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STUDIES ON PLATELETS AND HEPARIN DURING SENSITIZATION AND ANAPHYLAXIS

By

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In 1909, *Achard & Anyand* observed for the first time that platelets in the blood of a horse, immunized with dog serum, were agglutinated by dog serum, i.e. the corresponding antigen. *Rocha e Silva* in 1950 recorded that platelets were agglutinated and could be damaged by the antigen-antibody reaction and finally lysed in the capillaries. An indication of this damage is the release of 5-hydroxytryptamine from the platelets (*Humphrey & Jaques* 1955).

A correlation between the severity of the shock and the degree of thrombocytopenia in rabbits has been demonstrated by *Kopeloff & Kopeloff* (1941). A marked decrease in the number of circulating platelets has been observed in guinea pigs as early as five days after sensitization without administration of the anaphylactic shock dose (*Nilzén*, 1959). In earlier studies the author described the effect of heparin on platelet counts and as well as on the release of 5-hydroxytryptamine in anaphylactic shock, food allergies and burn injuries (*Johansson* 1960, a, b, c). The decrease in the number of circulating platelets after anaphylactic shock and burn injuries could be prevented by pretreating the animals with heparin. In human beings heparin was able to prevent the decrease in platelet count which accompanies allergic reactions elicited by a specific allergen. Heparin also inhibited the release of 5-hydroxytryptamine in anaphylactic shock and burn injuries in rab-

bits and most of the local response reaction to the intradermal injection of a specific allergen in man. *Blombäck, Johansson & Sjöberg* (1960) observed that most of the coagulation factors were consumed during an anaphylactic reaction. There are thus some indications that the changes observed during anaphylaxis might be due to an increased intravascular coagulation with consumption of coagulation factors. Also in the Saranelli-Schwartzman reaction a decrease in some of the coagulation factors has been noted (*McKay & Shapiro* 1958, *Kleinmaier et al.* 1959). This reaction was prevented if the animals were pretreated with heparin or high doses of dicumarol (*Good & Thomas* 1953, *McKay & Shapiro* 1958). *Phau, Lasch & Gunther* (1960) successfully treated a woman during shock probably due to a Saranelli-Schwartzman reaction with a state of hypocoagulability with heparin. There are a variety of pathological conditions in which the changes in coagulation factors present some similarities with those reactions mentioned above. Thus in liver cirrhosis periods of multiple deficiencies in thrombocytopenia are observed (*Bergström, Blombäck & Kleen* 1960). The present author in 1960 treated a woman with bleeding tendency due to liver cirrhosis during two bleeding crisis with heparin. A state of hypocoagulability was also found in this case (*Johansson* 1962). Also purpura fulminans has been successfully treated with anticoagulants as reported by *Little* (1959) and by *Bernstick & Hirson* (1960).

The object of this investigation was to study if heparin could prevent the changes in coagulation factors observed in connexion with anaphylactic shock.

MATERIAL AND METHODS

Rabbits of both sexes weighing 2.5–3.5 kg were used. The rabbits were sensitized with 1 ml horse serum intraperitoneally given daily for six days according to *Waalkes et al.* (1957) and *Sanyal & West* (1958).

Collection of blood samples. Blood was drawn from an ear vein or through a teflon catheter in a carotid artery. The first few ml of blood were discarded to prevent any possible effect of tissue juice in the experiments. Trisodium citrate $\times 2H_2O$ or heparin were used as anticoagulants.

Preparation of plasma. Nine volumes of blood were collected into plastic centrifuge containing one volume of 3.13 per cent trisodium citrate. In some experiments 0.1 μ g of heparin (Vitrum, Sweden) pr ml blood was used as anticoagulant.

Platelet-rich plasma was obtained by centrifuging heparinized blood at 185 g for 5 min. immediately after collection.

Platelet-deficient plasma was prepared by centrifuging platelet-rich plasma at 16000 g for 30 min. in plastic tubes at 4° C.

The supernatant plasma was then drawn off without disturbing the layer of blood cells. Mixtures of the two plasmas in different proportions were made to obtain the desired number of platelets.

All glassware was silicone treated (Siloxan 300, Uddeholms AB, Sweden).

Prothrombin-proconvertin (P-P) activity was determined according to *Owren & Aas* (1951). Human thromboplastin was used.

Fibrinogen was determined as described by *Bergström, Blombäck & Kleen* 1960.

Platelet counts were performed on blood drawn from an ear vein or through a catheter in a carotid artery. The first few ml of blood were discarded, because it has been stated by *Stefanini* (1950) that admixture of tissue juice to the blood may give irreversible aggregation of thrombocytes. One ml of blood was then collected into siliconized tubes containing 0.1 ml 2 per cent disodium ethylene diamine tetraacetic acid solution (EDTA) adjusted to pH 7.4. The blood was then aspirated into a silicone treated Ellermann pipette up to the 25 μ l mark and was then blown into a silicone treated tube, containing 475 μ of the reagent used by *Kristenson* (1927), (10 g urea, 2.5 g trisodium citrate 2H₂ 0.5 mercuric chloride, in 500 ml of distilled water).

The tube was rolled for a few minutes and ~~sedimented~~ for about 30 min. The suspension was transferred into a Bürker counting chamber and allowed to settle in a moist chamber for about 15 min. The number of platelets was counted in 16 rectangular square is 1/800 μ l. The counting was done with a dry lens magnification 500 of an ordinary microscope. The determinations of platelet were allways done in duplicate.

RESULTS

In 20 animals platelet counts were performed every second day from the 6th to the 21st day after the onset of sensitization. The results of the first group of 5 sensitized rabbits, which received 0.9 per cent saline intravenously into an ear vein are shown in Table 1 and Fig. 1.

A decrease in the number of circulating platelets was observed already a few days after the sensitization had been completed.

TABLE 1

Platelet Counts in Thousands per μ l during the First Period of Sensitization of 5 Untreated Rabbits.

Before sensitization		Days after the onset of sensitization							
		7 th	9 th	11 th	13 th	15 th	17 th	19 th	21 th
Rabbit 1	325	320	295	270	260	300	305	300	295
Rabbit 2	315	310	290	265	260	285	290	300	310
Rabbit 3	310	285	270	240	215	200	225	240	275
Rabbit 4	300	280	245	230	225	90	—	—	—
Rabbit 5	285	280	245	240	215	235	250	265	275
Mean values	307	295	263	249	237	220	268	276	289

TABLE 2

Platelet Counts in Thousands per μ l during the First Period of Sensitization in 5 Heparin Treated Animals.

Before sensitization		Days after the onset of sensitization							
		7 th	9 th	11 th	13 th	15 th	17 th	19 th	21 th
Rabbit 6	320	320	325	310	310	315	325	315	310
Rabbit 7	315	320	310	315	295	315	325	320	310
Rabbit 8	315	320	320	325	325	320	315	315	305
Rabbit 9	305	310	305	320	310	310	305	290	315
Rabbit 10	290	305	300	295	305	315	310	305	295
Mean values	309	315	312	313	309	315	316	309	307

In one rabbit which died on the 15th day with symptoms resembling those of anaphylactic shock, only 90,000 platelets per μ l was observed in the circulating blood.

A second group (5 sensitized animals) was treated in the same way except that they were given 10 mg of heparin (100 i.u. per mg) instead of 0.9 per cent saline into an ear vein.

The results of the platelet counts are shown in Table 2 and Fig. 1. In the heparin treated animals no decrease in the number of circulating platelets was found.

A third group (5 sensitized animals) received 10 mg of heparin intravenously on the 12th, 13th, 16th and 17th day after the onset of sensitization in order to study whether or

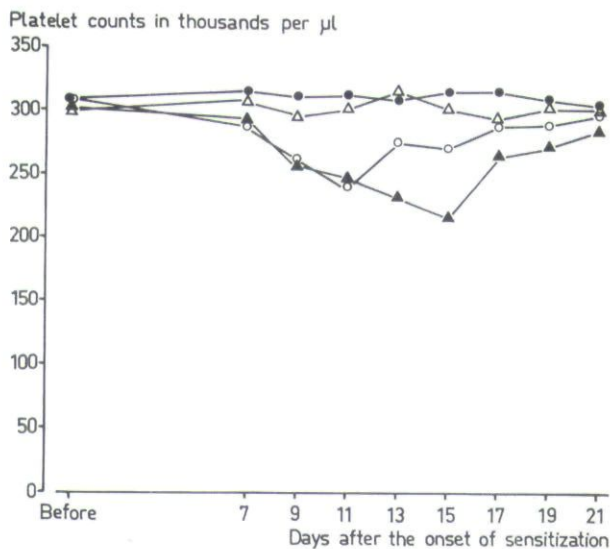


Fig. 1.

Mean values during the first period of sensitization in 5 untreated rabbits ▲——▲, 5 heparintreated rabbits ●——●, 5 rabbits which received 10 mg of heparin only on the 12th, 13th, 16th and 17th day ○——○, and in 5 nonsensitized control rabbits △——△.

not an increase in the number of circulating platelets could be detected following administration of heparin at intervals during the sensitization. A more rapid return to normal values seemed to occur in this group as compared with the untreated group. The platelet counts in these experiments are shown in Table 3 and Fig. 1. In five unsensitized control animals which only received 0.9 per cent saline intraperitoneally and intravenously no change in platelet counts was noted (Fig. 1).

In order to study the influence of heparin on the platelet count during anaphylaxis the following experiments were made. Blood from 5 sensitized and 5 unsensitized rabbits was drawn from ear veins six weeks after the sensitization when the fixation antibody titre was suspected to be high (*Waalke et al.* 1957). No difference in platelet count was observed in controls and sensitized animals 6 weeks after the sensitization

Nine volumes of blood were collected into plastic tubes containing 1 volume 3.13 per cent trisodium citrate.

TABLE 3

Platelet Counts in Thousands per μ l during the First Period of Sensitization in 5 Animals which Received 10 mg of Heparin only on the 12th, 13th, 16th and 17th Day after the Onset of Sensitization.

Before sensitization		Days after the onset of sensitization							
		7 th	9 th	11 th	13 th	15 th	17 th	19 th	21 th
Rabbit 11	325	305	270	230	280	275	285	290	290
Rabbit 12	315	285	270	260	285	285	295	290	300
Rabbit 13	310	300	245	230	265	260	285	290	295
Rabbit 14	300	275	260	255	265	260	290	295	305
Rabbit 15	295	280	275	235	285	275	285	280	300
Mean values	309	289	262	242	276	271	288	289	298

The blood was diluted with equal volumes of 0.9 per cent saline and platelet counts were performed. 2 ml of diluted blood was incubated at 37° C *in vitro* and 0.1 ml of a solution of heparin in 0.9 per cent saline was added. The tubes was swirled every 2nd min. After 10 min. of incubation 0.1 ml horse serum (diluted 1:10 with 0.9 per cent saline) or 0.9 per cent saline was added. The mixture was then incubated for 30 min. at 37° C and the tubes were swirled every 5 min. Platelet counts were made before and 30 min. after the diluted horse serum had been added to the diluted blood.

In Fig. 2 are shown the platelet counts of diluted whole blood from 5 sensitized animals and 5 controls after adding a specific antigen or 0.9 per cent saline to samples of diluted whole blood containing different concentrations of heparin. A concentration of less than 0.1 mg heparin per ml diluted blood was sufficient to prevent a decrease in the platelet count after addition of the antigen *in vitro*. In the blood samples without heparin a marked decrease in the number of platelets was observed. No change in platelet count was found in diluted blood from these two groups when 0.9 per cent saline was

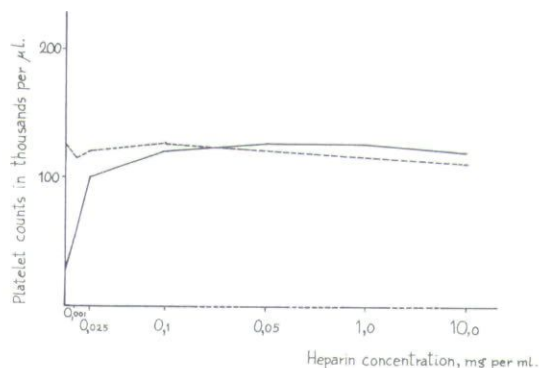


Fig. 2.

Platelet counts of plasma containing different concentrations of heparin after addition of horse serum *in vitro*.

- (—) Mean values from 5 sensitized rabbits.
 (----) Mean values from 5 normal rabbits.

added instead of the antigen *in vitro*. *In vivo* less than 0.1 and more than 0.01 mg heparin was necessary to prevent a decrease in the number of platelets during anaphylactic shock.

In order to study if an antigen-antibody reaction might influence on the coagulation time measurements experiments were performed *in vitro*. By the aid of a teflon catheter in the carotid artery blood was withdrawn from 5 sensitized rabbits on the 14th day after the onset of sensitization and from 5 control animals.

Three ml of blood was put into each of the silicone treated tubes, which contained 0.1 ml of horse serum or 0.1 ml of 0.9 per cent saline and the onset of clot formation was recorded. The effect of the specific antigen (horse serum) upon the coagulation time of blood from sensitized rabbits and control animals was studied.

The results are shown in Table 4. The coagulation time of blood from normal rabbits was not changed by different concentrations of horse serum and was of about the same magnitude as that observed in the experiments with 0.9 per cent saline. The sensitized group showed throughout a shorter coagulation time both with 0.9 per cent saline and with different

concentrations of the antigen. A shortening of the coagulation time was noted with rising concentrations of the antigen.

The role of platelets for the decrease in the coagulation time of heparinized blood from sensitized rabbits was studied in the following way.

TABLE 4
Effect of Horse Serum on the Coagulation Time (in Silicone) of Blood from Sensitized and Normal Rabbits.

	Number of rabbits	Coagulation time, minutes concentration of horse serum ($\mu\text{g/ml}$)				
		0 (NaCl)	0,01	0,1	1,0	5,0
Normal rabbits	5	38	34	34	32	30
		34	34	32	36	31
		32	30	30	31	30
		31	35	34	34	31
		30	30	30	30	28
Average		33,0	32,6	32,2	32,6	30,0
Sensitized rabbits	5	30	27	22	20	18
		30	24	23	23	19
		27	27	24	21	16
		24	21	20	18	16
		21	22	22	20	17
Average		26,4	24,2	22,2	20,4	17,2

To silicone treated tubes containing 0.1 ml of diluted horse serum or 0.9 per cent saline, 2 ml of platelet-rich plasma or platelet-deficient plasma was added. The onset of clot formation was recorded. The coagulation time of platelet-rich plasma obtained from sensitized animals was inversely proportional to the antigen concentration. The shortening of the coagulation time was significant compared to the measurements done with normal plasma and with platelet-deficient plasma from sensitized animals (Table 5 and 6).

For further coagulation studies one ml of horse serum was given to 10 sensitized animals (5 untreated and 5 heparin

treated) on the 14th and on the 15th day and to 5 control animals. Nine volumes of blood were collected from an ear vein into centrifuge tubes containing one volume of 3.13 per cent trisodium citrate at 1 hour before and at 1, 2, and 4 hours after the anaphylactic doses.

TABLE 5
Effect of Horse Serum on the Coagulation Time (in Silicone) of Platelet Rich Plasma and Platelet Poor Plasma from Five Normal Rabbits.
(0.1 μ g Heparin/ml used as Anticoagulant).

	Number of rabbits	Coagulation time, minutes Concentration of horse serum (μ g/ml)			
		0 (NaCl)	0.01	0.1	5.0
Platelet rich plasma	5	185	194	180	183
		183	184	186	186
		176	181	185	171
		171	172	183	169
		169	163	169	175
Average		178	179	180	177
Platelet poor plasma	5	198	193	190	186
		192	201	191	195
		189	174	179	171
		184	196	190	181
		173	182	181	184
Average		187	189	186	183

Counts of platelet rich plasmas were 160–200000 platelets/ μ and platelet poor plasma were 5–10000 platelets/ μ l.

Platelets, prothrombin-proconvertin and fibrinogen were determined. The results are shown in Fig. 3, and in Table 7, 8, 9, 10, 11, 12 and 13. A marked decrease in the number of circulating platelets in the untreated sensitized rabbits was noted within one hour after the intravenous dose of horse serum. The untreated sensitized animals showed lower platelet values before the horse serum injection than did the control animals. A slight decrease in the number of platelets

was also found in the controls. The decrease in the platelet count of the sensitized animals was significant compared with the controls.

TABLE 6

Effect of Horse Serum on the Coagulation Time (in Silicone) of platelet Rich Plasma and Platelet Poor Plasma from 5 Sensitized Rabbits.
(0.1 μ g Heparin/ml used as Anticoagulant).

	Number of rabbits	Coagulation time, minutes Concentration of horse serum (μ g/ml)			
		0 (NaCl)	0.01	0.1	5.0
Platelet rich plasma	5	168	164	160	142
		169	166	157	121
		157	145	148	137
		171	166	151	129
		172	160	148	134
Average		167	161	153	133
Platelet poor plasma	5	191	189	184	174
		187	194	186	182
		174	169	161	160
		172	176	174	169
		169	167	159	160
Average		179	179	173	169

Counts of platelet rich plasmas were 150-200000 platelets/ μ l and platelet poor plasmas were 5-10000 platelets/ μ l.

The prothrombin-proconvertin determinations are shown in Fig. 3 (Table 8). A decrease in the P-P level was noted already at one hour after the intravenous horse serum injections to untreated sensitized rabbits. The decrease at four hours was marked in all cases. The decrease noted in the control group was small. The fibrinogen levels in these experiments are given in Table 9. A slight decrease in the mean fibrinogen content was found at 4 hours after the first injection of the antigen.

After 24 hours the fibrinogen level had increased to values above the initial ones. A second dose of antigen again produced a small decrease within 4 hours.

TABLE 7

Platelet Counts in Thousands per μ l from 5 Sensitized Rabbits and 5 Control Rabbits before and at Intervals after Intravenous Injections of one ml of Horse Serum on the 14th and 15th Day after the Onset of Sensitization.

Sensitized rabbits	14 th day				15 th day			
	before hours after horse serum				before hours after horse serum			
	1	2	4		1	2	4	
Rabbit A	275	105	130	195	300	165	140	140
Rabbit B	275	170	165	205	265	90	120	165
Rabbit C	260	210	230	235	320	200	195	180
Rabbit D	295	123	200	290	270	120	195	225
Rabbit E	280	165	190	200	290	210	215	220
Average	277	155	199	225	289	157	173	186
Control rabbits	14 th day				15 th day			
	before hours after horse serum				before hours after horse serum			
	1	2	4		1	2	4	
Rabbit F	305	275	260	305	310	275	275	300
Rabbit G	295	290	275	270	290	285	270	295
Rabbit H	295	295	300	305	300	280	295	280
Rabbit I	315	290	290	300	300	310	270	305
Rabbit K	315	255	290	310	305	280	265	300
Average	305	284	283	298	302	286	275	292

TABLE 8

Prothrombin-Proconvertin Activity per cent of Plasma from 5 Sensitized Rabbits before and at Intervals after Intravenous Injection of one ml Horse Serum on the 14th and 15th Day after the Onset of Sensitization.

Sensitized rabbits	14 th day				15 th day			
	before hours after horse serum				before hours after horse serum			
	1	2	4		1	2	4	
Rabbit A	112	64	62	61	104	62	57	53
Rabbit B	106	79	65	67	93	68	61	64
Rabbit C	100	58	56	52	99	42	46	46
Rabbit D	98	51	54	53	101	57	59	52
Rabbit E	91	76	69	61	97	71	68	63
Average	101	66	60	59	100	69	58	56

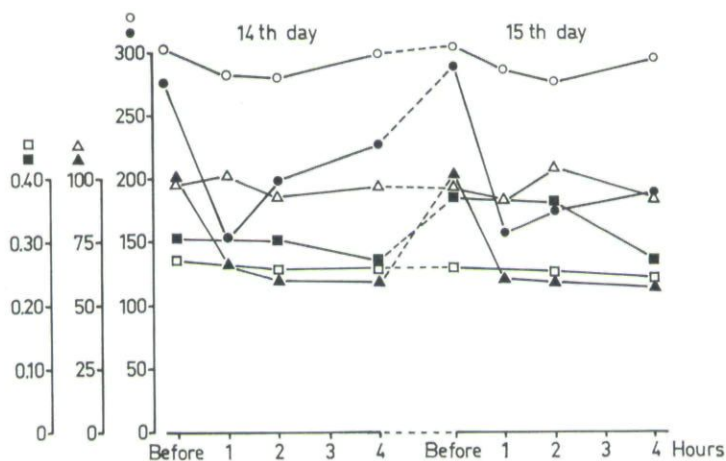


Fig. 3.

Mean values in 5 sensitized rabbits and in 5 control rabbits of platelet counts (10^3) (● — sensitized, ○ — controls), prothrombin-proconvertin activity (per cent) (▲ — sensitized, △ — controls) and in fibrinogen content (g/100 ml) (■ — sensitized, □ — controls) before and after i.v. injection of horse serum on the 14th and 15th day after the onset of sensitization.

TABLE 9

Fibrinogen Content (g per 100 ml) of Plasma from 5 Sensitized Rabbits before and at Intervals after Intravenous Injection of one ml of Horse Serum on the 14th and 15th Day after the Onset of Sensitization.

Sensitized rabbits	14 th day before hours after horse serum			15 th day before hours after horse serum		
	1	2	4	1	2	4
Rabbit A	0,29	0,28	0,27	0,36	0,35	0,27
Rabbit B	0,34	0,32	0,32	0,42	0,39	0,30
Rabbit C	0,23	0,24	0,22	0,34	0,29	0,22
Rabbit D	0,36	0,34	0,27	0,39	0,38	0,27
Rabbit E	0,38	0,33	0,26	0,36	0,37	0,29
Average	0,31	0,30	0,27	0,37	0,36	0,27

Control experiments were made under identical conditions but omitting the antigen injections. Small changes in fibrinogen were noted (Table 10).

TABLE 10
Mean and Range Values of Prothrombin-Proconverin and Fibrinogen from 5 Control Rabbits before and at Intervals after Intravenous Injection of Horse Serum on the 14th and 15th Day after the Onset of Intraperitoneal 0.9 per cent Saline Injections.

		14 th day				15 th day					
		Before		1	2	4	Before		1	2	4
P.P	Mean	98		101	93	97	96		91	104	93
per cent	Range	108-91		116-89	104-93	105-90	104-90		102-86	113-91	104-87
Fibrinogen	Mean	0.27		-	0.26	0.26	0.26		-	0.25	0.24
g per 100 ml	Range	0.32-0.23		-	0.31-0.24	0.31-0.24	0.32-0.24		-	0.32-0.22	0.30-0.31

TABLE 11

Platelet Counts in Thousands per μ l of Blood from 5 Heparin Treated Sensitized Animals before and at Intervals after Intravenous Injection of a Specific Antigen on the 14th and 15th Day after the Onset of Sensitization.

Sensitized rabbits	Before hours after horse serum				Before hours after horse serum			
	1	2	4		1	2	4	
Rabbit L	295	270	260	275	280	270	285	275
Rabbit M	265	270	260	255	275	245	265	280
Rabbit N	250	255	265	265	260	250	265	270
Rabbit O	265	260	250	240	275	265	260	250
Rabbit P	240	265	250	245	270	260	265	245
Average	263	264	257	256	272	258	272	264

TABLE 12

Prothrombin-Proconvertin content (per cent) of Plasma from 5 Heparin Treated Rabbits before and at Intervals after Intravenous Injection of a Specific Antigen.

Sensitized rabbits	Before hours after horse serum				Before hours after horse serum			
	1	2	4		1	2	4	
Rabbit L	89	93	86	85	95	89	84	81
Rabbit M	94	90	87	89	87	81	80	80
Rabbit N	99	93	90	86	97	91	90	84
Rabbit O	92	94	90	83	104	97	96	92
Rabbit P	101	94	83	80	98	95	93	89
Average	95	93	87	85	96	91	89	85

TABLE 13

Fibrinogen content (g per 100 ml) of Plasma from 5 Heparin Treated Sensitized Rabbits before and at Intervals after Intravenous Injection of one ml of Horse Serum on the 14th and 15th Day after the Onset of Sensitization.

Sensitized rabbits	14 th day Before hours after horse serum			15 th day Before hours after horse serum		
	2	4		2	4	
Rabbit L	0,23	0,25	0,23	0,22	0,21	0,20
Rabbit M	0,28	0,26	0,24	0,24	0,23	0,23
Rabbit N	0,34	0,29	0,30	0,32	0,33	0,32
Rabbit O	0,32	0,32	0,33	0,30	0,30	0,29
Rabbit P	0,29	0,31	0,30	0,32	0,30	0,29
Average	0,29	0,29	0,28	0,28	0,27	0,27

In the heparin treated group no significant changes of platelet count, prothrombin-proconvertin and fibrinogen was noted (Table 11, 12, 13).

DISCUSSION

It is well known that in anaphylactic shock the number of circulating platelets decreases. According to *Rocha e Silva* this decrease depends on at least two factors: 1. Trapping of platelets into the capillary beds—a property restricted largely to the lung. 2. Damage and lysis of platelets by the antigen-antibody reaction. Antigen-antibody reactions have also been shown to cause clumping or agglutination of the platelets *in vivo* (*Rocha e Silva* 1950, *Stetson* 1951) as well as *in vitro* (*Miescher & Strassle* 1956).

A variety of agents such as polysaccharides, endotoxin, trypsin, peptone and a large number of drugs injected intravenously will produce a drop in the number of circulating platelets (*Scroggie, Jaques & Rocha e Silva* 1947, *Rocha e Silva* 1950, *Weil & Spink* 1957, *Acroyd* 1955). In human beings *Nilzén* (1953, 1958) and *Storck, Hoigné & Koller* (1955) have reported that intravenous injections of an antigen into patients sensitive to this antigen will cause a fall in the number of platelets in their blood.

It would therefore be of importance to establish the role of the decrease in circulating platelets due to various agents and antigen-antibody reactions. If destroyed the platelets distribute substances eg. platelet factor 3 to the blood which contribute to the clotting mechanisms (see synopsis by *van Creveld* 1954, *Johnstone & Seegers* 1958).

Heparin prevents the decrease in circulating platelets during anaphylaxis and burn injuries in the experimental animals (*Johansson* 1960 a, b, 1961) and is also considered to have a protective action in anaphylactic shock in burn injuries and the Saranelli-Schrawtzmann reaction (*Kyes & Strausses* 1927, *Elrod, Mc Cleerly & Ball* 1951, *Hallberg & Pitzele* 1959).

The antithrombic effect of heparin is well known. A hypothesis regarding the physiological role of heparin is according

to *Mellanby* in 1934, that heparin is secreted locally by the mast cells to prevent intravascular clotting due to entry of thromboplastin into the blood due to tissue damage (c.f. *Jaques* 1955). The platelets contain substances which can trigger blood clotting when released. In the anaphylactic reactions such substances are most probably released from the platelets and would therefore be able to initiate a transient intravascular coagulation of blood. The beneficial effect of heparin in anaphylactic shock might then be due to inhibition of the sequence of reactions leading to intravascular clotting.

The mode of action of heparin, as an anticoagulant, is controversial. It inhibits the agglutination and desintegration of blood platelets (*Best, Campbell & Mclean* 1938, *Zucker* 1951). Low concentrations of heparin prevent the formation of blood thromboplastin from its precursors (*Biggs, Douglas & McFarlane* 1953). According to *Jorpes* heparin as a consequence of its strong negative electric charge in some way inhibits all stages in blood coagulation. Heparin is also together with a cofactor in plasma a strong antithrombin. *O'Brien* (1958) suggested that heparin acts by combining with Christmas factor (IX). Whether heparin acts directly on the platelets or indirectly via plasma factors such as Hageman factor (XII), plasma thromboplastin antecedent (XI) is not known. Evidence have been put forward (*Lüscher* 1956) that thrombin is necessary for producing "viscous metamorphosis" of the platelets. However, heparin also produces a marked increase in the negative charge of platelets in citrate buffer (*Ross & Ebert* 1959), which diminishes their ability to agglutinate.

In a preliminary work (*Blombäck, Johansson & Sjöberg* 1960) found that coagulation factors such as platelets, fibrinogen, factor V, Christmas factor and prothrombin and/or proconvertin were consumed during an anaphylactic reaction in rabbits. The findings were compatible with the view that the disintegrated platelets released substances initiating a diffuse intravascular coagulation, which was of such a magnitude as to cause consumption of most coagulation factors.

Some of the results in this investigation are compatible with

the hypothesis of an intravascular coagulation. 1. The decrease found in the number of platelets when a specific antigen was given to the animals on the 14th and on the 15th day after the onset of sensitization. 2. A rapid decrease in prothrombin-proconvertin activity was observed following the anaphylactic shock. This decrease may well be associated with coagulation. Another possibility would be a depressed synthesis in the liver due to oedema. But in view of the rapidity of which the decrease occurred this does not seem to be very likely. 3. A decrease in the fibrinogen level of blood following the anaphylactic reaction. 4. Pretreatment of the animals with heparin prevented the decrease in platelets, prothrombin-proconvertin and fibrinogen.

It is known that aggregation of the white cells of blood occurred during anaphylaxis in rabbits. This could explain the rapid occurrence of thrombocytopenia after an anaphylactic reaction. The aggregated blood cells in the capillaries disintegrate and release thromboplastic substances to the blood and the subsequent coagulation is started with consumption of the coagulation factors. Similar defects in the coagulation have been described in other conditions with intravascular aggregation, such as hypothermia (*Ellis et al.* 1957), crush injury (*Bergentz & Nilsson* 1961) Schwartzmann reaction (*Pfau, Lasch & Günther* 1960), and after injection of bacterial endotoxin (*Shapiro & McKay* 1958).

The present investigation has further shown that the coagulation time in silicone of blood drawn from sensitized animals is shorter than that of blood from normal rabbits. This study shows that the platelets may be involved in the processes which shorten the coagulation time of the blood from sensitized rabbits. The antigen further had no ability to affect the coagulation time of heparinized platelet-deficient plasma from sensitized animals. During a sensitization period a continuous decrease of the platelet count occurred for several days after the last injection of specific antigen. These results are in good accordance with those found in guinea pigs (*Nilzén*, 1959). To exclude the possibility that the decrease in the platelet

count occurring during sensitization was due to an infection, control animals were given daily injections of 0.9 per cent saline into an ear vein during 3 weeks. No change in the number of circulating platelets was observed.

In an earlier study, the present investigator has showed that heparin prevented the decrease in the platelet count during anaphylaxis and allergic reactions (Johansson, 1960 a, b). The present study is in accordance with these results. Heparin given to the animals during sensitization prevented the decrease in the platelet count. This work shows that less than 0.1 mg heparin per ml diluted blood is sufficient to prevent the decrease in the platelet count after addition of a specific antigen *in vitro*. A result in accordance with an earlier investigation (Johansson, 1961) is that heparin also inhibited the release of 5-HT when an antigen was added to whole blood from sensitized *in vitro*, and the release of 5-HT seems to be a good indication of platelet damage by an antigen-antibody reaction (Humphrey & Jaques 1955).

SUMMARY

During a sensitization period and after an anaphylactic shock a decrease in the number of circulating platelets was found in experiments on rabbits. If the animals had been pre-treated with heparin 1 hour before the anaphylactic shock was initiated, no decrease in the platelet count was observed. 0.1 mg of heparin per ml blood was sufficient to prevent *in vitro* and *in vivo* clumping of platelets after addition to the specific antigen to blood from sensitized animals. The coagulation time, measured with silicone technique, was found to be shorter in sensitized rabbits than in control rabbits. A shortening of the coagulation time, varying with the antigen concentration, was observed in whole blood and in platelet-rich plasma from sensitized rabbits. In platelet-deficient plasma from sensitized rabbits, as well as in whole blood and in platelet-rich and platelet-deficient plasma from normal rabbits, no change in the coagulation time was observed. A decrease in the number of

platelets, in prothrombin-proconvertin activity and in fibrinogen was noted after injection of the specific antigen *in vivo* at 2 weeks after onset of sensitization. Pretreatment of the rabbits with heparin inhibited the decrease in fibrinogen, prothrombin-proconvertin and in number of platelets. It is suggested that a transient intravascular coagulation may occur during anaphylaxis.

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