agranulocytic angina does develop, penicillin, which combats infection until there is spontaneous regeneration of the granulocytes, is in our opinion the drug of choice. Although liver does not prevent granulocytopenia we believe that its daily oral administration lessens the incidence of the milder thiouracil side-effects.

We are indebted to Lederle Laboratories and to Wilson Laboratories who supplied us with thiouracil and liver respectively for this work.

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RÉSUMÉ

Deux séries d'observations démontrent que le foie de veau ne protège pas complétement contre l'agranulocytose chez les malades soumis au thiouracil. Il s'agit chez ces malades d'observer rigoureusement l'hémogramme, de continuer l'administration du foie et de diminuer ou de cesser à temps l'usage du thiouracil. Dans les cas observés au tout début, la pénicilline semble être l'agent de choix pour juguler l'infection jusqu'à ce que les granulocytes se régénèrent spontanément. Le foie de veau n'empêche pas l'agranulocytose mais il en diminue la fréquence.
JEAN SAUCIER

SILICONES AND BLOOD COAGULATION

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THE clotting of blood is markedly influenced by the nature of the surface with which it comes in contact. Following the discovery by Freund[1] in 1886, and by Bordet and Gengou[2] in 1901, that coating of vessels with vaseline or paraffin markedly delays clotting, it has been generally assumed that an important factor initiating clotting is contact of the blood with a wettable surface. However, there has been no available material sufficiently effective and sufficiently adaptable to be of general practical use in delaying the clotting of mammalian blood. Recently certain plastics, the silicones, have been developed which possess water-repellent properties.[3, 4, 5] These substances are polymers derived from organic silicon compounds, and a wide variety of types is available. It has been found that one of these compounds (General Electric Dri-Film No. 9987) has valuable properties in delaying the clotting of blood.

METHODS

All glassware, etc., was scrupulously cleaned and dried before use. To obtain an effective film, General Electric Dri-Film No. 9987 (methyl-chloro-silane) was applied in liquid form by pouring it into or through the glassware and needles used. The methyl-choloro-silane was left in contact with the object for a few seconds and the excess was poured off. The object was then rinsed thoroughly with distilled water. This formed the silicone by hydrolysis and fixed it on the material. The last traces of hydrochloric acid liberated were removed by rinsing with very dilute ammonia water followed by copious washing with distilled water, and the articles dried in hot air. This procedure gives a film much heavier than the unimolecular film recommended by the manufacturers. In our experience this heavier film is essential for use in a study of the clotting system.

A fresh application of the silicone is made each time the glassware, etc., is used. It is our impression that the surface is improved with the second and third coatings, so that we now subject all new glassware and needles to three or four treatments. (Some etching of the needles has been observed). For aseptic use, the prepared syringes and needles are placed in boiling distilled water for 1½ to 2 minutes.

Clotting times were determined in standard 8 mm. pyrex test tubes at 20° C., the formation of a complete gel being taken as the end point. Platelet counts were made from samples taken into a formalin citrate mixture as described previously.[6] Blood samples were taken from the antecubital vein. Rubber tubing clamped in a hæmostat was used as a tourniquet and the pulse palpated to make sure of a good arterial flow.

RESULTS

Effect of silicone surfaces on clotting time.—In order to test the effect of the silicone treatment on clotting, 5 c.c. blood samples were removed with a needle and syringe previously treated with the silicone. The needle was removed and 1 c.c. of blood placed in treated and untreated tubes. The results are shown in

Table I. In all cases there was a marked lengthening of the clotting time in the silicone tube to about twice the clotting time of the control. In a few tests with a paraffin coating a similar effect was observed, but it was much less than with the silicone.

In a different experiment (Table II) the effectiveness of silicone in preventing coagulation was further demonstrated, and another important factor affecting clotting time was studied. Fifteen c.c. of blood were taken with a silicone-coated syringe and needle, the needle removed and 5 c.c. of blood placed in each of two centrifuge tubes. The remaining 5 c.c. were then divided in 1 c.c. portions between small tubes and the clotting time determined at 20° C. Tube 5 represented the blood last out of the syringe, and presumably first out of the body; tube 4, the second last, and so on. The clotting time in silicone of tube 5 was 61 minutes, of tube 4, 70 minutes, of tubes 2 and 1, 2 hours. The much shorter clotting time of the last 1 c.c. of blood in the syringe is a fairly consistent finding. We believe that this is due to the small amount of tissue juice or thromboplastin which enters the needle when passing through the vein wall and overlying tissues, since we found that any more extensive damage will cause the sample to clot in 20 minutes or less. Thus, samples where the needle caught in a valve or where the vein wall collapsed against the needle, or when a hæmatoma developed, all clotted in a short time. Evidently silicone cannot protect against the clotting activity of damaged tissue. However, by avoiding eddy currents which would cause mixing, the slight amount of thromboplastin added to the blood on inserting the needle can be confined to the first 2 c.c. of blood. To improve control of this factor, in all further experiments 5 c.c. of blood were left in the syringe. As a check, the clotting times were determined serially for the 5 c.c. as in Table II.

TABLE I.
COMPARISON OF EFFECT OF TREATMENT OF TUBE.

		Clotting time of blood, mins.		
		Plain tube	*Silicone*	*Paraffin*
Dog............	1	15	29	24
	2	21	35	
	3	13	31	
	4	18	30	
Rat.............	1	6	13	
	2	2	4	2
	3	4	6	
Man............	1	10	18	8
	2	11	31	15

TABLE II.
CLOTTING OF BLOOD IN SILICONE

Tube		*Clotting time mins.*
1	......	133
2	......	116
3	......	>> 52 (Glass, 13)
4	......	70
5	......	61
Plasma........		20 (Glass)
		120 (Silicone)

Tube 3 is of interest. After standing 52 minutes the blood was still completely fluid with no sign of clotting. It was therefore transferred to a glass tube, using a dry syringe and needle, and gave a clotting time of 13 minutes which is a normal value.

The blood in the centrifuge tubes was spun to obtain plasma. The clotting time of this plasma was 20 minutes in glass, 2 hours in silicone. Further discussion of this point will be given later.

Preparation of normal plasma.—It is evident, therefore, that with suitable precautions to prevent contamination with thromboplastin we can, by the use of silicone, delay clotting of blood up to two hours at room temperature. By this means it is possible to obtain plasma without the use of anticoagulants, and without modifying the clotting system in any way. After obtaining the plasma it can be stored in the refrigerator. We have stored samples of plasma for periods up to six weeks. They were quite fluid and when brought back to room temperature they clotted like normal plasma.

Preservation of platelets.—The formed elements of blood were studied, using the technique outlined above. Ten c.c. of blood were placed in a silicone-coated beaker at room temperature and samples were removed at

TABLE III.
CELL COUNTS AND CLOTTING TIME OF BLOOD STORED IN SILICONE

Time mins.*	*Red blood cells /mm.^{3}*	*White blood cells /mm.^{3}*	*Platelets /mm.^{3}*	*Clotting time mins.*
1.......	4,100,000	6,200	253,000	39
15.......	4,344,000	7,900	263,000	34
30.......	4,050,000	5,200	271,000	31
45.......	5,319,000	4,700	139,000	19
64.......	....	....	....	10
73.......	....	....	....	6
79.......	clotted	....	....	..

*Time from removal of needle from vein.

intervals for the estimation of cells and clotting time. The results are shown in Table III. After 30 minutes the platelets still gave a normal count with no sign of agglutination. The clotting time (31 minutes) also was only slightly shortened. Samples taken after 45 minutes outside the body showed a marked reduction (50%) in the platelet counts and the clotting time was also reduced to half the original value. It is of interest that some decrease in the white cell count was also observed at this time while, due to sedimentation, a rise in the red cells had occurred. The preservation of the platelets for 30 minutes is remarkable, since even with large doses of heparin, platelet agglutination and disintegration is very rapid in blood samples taken by ordinary techniques without oxalate or citrate.

The significance of the platelets in clotting.— We plan to use this technique to study the mechanism and the factors involved in the early stages of clotting. The first problem investigated, the results of which we wish to report at this time, is the influence of the platelets. It is usually stated that plasma within the blood vessels does not clot because the initiation of clotting requires another factor present in cells and particularly in the platelets. Recently Lozner, Taylor and MacDonald[7] have revived the experiment of Bordet and Gengou to show that plasma will clot in the absence of cells and deny that the platelets have any significance. We have therefore studied the influence of platelets by the silicone technique. The results are shown in Table IV. In seven experiments, blood removed with the usual precautions was placed in two centrifuge tubes and centrifuged at different speeds to give plasmas of different platelet content. In the last two experiments the blood was placed in one tube and the plasma decanted after different periods of time in the centrifuge. In all samples the platelet count and clotting times in glass and silicone were determined.

We will first consider the experiments which were not successful. These results must be discarded in assessing the influence of platelets. However, they do demonstrate the great activity of the tissue factor which was mentioned earlier. In experiment 30, a hæmatoma developed while the sample was being taken. Very short clotting times were obtained, particularly in silicone, so that much of the difference between glass and silicone observed in the other samples disappeared. In experiment 34, the flow of blood was slow and the vein collapsed during removal of the last 5 c.c., allowing the needle to touch the intima of the vein momentarily. This portion of the blood, which was first out of the syringe, clotted in the centrifuge, whereas the rest of the sample obtained in the normal way gave the normal long clotting time, and was therefore included in the series.

TABLE IV.

PLATELET COUNTS AND CLOTTING TIMES OF PLASMA

Experiment	*Subject*	*Sample*	*Platelet count per mm.³*	*Centrifuge time mins.*	*Clotting time, mins.*	
					Glass	*Silicone*
21	L.B.J.	A	158,000	5	20	118
		B	15,000	7	85	205
23	E.F.	A	552,000	5	19	84
		B	77,000	13	38	130
30	E.F.	A	378,000	8	(21)	(29)
		*B	3,300	25	(12)	(14)
31	A.M.	A	148,000	5	19	105
		B	6,900	21	47	93
34	L.B.J.	**A	clot			
		B	103,000	11	64	134
35	L.B.J.	A	58,000	7	60	249
		B	22,000	20	47	236
62	E.F.	A	86,000	7	38	86
		B	2,500	18	71	116
66	E.F.	A	600,000	3	59	99
		B	8,000	13	97	145
67	L.B.J.	A	120,000	6	96	396
		B	5,200	16	55	120

Part A centrifuged in the International at 1,250 r.p.m. Part B in the Angle centrifuge at 3,000 r.p.m. B (62, 66 and 67) in International at 2,000 r.p.m. *Hæmatoma developed. **Vein collapsed.

In examining the main data of the table, it can be observed that the difference between clotting times in untreated ("glass") and silicone-treated ("silicone") tubes is equally marked with plasma. Further, if we examine the pairs of samples for their clotting times in glass we observe that in five of seven experiments the clotting time is longer in the portion with the lower platelet count. Also, plotting the clotting time in glass against the platelet count on logarithmic paper for all the samples gave an approximate straight line, and on calculation a correlation coefficient of –0.45 was obtained. The possibility of this occurring by chance alone was 1:10. Though this correlation is not good, it suggests that an effect of the platelets on the clotting time ought not to be completely discounted.

We can also compare the clotting time of the sample in silicone with the platelet count. In experiment 21 there was a much longer clotting time with the B sample. However, the difference was slight in 23; and in 31 where B showed a count of only 6,900 it had a shorter clotting time than A. Likewise determining the correlation on all the data gave a correlation coefficient of –0.10, which is ascribable to chance alone. Hence the clotting time in silicone shows no dependence on the platelet concentration and the variations in clotting time suggest the influence of some other factor.

If the platelets were the only factor involved in initiating clotting, it should be possible, by sufficiently reducing the platelet count, to render the plasma incoagulable. This we have attempted by taking the upper portion of plasma after centrifuging at high speed. The results are shown in Table V. It is evident that a further reduction of the platelet count to as low as 3,000 or 4,000/mm.^{3} did not markedly increase the clotting time. The average clotting time in glass was 37 minutes, and in silicone 140 minutes. Inspection of the data in Tables IV and V suggests that even at low levels of the platelets, they should still be considered a factor in determining the clotting time in glass, though they are of much less significance in silicone. The plasma did not become incoagulable with the lowest platelet count obtained. Evidently some other factor is operative in causing clotting. The fact that the clotting times in both glass and silicone fail to correspond with the level of the platelet count also indicates this. For example, experiments 21 B, 34 B and 62 B show similar clotting times in glass (85′, 64′, 71′), yet the platelet counts are 15,000, 103,000 and 2,500/mm.^{3}, and the clotting times in silicone are 205′, 134′ and 116′.

Mechanical agitation, as from pouring or from vibration in the centrifuge, and contamination by dust, appear to be of minor importance. But in experiment 67 some such factor might explain why the first sample of plasma poured off should have a clotting time in both glass and silicone very much longer than that of the second sample, yet the platelets had been greatly reduced in the latter. As a measure of the factor involved in manipulation of the samples, a comparison is made in Table V of the longest time in silicone of the residual blood left in the syringe with the clotting time of the plasma in silicone. The difference between the clotting time in silicone of plasma and blood is partly due to the fact that the twenty minutes in the centrifuge is not included in the plasma time. When this is taken into consideration, four samples showed a longer clotting time with the blood and the other four showed a longer clotting time with the plasma.

TABLE V.
PLATELET COUNTS AND CLOTTING TIMES IN GLASS AND SILICONE

			Clotting time, mins.		
Experiment	*Subject*	*Platelet count /mm.^{3}*	*Plasma in glass*	*Plasma in silicone*	*Blood in silicone*
36	L.B.J.	4,000	37	195	141
37	M.B.	27,000	35	121 131	137
38	E.F.	16,000	30	85	218
39	L.B.J.*	4,000	(14)	(39)	175
40	A.M.	3,600	19	105 97	245
43	L.B.J.	6,400	58	118	218
44	A.M.	3,400	45	212	138
	Average......		37	140	182

Blood centrifuged 20′ at 2,000 r.p.m. Plasma decanted off into silicone. *Fat globule centrifuged out of plasma.

An interesting point was provided by experiment 39. This was a good blood sample as judged by a clotting time of 175 minutes for the blood in silicone, and on centrifuging the platelet count was reduced to 4,000/mm.^{3} However, during the centrifuging a small amount of fat separated out and it could be seen that the fat-plasma interface provided a nucleus for clotting. Hence the short clotting times of 14 minutes for glass and 39 minutes in silicone.

Discussion

It is evident from the results reported that the use of the silicone coating (Dri-Film) gives a surface which can effectively preserve blood from clotting for several hours. In such stored blood the platelets are preserved from agglutination and disintegration up to 30 minutes. Thus it is possible to obtain normal plasma without the use of anticoagulants. Such plasma can be stored at 0° C. for several weeks, and clots on being returned to room temperature. The normal clotting times reported for glass are considerably longer than that usually found. Preliminary tests indicate that this is due to the technique of removal of the blood. It has been found that the use of a silicone-treated syringe, the removal of the needle before emptying the syringe and the discarding of the last blood in the syringe are equally important factors contributing to this.

However, the silicone is ineffective in the presence of tissue damage. The slightest trace of thromboplastin, such as that picked up by the needle in its passage through the skin and vessel wall or by close contact with the intimal surface, is sufficient to clot the blood promptly. By suitable precautions it is possible to obtain blood samples apparently free of such contamination. Plasma obtained from such blood clots in 20 to 100 minutes in glass, 1½ to 6½ hours in silicone. The clotting time in glass is affected by the number of platelets in the plasma. On the other hand, the clotting time in silicone is less affected by the platelet count. This suggests that the effect on clotting of contact of plasma with glass is due either to the direct action of the glass on the platelets or on some factor which influences clotting through its effect on platelets.

While apparently the platelets are preserved in silicone, the plasma still clots independently of their action. Of course, once clotting starts, platelet alterations will take place as a result of the clotting. The initiation of clotting of plasma in silicone must be attributed to some other factor in the plasma. Wooldridge,[8] Lozner, Taylor and MacDonald, and others, have suggested that plasma contains a soluble factor which will initiate clotting. Its nature is not known. The possibility that this is thromboplastin present in circulating blood and derived from cell destruction or from products of digestion, etc., does not appear to have been considered. The extremely small amounts of thromboplastin from tissue damaged by the needle, which will affect the clotting of native plasma, has been demonstrated in these experiments and lends support to this view. The results of the recent experiments of Chargaff,[9] who removed the active factor from decalcified plasma with the ultracentrifuge, also suggest a similar agent.

Since such minute amounts of thromboplastin are effective in hastening the clotting of normal plasma, it may be urged that it is impossible to obtain a sample of blood completely free from significant amounts of tissue juice and tissue thromboplastin, and that we have simply reduced such contamination. While conceding the force of this argument may we point out that, on the same basis, it is equally unreasonable physiologically not to consider the presence in the circulation of a substance as ubiquitous in the body as thromboplastin. Presumably those variations in the data reported in Tables IV and V which did not parallel the platelet count were due to variations in thromboplastin in the sample, in part from admixture on obtaining the sample, and in part due to variations in amount in the circulating blood. Whether the proposed factor in plasma responsible for the clotting in silicone is affected by surface is not definitely known. If it is not, by removing the platelets it should be possible to obtain clotting times in glass approaching those in silicone. Lowering the platelet count to 3,000 to 4,000/mm.^{3} was not sufficient to achieve this.

It will be observed that the conclusions drawn are in agreement with the generally accepted views on blood coagulation. However, the use of silicone makes it possible to secure information which demonstrates these factors in a more convincing manner than has previously been possible. It should be emphasized that in the initiation of clotting, the platelets are a relatively minor factor compared to thromboplastin from damaged tissue, and it is only when the latter is kept to a minimum by careful technique that an effect due to platelets can be observed.

Summary

1. The use of a silicone coating (General Electric Dri-Film) on glassware and needles gives a surface which preserves blood from clotting for several hours. In this blood the

platelets are preserved from agglutination and disintegration up to half an hour.

2. By the use of such coatings it is possible to obtain normal plasma without the use of anticoagulants. Such plasma clots in 20 to 100 minutes in glass, 1½ to 6½ hours in silicone. The length of clotting time in glass is affected by the number of platelets remaining in the plasma.

3. Contamination of blood samples with minute amounts of substances from damaged tissue will clot this plasma rapidly. The effect of these substances on the clotting time of plasma is not influenced by the nature of the surface to which it is exposed.

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THE PRINCIPLES AND PRACTICE OF INGUINAL HERNIORRHAPHY

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SURGICAL repair of inguinal hernia is one of the most commonly performed operations in any modern hospital, but often one of the most poorly executed procedures in the operating room.

If one is to blame the literature, it is mainly in the direction of lack of uniformity, with too much emphasis on one or other "technique" or "method", usually with somebody's name attached. What is all too often understressed or omitted, both in the textbooks and the journals, is an adequate attention to the finer points of surgical anatomy: there is still a very definite need for systematizing the treatment of inguinal hernia on a basis of certain clear-cut principles of anatomy and surgery.

An inguinal hernia consists essentially of the protrusion of abdominal contents within a peritoneal pouch through a hiatus in the transversalis fascia, into the inguinal canal and often beyond it. This hiatus may occur at the internal abdominal ring, medial to that ring, or lateral to it.

The extent of the surgical repair is governed mainly by the size of the defect in the transversalis fascia and not by the size of the sac. The numerous operations which are described in most surgical textbooks are based upon or modified from Bassini's original technique and rely upon reinforcements of the inguinal canal by the construction of an artificial musculo-aponeurotic barrier, rather than a painstaking reconstruction of a firm layer of transversalis fascia. In considering such operations certain disadvantages must be noted.

In the first place it will usually be found that in order to suture the internal oblique and transversus muscle down to Poupart's ligament a fair amount of tension is unavoidable. The operator undertakes to suture these structures together under adequate tension, and yet not tightly enough to cause necrosis of the muscle fibres. Also he is attempting to obtain good physiological union between the smooth convex edge of muscle and the smooth concave surface of inguinal ligament. In operating upon hernial recurrence cases following previous Bassini repairs I have in all cases found that non-absorbable sutures, such as silk, linen or cotton, were not used, that the internal oblique and transversus muscles had separated partially or completely from Poupart's ligament, and that usually a defect of varying size was still present in the transversalis fascia. Such a separation of these muscles can hardly be blamed on coughing and straining, as these acts cause the curved lower fibres of the inguinal muscles to contract and become straighter, which in fact tends to approximate them to Poupart's ligament.

Several factors are probably responsible. In the first place there is the unrelieved upward and medial drag of the rectus sheath border on the conjoint tendon which is attached closely to it. Secondly, there is frequently some degree of ischæmic necrosis in the lower muscle fibres, due to strangulation by sutures which have been tied too tightly in order to obtain adequate apposition of muscles to liga-