STUDIES IN EXPERIMENTAL EXTRACORPOREAL THROMBOSIS.

V. Influence of Certain Chemical Substances on Extracorporeal Thrombosis with Special Reference to the Efficacy of a Combination of Heparin and Magnesium Sulfate.

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With the method of studying experimental extracorporeal thrombosis already described, the influences of certain chemical substances, such as distilled water, bile salts, *d*-glucose, urea and calcium chloride, have been examined.

The Effect of Distilled Water.

It is well known that shed or hemolyzed blood, when injected into the circulation, may cause the liberation of fibrin and subsequently intravascular clotting. On the other hand, it has been stated by Reed that heparinized blood, despite its incoagulability, can clot very soon after the addition of distilled water. In this connection, I have carried out a series of experiments and have verified this fact, as will be seen from the results.

Experiment 1.—A rabbit weighing 2100 gm. was given 50 mg. of heparin (Hynson, Westcott and Dunning) intravenously. Blood was drawn immediately from the carotid artery into a paraffined vessel; 1 cc. of the blood was put into each of a series of small test-tubes and various amounts of distilled water were added to each and clotting time was recorded (Table I). Accordingly I attempted to see what the effect would be of distilled water placed outside of the collodion membrane.

Experiment 2.—When the collodion tube of the circulatory loop was surrounded by distilled water, the latter passed through the membrane into the circulation, causing, from 5 to 10 minutes later, hemolysis of the heparinized blood flowing along the wall of the collodion tube. 30 minutes or more later, fibrin formed, and

coagulation of hemolyzed blood followed. In the serial sections this clot looked almost entirely homogeneous, and neither structure nor formed elements could be differentiated because of hemolysis.*

Two reasons are advanced to explain this phenomenon: one that tissue extract or fibrinogen and hemoglobin set free by hemolysis neutralize the heparin action, and the other, dilution of the anticoagulant. According to Howell, the latter explanation is less probable.

TABLE I.

Clotting Time of Heparinized Blood in Water.

	5 min.	7 min.	12 min.	20 min.	40 min.	60 min.	18 hrs.
1 cc. of heparinized blood in 0.1 cc. water	−	−	−	+	+	++	++
1 cc. of heparinized blood in 0.2 cc. water	±	+	+	+	+	++	++
1 cc. of heparinized blood in 0.5 cc. water	+	+	+	++	+++	+++	+++
1 cc. of heparinized blood in 1.0 cc. water	+	+	+	++	+++	+++	+++
1 cc. of heparinized blood in 2.0 cc. water	−	−	−	+	+	+	++
1 cc. of heparinized blood in 3.0 cc. water	−	−	−	−	−	±	±
Control	−	−	−	−	−	−	−

The Influence of Bile Salts on Clotting in the Heparinized Animal.

For a long time it has been known that jaundice tends to disturb the clotting process of the blood. This fact was also verified by Minot and his associates, who found, by using Howell's recalcification method, that the coagulation time was increased in a series of cases of jaundice. Haessler and Stebbins demonstrated that the clotting time of the blood, to which bile salts have been added, is proportional to the quantity of bile salts present and that the bile interferes with the conversion of fibrinogen into fibrin and not with the formation of

* It will have been observed that the tenacity of both red and white thrombi has varied under different experimental conditions. This may depend on variation in the character of the agglutination of the elements or on the amount and nature of the fibrin deposited. Since thrombi *in vivo*, under different conditions, show similar variation in tenacity, the relation of these phenomena to embolism assumes prime importance. Pathologically the question is not only why thrombi form, but why emboli are dislodged in some cases and not in others. In this connection it is to be remembered that fibrin deposited from fibrinogen *in vitro* by any other than biologic methods is not crystalline (Howell).

thrombi. To my knowledge, no one has yet investigated *in vitro* or *in vivo* the influences of bile salts on heparinized blood. I carried out some experiments with a rabbit, whose blood had been rendered less coagulable by intravenous injections of heparin, also some *in vitro* with the blood of normal rabbits, to which heparin was added (16 mg. to each 40 cc.) and found that in both instances more than 1 drop of 1 per cent solution of bile salts to each cc. of blood caused hemolysis, but no decrease of the clotting time.

Experiments were carried out by means of the extracorporeal loop after the injection of heparin. Bile salts, dissolved in physiologic sodium chloride solution (1 or 2 gm. for each 100 cc.) were placed in the container surrounding the collodion tube of the apparatus, and also, in other cases, introduced directly into the circulatory loop through the rubber tube connecting the arterial cannula and the side tube. Thus the simultaneous influence of bile salts and of heparin on experimental thrombosis was observed.

In the former case, that is with bile salts in the dialyzing container, so long as no coagulation or no obstruction occurred the white thrombi not only appeared in abundance in the venous half of the apparatus (the glass tube, paraffined part and collodion tube) but they grew more rapidly than when physiologic sodium chloride solution was used. Radiation, or fan formation, was less pronounced. In the structure of this thrombus, the platelets, of course, play a great part, but the rôle of the leucocytes as a constituent of the thrombus should not be overlooked. The size of each white thrombus at the end of 1 hour or 3 hours was many times larger than when physiologic sodium chloride solution was used for a corresponding period. They appeared as cauliflower-like excrescences.

The thrombi obtained after injection of bile salts were also unquestionably larger than when physiologic sodium chloride solution alone was used. Furthermore, the white thrombi appeared in the arterial half of the apparatus, especially near the place of injection, in the arterial corner tube and in the arterial end of the collodion tube, despite the speed of the stream. The arterial corner tube was nearly obstructed after 3 hours, in spite of the fact that its lumen was many times greater than that of the narrow part of the cannula.

In both cases, when the solution of bile salts was placed outside of

the collodion tube and also when it was injected into the circulation, the mass of white thrombi was loose, soft and fragile, being easily broken up into the individual platelet thrombi. There appeared to be less contractility, suggesting that fibrin was absent or if present was abnormal or of a poor quality. Even when complete obstruction

TABLE II.

Size and Number of White Thrombi with 1 or 2 Per Cent Bile Salts Placed Outside of the Collodion Tube.

Date	No.	Time	Length	Width	No. in one field	Remarks
1926			*mm.*	*mm.*		
Mar. 22	9	3 hrs.	0.60	0.50	2	No obstruction
			0.50	0.50	3	
May 6	22	3 hrs.	1.40	1.20	2	
			0.50	0.40	5	
			0.08	0.10 or	7	
			0.40	0.20	8	
			0.20	0.20	5	
May 13	25	4 hrs., 20 min.	1.40		Many	Long ranges of hillocks. No obstruction because the white thrombi gathered mostly in the wide part of the apparatus in the corner tube
June 28	52a	3 hrs.	0.10	0.08	More than 20	Two ranges of hillocks
			0.20	0.20		
			1.0	0.8		
June 28	52b	2 hrs., 30 min.	3.0	4.0		Large white thrombus in the corner tube obstruction
			0.06	0.08		

by a thrombotic mass occurred, no clotting followed. These phenomena are in part due to the action of the heparin, but it seems reasonable to consider that the bile salts also disturb the coagulation of heparinized blood, since such extreme results were not found with heparin alone.

The fact that white thrombi are readily stimulated by bile salts and the red thrombi formed through the influence of bile salts are fragile, might explain why postoperative pulmonary embolism is found in a relatively large number of cases of hepatic disease and gall stones (Wilson).

Typical protocols from a series of experiments follow, and the results of the series are shown in Tables II and III.

Experiment 3 (Bile Salts by Dialysis).—Rabbit 52a weighing 1800 gm. was injected intravenously with 40 mg. of heparin at 12.20 p.m. At 12.40 p.m. the apparatus was connected and 2 per cent bile salts in physiologic sodium chloride solution put outside the collodion tube. The blood stream was very rapid, pulsation of the collodion tube and the jugular vein being marked. The clotting time was more than 30 minutes. At 3.40 p.m. the collodion tube was removed. Throughout the whole apparatus many rather large white thrombi were seen, most of them confluent and constituting an inner coat or cast on the inner surface of the tubes. The individual thrombi were connected by abnormal appearing fibrin threads. In the collodion tube, similar conditions prevailed. There were innumerable small thrombi but a few were very large. The smaller types fused together to form the thick membrane composed of platelet clumps with numerous leucocytes. In one field more than twenty of the white thrombi measured 0.10 by 0.08 mm., and seven measured 0.20 by 0.20 mm. The large variety were many times larger than those observed in the case of physiologic sodium chloride solution in the heparinized animal. Microscopically large high hillocks of platelet clumps were seen with numerous leucocytes in them. The hillocks measured 1.0 by 0.8 mm., with sometimes a range of hillocks of 0.5 mm. width with valleys intervening.

TABLE III.

Size and Number of Thrombi with 2 Per Cent Solution Bile Salts Injected into the Vessel.

Date	No.	Time	Length	Width	No. in one field
1926		*hrs.*	*mm.*	*mm.*	
June 1	39a	3	1.00	0.80	3
			0.54	0.50	2
			0.40	0.40	3
June 1	39b	3	0.56	0.40	3
			0.44	0.40	3

Experiment 4 (Bile Salts Injected into the Arterial Side of the Apparatus).—Rabbit 39a weighing 1020 gm. was given 40 mg. of heparin intravenously at 11.30 a.m. At 12.00 the apparatus was connected and 3 cc. of 1 per cent solution of bile salts was injected into the arterial side of the extracorporeal loop. At 1.00 p.m. 1 cc. of solution of bile salts was injected in the same way. There was a violent stream and marked pulsation in the artificial vascular loop. At 2.00 p.m. 1 cc. of solution of bile salts was injected. At 3.00 p.m. the apparatus was detached; the blood in it remained incoagulable. In the arterial corner tube there were many white thrombi which obstructed the lumen. In the arterial end of the collodion tube a good many large white thrombi had gathered, almost obstructing it. Also, in the outer lower part and bottom of the collodion tube there were numerous rather large white thrombi, each hillock being high. Fern-like radiating figures were also seen. In one field, two or three white thrombi measured 1.00 by 0.88 mm., two measured 0.54 by 0.50 mm. and three measured 0.40 by 0.40 mm.

TABLE IV.

Size and Number of White Thrombi with 1 or 2 Per Cent d-Glucose Solution Outside of the Collodion Tube.

Date	No.	Time	Length	Width	No. in one field	Remarks
1926			*mm.*	*mm.*		
May 7	23b	1 hr.	0.10	0.08	More than 20	In the figure of radiation
			0.04	0.04	More than 25	
June 21	48a	3 hrs.	0.26	0.024	4	
June 21	48b	3 hrs.	0.20			Irregular ranges of hillocks
			0.40	0.40		
			0.20	0.20	Many	Radiation
June 21	48c	2 hrs.	0.10	0.10	12	Radiation
July 19	61a	2 hrs.,	0.24	0.10	6	
		30 min.	0.12	0.10	5	
July 19	61b	30 min.				Blood coagulable
July 21	65	2 hrs.	0.16	0.16	6	Radiation
			0.10	0.08	4	

Influence of d-Glucose.

Changes in blood sugar content may cause only slight fluctuations in the clotting time of the blood. It was desirable to investigate the

influence of *d*-glucose on thrombosis, because there is a high blood sugar content, not only in diabetes, but also after *d*-glucose has been introduced as a therapeutic measure. The essential data from twelve experiments on the effect of *d*-glucose on heparinized blood *in vivo* are presented. When *d*-glucose dissolved in physiologic sodium chloride solution (1 or 2 gm. for each 100 cc.) was placed in the container outside of the collodion sac, numerous tiny white thrombi appeared in the course of time (Table IV). The size of each at any given time was relatively smaller than those seen with physiologic sodium chloride solution in the container. The thrombi in the

TABLE V.

Size and Number of Thrombi with 10 Per Cent Glucose Injected into the Vessel.

Date	No.	Time	Length	Width	No. in one field	Remarks
1926			*mm.*	*mm.*		
June 23	50a	3 hrs.	0.08	0.04	1 or 2	
June 26	51a	3 hrs.	0.08	0.06	9	
June 26	51c	3 hrs.	0.08	0.06	1 or 2	
June 26	51b	30 min.	No thrombi			Obstruction of carotid artery
June 23	50b	1 hr., 5 min.	6.0	3.0		Red cell clumps and clot

collodion tube tended to assume radiating figures in the majority of cases. They were composed chiefly of platelets, and, on and around them, there were in the earlier period a very few leucocytes but in the later stages a considerable number. Even in an experiment of three hours duration obstruction and consequent stopping of the stream was rare, even though, in the constricted part of the cannula, and under certain conditions elsewhere, the thrombi congregated, making rather a loose mass which could be broken easily; fibrin formation seemed to be delayed and abnormal or poor, while with physiologic sodium chloride solution, despite ample action of the heparin, fibrin did appear in rather a short time, and contributed to the obstruction.

Despite these changes, the *d*-glucose did not appear to shorten the clotting time of heparinized blood.

When the *d*-glucose solution (10 per cent) was injected into the artificial circulatory loop through the gum tube between the arterial cannula and side tube, white thrombus formation was rather rare throughout the apparatus (Table V). These findings suggest that hypertonic sugar solution tends to make platelets and leucocytes conglomerate less readily; hence the thrombotic elements do not precipitate readily nor adhere to the vessel wall so that they are carried away. These results were constant for the series of experiments although they are difficult to explain at present.

Influence of Calcium Chloride.

The exact significance of calcium in fibrin formation is still unsettled. Blood from which the calcium has been precipitated by oxalate or citrate will not coagulate, but the addition of calcium salts will promptly cause it to coagulate. As to the influence of calcium on blood clotting, the hypothesis usually accepted is that calcium ions are necessary for the transformation of prothrombin into thrombin. Howell considers that no kinase is necessary, the calcium activating the prothrombin whenever it is not inhibited by heparin. On the other hand, it is known that the presence of calcium in high concentration in the blood prolongs the coagulation time (Freund, Rosenmann and Loewenstein, and others).

Furthermore, Howell and Holt state that no amount of calcium causes clotting of heparinized blood, showing that the heparin does not act by decalcifying the blood. Considering the various rôles of calcium, such as the calcification of vessels, the field of blood clotting, or its wide intravenous use therapeutically, it is of considerable importance to investigate the influence of calcium on extracorporeal thrombosis. Therefore, eight experiments were performed after the preliminary injection of heparin.

Experiment 5.—Rabbit 26 weighing 1950 gm. was given 40 mg. of heparin intravenously at 1.30 p.m. The apparatus was connected at 1.40 p.m. and the collodion tube surrounded with 1 per cent of calcium chloride in physiologic sodium chloride solution, kept at body temperature. The blood stream had been kept vigorous by twice removing the obstruction of the venous cannula. At

TABLE VI.

Size and Number of Thrombi with 1 Per Cent Solution of Calcium Chloride Placed Outside of the Collodion Tube.

Date	No.	Time	Length	Width	No. in one field	Remarks
1926			*mm.*	*mm.*		
Mar. 11	4a	4 hrs.	Small, white thrombi covered by red clot			Within 30 min. obstruction and sedimentation (4 hrs. and 20 min. after 50 mg. heparin injection); clotting time 5 min.
Mar. 12	5	2 hrs., 10 min.	0.30 0.16	0.24 0.12	5 8	Obstruction one time; removed Fibrin network
Mar. 15	6a	30 min.	0.16 0.10	0.14 0.06	2 15	
Apr. 25	13b	30 min.	0.10 0.08	0.06 0.06	2 3	
May 15	26	3 hrs.	5.00 2.40 1.20 0.40 0.14	1.5 1.20 1.00 0.30 0.12	1 1 4 4 20	Thrombus 1 mm. thick. Venous cannula cleaned up twice as obstruction occurred

TABLE VII.

Size and Number of Thrombi with Hourly Injections of 2 Per Cent Solution of Calcium Chloride.

Date	No.	Time	Length	Width	No. in one field	Remarks
1926		*hrs.*	*mm.*	*mm.*		
June 4	41a	3	1.10 0.16	0.40 0.12	4 15	
June 4	41b	3				All over the apparatus white thrombi causing obstruction, followed by red thrombi. 6 hrs. and 30 min. after 50 mg. heparin (1600 gm. body weight) injection, the clotting time was 5 min., but 20 min. later it was 2 min.

4.40 p.m. the stream was still violent; the clotting time *in vitro* was more than 30 minutes. The apparatus was disconnected. White thrombi in the tubes were very large and numerous (Table VI), especially in the venous half of the apparatus. The venous corner tube was thickly coated. The collodion tube contained large white thrombi in its venous half, while there were very few in the arterial half.

Experiment 6.—Rabbit 41a weighing 1600 gm. was given intravenously 50 mg. of heparin at 11.00 a.m. At 11.20 the whole apparatus was connected, and the collodion tube surrounded with physiologic sodium chloride solution, kept at body temperature; 2 cc. of 2 per cent solution of calcium chloride was injected very slowly into the annular circulation through the rubber tube, which unites the arterial cannula and the arterial side tube; after that 1 cc. of calcium solution was injected every hour in the same way. By 11.40 a.m., from the place of the injection down to the venous corner tube, numerous white thrombi had appeared; the stream was still vigorous. At 2.40 p.m., after 3 hours, the blood remained non-coagulable; the circulation had not been impeded. 3 hours after connection, the apparatus was detached; in the paraffined tubes a goodly number of large white thrombi adhered to the inner wall, making a thick coat (Table VII). Nevertheless, there remained a tiny tunnel through which the blood had been steadily circulating. In the collodion tube, white thrombi were connected with one another irregularly.

When the collodion tube of the apparatus is surrounded by calcium chloride in physiologic sodium chloride solution (1 or 2 gm. calcium chloride to 100 cc. physiologic sodium chloride solution) and at body temperature, the white thrombi appear chiefly in the venous half of the apparatus (the paraffined part of the glass tube and the collodion tube), and as long as the incoagulable blood flows, thrombi grow rather more rapidly than when physiologic sodium chloride solution is in the container; and the narrow part of the venous cannula is easily obstructed by masses of white thrombi, made up of clumps of platelets and leucocytes, interwoven by fibrin threads; the thrombi are not so fragile as when bile salts or *d*-glucose solutions are used. Sedimentation of erythrocytes readily follows, and fibrin seems to form easily. When the obstructing plug of the narrow part is removed as formed, the thrombi in the venous corner tube and collodion sac develop more speedily than in the case of sodium chloride solution and in the course of 2 or 3 hours the lumen of the corner tube which is many times wider than that of the narrow part of the cannula, is nearly obstructed by a thick coat. Otherwise on account of the prompt plugging of the narrow part, a further supply of hematogenic throm-

botic elements is lacking. As a result in the collodion tube, small white thrombi form, which are almost always covered by sedimented erythrocytes. Similar findings are encountered when 2 per cent calcium chloride solution is injected into the circulation.

Effects of Magnesium Sulfate on Extracorporeal Thrombosis.

The foregoing experiments probably shed some light on the problem of thrombosis; however, the ultimate object of these investigations was the prevention of thrombosis, especially of postoperative pulmonary embolism. For this purpose no special prophylactic methods are commonly adopted. On the basis that the slowing of the blood stream probably constitutes one of the most important causes, exercise and movement of the extremities, and massage have been advised by some in order to accelerate the rate of circulation. Similarly, desiccated thryoid has been employed by Walters.

Mason's attempts to protect experimentally against intravascular clotting with heparin are praiseworthy. But, as my numerous experiments show, the formation of white thrombi on the pathologic lining of the vessels was not prevented by a single dose of the heparin employed. The experiments with the injection of hypertonic *d*-glucose solution suggest that the introduction of hypertonic solution into heparinized blood will tend to combat white thrombus formation. Magnesium sulfate also comes into consideration. It has been employed by Fonio in an indirect method of counting blood platelets, to protect against the cohesive properties of the platelets. Some authors believe that there is a close relationship between the earlier stages of thrombosis and agglutinability of platelets. Recently, Hans Schulte investigated the influence of hydrogen ion concentration on agglutination of isolated blood platelets over a period of 12 hours, and reported that it is most marked at a pH of 4.3 to 3.5. He could recognize no influence of sodium chloride and calcium on the process. On the other hand, the results of my studies on experimental thrombosis show that calcium chloride solution seems to stimulate the appearance and development of white thrombi. One might expect some relationship between the presence of calcium chloride in the blood and the development of white thrombi. If, from the colloid-chemical viewpoint, the thrombus formation is closely related to the

agglutination of platelets as colloidal precipitation, magnesium ions and other bivalent positive ions should precipitate the platelets in the same way as calcium does. Thus the influence of magnesium sulfate on thrombosis would appear to be interesting and perhaps of considerable significance. One protocol will suffice.

Experiment 7.—Rabbit 38 weighing 1010 gm., was given 40 mg. of heparin intravenously at 10.25 a.m. At 11.10 a.m. the apparatus was connected and the collodion tube surrounded by 1 per cent magnesium sulfate in physiologic sodium chloride solution. During 3 hours there was a violent stream and marked pulsation in the collodion tube and jugular vein. Clotting time was more than 30 minutes. There was no clotting or obstruction. At 2.10 p.m. the apparatus was detached; there were very few tiny white thrombi in the arterial corner tube. In one place in the upper part of the venous half of the collodion tube an occasional tiny white thrombus was seen, which microscopically consisted of platelets; on each of the thrombi a few scattered leucocytes were recognized. Of twelve thrombi in one field, ten measured 0.08 by 0.04 mm., and two measured 0.10 by 0.06 mm.

From four experiments it was concluded that when the collodion tube was surrounded with 1 per cent solution of magnesium sulfate only a few small white thrombi appeared in the arterial half of the apparatus, while in the venous half very few or no white thrombi were found even when the rate of flow was slow enough to favor adequate formation of thrombi. In the collodion tube, which is the lowest and widest part of the circulatory loop, small white thrombi, often of microscopic size, were seen after 3 hours. The total number of such thrombi was very small in comparison with other experiments. Throughout a 3 hour experiment no obstruction had occurred, neither did any thrombi adhere to the glass cannulas. The action of magnesium sulfate on thrombosis is different from the action of calcium chloride. Magnesium appears to protect definitely against thrombosis, probably by the precipitation of small amounts of calcium as calcium sulfate.

The influence of the intravascular injection of magnesium sulfate solution was next examined. In two experiments the effect of the intravenous injection of magnesium sulfate solution (50 mg. for each kilo of body weight an hour) was tried without the use of any other anticoagulant. The coagulation time of the blood was increased from two to three times normal and thereby clotting in the artificial vascular loop was prevented for from 25 to 30 minutes. White thrombus formation was markedly reduced. Thin layers of blood clot were observed in the collodion tube, and mixed thrombi formed on the irregular surfaces of the apparatus and also in the jugular vein. The venous cannula, and a little later the arterial cannula, were finally obstructed by small masses of white thrombi and fibrin. Despite this, most of the blood in the loop remained fluid.

In the next set of six experiments, the intravenous use of a combination of magnesium sulfate and heparin yielded still more interesting results.

Experiment 8.—Rabbit 7 weighing 2250 gm., was given 20 mg. of heparin intravenously at 3.30 p.m. At the same time 1 cc. of 10 per cent magnesium sulfate was administered intravascularly into the ear vein. At 4.00 p.m. the apparatus was connected and the collodion tube surrounded by physiologic sodium chloride solution of body temperature. At 4.30 p.m. 1 cc. of 10 per cent magnesium sulfate solution was injected intravenously, and at 6.30 p.m. 1 cc. was again injected intravenously. At 7.00 p.m., after 3 hours experiment, the streaming was vigorous; no thrombi were recognized anywhere. The apparatus was detached. In the collodion tube and glass tubes no thrombi were found, even by microscopic examination. The clotting time of the blood was more than 30 minutes.

When a large quantity of 10 per cent magnesium sulfate solution was injected into the annular circulation, or introduced into the general circulation, after intravenous administration of heparin, no thrombi appeared for a certain period in any part of the apparatus. When physiologic sodium chloride solution was used around the outside of the collodion tube, without injection of magnesium solution, white thrombi appeared in 15 minutes in the venous corner tube; 2 cc. of 10 per cent magnesium sulfate solution to 2250 gm. of body weight protected against the occurrence of the first stages of thrombosis for a period longer than 3 hours; 3 cc. of the solution to 2100 gm. of body weight for longer than 6 hours. A few platelet clumps were formed despite the action of magnesium, and sometimes they appeared in the narrow part of the venous cannula, constituting a kind of dam. But when 1 cc. of 10 per cent solution of magnesium sulfate was injected every hour into the circulation of the rabbit, no thrombi appeared. Besides, throughout the experiments, neither obstruction of the circulation nor hindering of the action of heparin has been observed. 3 cc. of 10 per cent solution of magnesium sulfate to 2100 gm. of body weight appears to be almost a lethal dose. This toxicity is at any rate a weak point from the standpoint of clinical application, even though magnesium sulfate solution can prevent the first stages of thrombosis.

Effects of a Combination of Magnesium Sulfate and Bile Salts.—In order to see the antagonistic action between magnesium ions and bile

salts, a series of experiments was performed. When the magnesium sulfate solution, in four experiments, was placed outside of the collodion tube, despite intravascular injection of bile salts, no thrombi appeared in the collodion tube, or if they did, they were scarce and very small. It appears certain that magnesium ions went through the membrane into the circulation, and acted on the platelets and leucocytes in such a manner that the cellular thrombotic elements, passing down along the wall of the sac, were rendered less prone to agglutinate, and were carried away before they were precipitated, also the magnesium sulfate may act to destroy the platelets. When a solution of bile salts surrounded the collodion sac and magnesium sulfate solution was injected into the circulation, the formation of white thrombi was undoubtedly retarded to a certain degree, in regard to number and size, in spite of the stimulating action of bile salts.

This suggests that magnesium sulfate in small amounts will prevent thrombotic elements, especially platelets, from adhering to a pathologically changed vessel wall, partially owing to the platelet-destroying action of magnesium. This may serve to prevent thrombus formation. Possibly the phenomena may be ascribed to changes in surface tension.

CONCLUSIONS.

1. Distilled water dialyzes through the collodion tube and causes hemolysis. Clotting of the hemolyzed blood in the collodion tube occurs later.

2. Bile salts accelerate the appearance and development of white thrombi in the heparinized animal. The masses of white thrombi are very loose, soft, fragile and easily broken into clumps of platelets. They are poor in fibrin or the fibrin is abnormal, since they have less retractility than those obtained from the use of physiologic sodium chloride solution or calcium chloride or serum. Despite the increase in number and size of white thrombi, normal clotting does not occur.

3. When the collodion tube is surrounded by 1 or 2 per cent *d*-glucose solution, tiny and numerous white thrombi appear as radiating figures. The masses of white thrombi are rather loose and fragile. The clotting time of the heparinized blood does not appear to be shortened.

4. When a 1 to 2 per cent solution of calcium chloride is used as the dialyzing fluid outside the collodion tube, or when it is injected into the circulation, the formation of white thrombi is accelerated. They grow very rapidly. In spite of the action of heparin, the white thrombi formed are not so fragile as when bile salts are placed outside of the collodion tube. Fibrin seems to form easily. Obstruction of venous cannula takes place speedily and if the clots in the cannula are not removed, the white thrombi in the collodion tube remain small and become red by sedimentation of red cells.

5. Intravenous use of 10 per cent solution of magnesium sulfate without heparin retards the coagulation of circulating blood and permits the blood to flow through the extracorporeal loop from three to four times as long as normal. The formation of white thrombi, as well as red, is retarded. Magnesium sulfate (1 per cent) in physiologic sodium chloride solution placed outside the collodion tube markedly retards the formation of white and red thrombi in the heparinized animal. Magnesium sulfate (10 per cent), 50 mg. for each kilo of body weight each hour, administered intravascularly in the heparinized animal definitely prevents the first stages of thrombosis, and consequently prevents clotting.

6. It is possible by the combined use of adequate amounts of magnesium sulfate and of heparin intravenously to prevent all stages of thrombus formation for from 1 to 3 hours.