagulation Time Following Intravenous Administration of Methylcellulose and Other Colloids*

Colloid	Dog No.	Coag. time (min.) in glass			Coag. time (min.) in silicone		
		Before drug	After drug	Time (hr.) after drug	Before drug	After drug	Time (hr.) after drug
Control—no colloid	6-7	15			45		
		18(1)*			50(1)*		
		18(3½)			51(3½)		
Methylcellulose	4-4	9	9	½	21	30	½
		10(2)	10	5	25(2)	35	5
Methylcellulose	4-11	12	12	1	19	22	1
			12	6		28	6
Methylcellulose	4-24	12	14	½	25	33	½
Carboxymethylcellulose	4-27	14	13	¾	37	40	¾
Dextran	5-2	17	17	¾	30	38	¾
Polyvinylpyrrolidone	5-2	18	21	¾	44	50	¾

* Figure in parenthesis is time interval, in hours, between successive control readings.

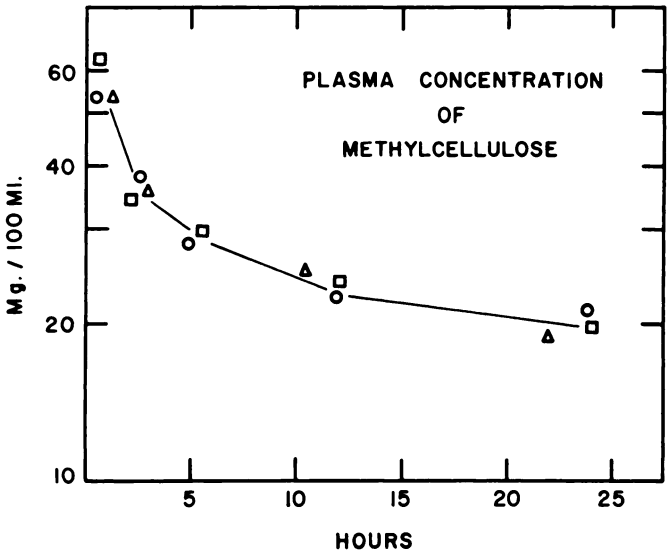

FIG. 4.—Plasma concentration of methylcellulose. Intravenous injection at zero time. △ Sheppie, 37 mg./Kg.; □ Sheppie, 50 mg./Kg.; ○ Methyl, 50 mg./Kg.

prolongation of clotting time in silicone was observed in two anesthetized animals even in the absence of colloid injection, making it difficult to appraise the significance of the methylcellulose effect.

Methylcellulose levels in blood plasma, following intravenous injection in dogs, are shown in the graph (fig. 4). The plasma level that would have been attained by equilibration immediately after injection was evaluated by extrapolating back to zero time from the one-half and the two and one-half hour readings. From this data and the known injected dose the volume of distribution was calculated. It turned out to be 6 per cent and 7 per cent of the body weight in two separate experiments on the dog, Sheppie, and 9.4 per cent in Methyl. Since blood plasma is considered to occupy 5 per cent of body weight, these figures would be consistent with retention of a major part of the drug in the blood stream. The fall in plasma concentration was relatively rapid during the first hours, then assumed a slower rate. The drop was not linear even when concentration was plotted on a logarithmic scale as shown in the graph. This indicates that the drug does not leave the blood stream at a rate directly proportional to concentration. It suggests perhaps a more rapid elimination, initially, of the smaller molecular species.

Discussion

The conversion of the surface of plasma clot from a hydrophobic to a hydrophilic state has been obtained after intravenous administration of methylcellulose, just as previously observed with in vitro application. The clot-wetting action remained at a high level in dogs for 6 or 7 hours and was still demonstrable at 24 hours. Other colloid additives, which had been found inactive in vitro, were similarly ineffective when introduced into the blood stream of the intact animal.

A change in surface tension characteristics was also evident following methylcellulose injection. There was a striking alteration in the pattern of the surface tension-time curve. The initial reading, or dynamic tension, was reduced; the slope of successive readings was changed from a negative to a positive value. The finally attained stationary level, or static tension, was elevated above the initial dynamic reading; in control plasma the reverse was the case. The inactive colloids, carboxymethylcellulose, dextran, and polyvinylpyrrolidone, gave curves indistinguishable from the pre-injection controls.

The duration of methylcellulose activity was in keeping with the persistence of the drug in the blood stream, as determined by direct chemical analysis. This material is a polymer of large molecular dimensions, so that once introduced intravenously it should leave the plasma at a relatively slow rate. Though of high average molecular weight, there is considerable heterogeneity. This dispersion of molecular weights is very likely the basis for the more rapid elimination of drug from the blood stream in the earlier hours, the smaller molecules being first removed at a more rapid rate.

The extensive surface tension measurements in man by Künzel[6] and by Kaunitz and Schweiger[7] failed to disclose deviations in disease of sufficient magnitude and consistency to warrant search for corrective measures. No clear-cut association was established with specific disease states, except in the presence of icterus where there was some depression of the surface tension values. In an in vitro experimental study, Orma[8] obtained a reduction in surface tension of

human plasma with the detergent, Tween 20 (polyoxyalkylene sorbitan laurate). In the absence of direct indications for therapy directed against surface tension abnormalities, methylcellulose would not be expected to assume prominence as a surface tension alterant.

In the field of blood clotting, however, there may exist an appropriate role for the surface effects of this agent. Here, the surface tension changes assume importance only insofar as they indicate the accumulation of methylcellulose in a surface film. The establishment of a hydrophilic interface should facilitate the spread of fluid plasma over a moist tissue surface. With improved wetting, there is usually associated an increase in adhesion. After the plasma undergoes coagulation, improved bonding should persist between clot and tissue. It is very likely that further buildup of clot is accomplished by a process of accretion, rather than by coagulation in bulk. Enhanced wetting of each clotted layer should promote better adhesion to the superimposed film of fluid plasma. As successive films undergo coagulation there should ensue more intimate fusion between the stratified layers, resulting in improved structural properties of the whole clot.

Methylcellulose is a material that can be introduced into the blood stream without acute reaction. It is of such low acute toxicity that its use as an emergency plasma volume extender was proposed by Hueper, Martin, and Thompson.[9] Upon repeated injection, this polymer was found by Hueper[10] to accumulate and to remain permanently stored in various organs and in endothelial tissues. It is this type of chronic toxicity, shared in common with other nonmetabolized macromolecular substances, that would appear to be the limiting factor in any proposed use of methylcellulose.

Summary

Methylcellulose, injected intravenously in dogs, altered the surface properties of blood plasma, as measured both in the fluid and clotted state. In fluid plasma, it altered the pattern of the surface tension-time curve, instituting the following changes: a reduction in the dynamic value of surface tension, a progressive rise in value contrasted to the normal decline, an elevation of the final static level above the pre-injection value. In clotted plasma, methylcellulose converted the normal water repellent surface to one that was water attracting, or hydrophilic. There was no change of coagulation time in glass, but possibly some prolongation in siliconed tubes. No surface tension or hydrophilic changes were induced by the other colloids tested; namely, dextran, polyvinylpyrrolidone and carboxymethylcellulose. The physical changes induced by methylcellulose are of a type that might favorably influence clot structure and adhesion.

Summario in Interlingua

Methylcellulosa injicite per via intravenose in canes alterava le proprietates de superficie del plasma sanguinee. Le alterationes esseva manifeste in mesurationes con plasma fluide e con plasma coagulate. In plasma fluide, le agente alterava le forma del curva de tension de superficie e tempore per producer le sequente effectos: Un reduction del valor dynamic del tension superficial, un augmento progressive del valor in contrasto con le declino normal, e un elevation del static nivello final a un nivello supra illo del valor existente ante le injection.

In plasma coagulate, methylcellulosa converteva le superficie, que es normalmente un repulsor de aqua, in un attractor de aqua, i.e. le superficie deveniva hydrophile. Esseva notate nulle alteration del tempore de coagulation in vitro, sed in tubos revestite de silicium ille tempore esseva possibilemente prolongate. Nulle alterationes del tension superficial o del stato de hydrophilia esseva inducite per le altere colloides probate. Istos esseva dextrano, polyvinylpyrrolidona, e carboxymethylcellulosa. Le alterationes physic que es inducite per methylcellulosa representa un genere de effectos probabilemente favorabile super le structura del coagulo e super le adhesion.

REFERENCES

[1] ROSENFELD, M.: Methylcellulose as a wetting agent in blood clot. Bull. Johns Hopkins Hosp. *99:* 239, 1956.

[2] SAMSEL, E. P. AND DELAP, R. A.: Colorimetric determination of methylcellulose with anthrone. Anal. Chem. *23:* 1795, 1951.

[3] SCOTT, T. A., JR. AND MELVIN, E. H.: Determination of dextran with anthrone. Anal. Chem. *25:* 1656, 1953.

[4] JACKSON, D. P., KUHL, W. J., JR. AND IRVIN, J. L.: The determination of aromatic amidines in plasma and urine. J. Biol. Chem. *167:* 377, 1947.

[5] TOCANTINS, L. M.: The Coagulation of Blood. Methods of Study. New York, Grune & Stratton, 1955.

[6] KÜNZEL, O.: Untersuchungen der oberflächenspannung im normalen und pathologischen serum. Ztschr. f. Klin. Med. *133:* 607, 1938.

[7] KAUNITZ, H. UND SCHWEIGER, E.: Weitere untersuchungen über die klinische bedeutung der oberflächenspannung des blutserum. Ztschr. f. klin. Med. *132:* 584, 1937.

[8] ORMA, E.: The effect of increasing concentrations of Tween 20 (polyoxyalkylene sorbitan laurate) on the surface tension of plasma. Acta Physiol. Scand. *31:* 230, 1954.

[9] HUEPER, W. C., MARTIN, G. J. AND THOMPSON, M. R.: Methyl cellulose solution as a plasma substitute. Am. J. Surg. *56:* 629, 1942.

[10] ——: Experimental studies in cardiovascular pathology. IV. Methyl cellulose atheromatosis and thesaurosis. Arch. Path. *33:* 1, 1942.