

BLOOD COAGULATION DEFECT INS MASUGI NEPHRITIS

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The platelet count and the coagulation pattern have been studied on the basis of nine laboratory tests in Masugi nephritis of the delayed type.

Platelet consumption was found to constitute the initial manifestation of Masugi nephritis. The pre-nephritic stage and the manifestation of nephritis were invariably associated with hypercoagulability, as reflected by a loss of the spontaneous fibrinolytic activity of plasma, together with an elevation of the fibrinogen level and an increase in maximal thrombus elasticity. At the onset of nephritis the platelet count declines again. The hypercoagulability is attributed to intravascular coagulation in the kidney.

Introduction

It has been shown earlier [3—6, 19, 20] that nephropathies of immunological origin are accompanied by hypercoagulability, as reflected by a reduction in the fibrinolytic activity of blood plasma, together with an elevation of the fibrinogen level and an increase in maximal thrombus elasticity. Though we could perform coagulation studies in a case 17 days after onset of the disease, insight into the coagulation defects in the incipient phase rests with the evidence of animal studies.

The best experimental counterpart of human glomerulonephritis is Masugi nephritis of the delayed type [18, 32]. This was the model we had selected for the study of coagulation defects preceding the manifestation of nephritis.

Materials and methods

Potent antirabbit kidney sera (nephrotoxin) were raised in ducks, using the original technique of MASUGI [32] with slight modifications.

Of 24 rabbits eleven were treated intravenously with nephrotoxic duck serum (1.5 ml per kg body weight), six animals with normal duck serum, and seven with 1.5 ml/kg physiological saline.

The animals were checked daily for blood pressure, body weight and urine. Blood pressure was measured by the method of GRANT and ROTSCILD [12]. The onset of nephritis which ensued in all nephrotoxin treated animals was marked by proteinuria, haematuria and cylindru-ria.

Nine ml blood was drawn from the lateral ear vein in a silicon-coated tube containing 3.8% Na citrate on two occasions before administration of the antirabbit kidney serum, then

at 30 min and 4 days subsequent to it, finally on the day following onset of nephritis (i. e. on the 8th or 9th day of the study). The samples were taken from the controls in the same manner and at the same intervals.

In the coagulation and platelet studies the following methods were used.

- 1) Partial thromboplastin time, according to PROCTOR and RAPAPORT [40];
- 2) coagulation time of recalcified citrated whole blood, according to HOWELL [39];
- 3) heparin tolerance time, according to MARBET and WINTERSTEIN [31];
- 4) plasma fibrinogen [11];
- 5) assay of plasma for spontaneous fibrinolytic activity by gravimetry [11];
- 6) platelet count, according to BRECHER and CRONKITE by phase contrast microscopy [7];
- 7) platelet aggregation time under the effect of ADP, according to MITCHELL and SHARP [34];
- 8) platelet-factor 3 availability, according to HARDISTY and HUTTON [14];
- 9) thromboelastography after recalcification of citrated whole blood, according to HARTERT [15].

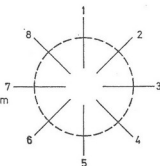
Results were evaluated statistically by Student's one sample *t*-test.

In the illustrative cases the results were represented on a coagulogram according to GERENDÁS [11]. The normal coagulation values are represented on of radially arranged scales in a manner to occupy the circumference of a circle which represents the normal range of values (Fig. 1). Normal values were established on the grounds of 48 examinations in 24 rabbits. Data outside the circle indicate hypocoagulability, those inside the circle a shift towards hypercoagulability. Angular projection of the circle: hypocoagulability; impression of the circle: hypercoagulability. Figure 1 shows that the thromboelastogram of citrated blood in rabbits differ from that in man: the *r* and *k* times are shorter, the maximal amplitude, thus also the maximal elasticity (*me*) calculated therefrom, are greater.

The day after onset of nephritis the animals were killed by an overdose of phenobarbital and the kidneys were processed for microscopic study.

METHODS

	Normal values
1. Partial thromboplastin time	28 - 127 sec
2. Recalcification time	60 - 110 sec
3. Heparin tolerance time	75 - 200 sec
4. Fibrinogen	200 - 300 mg/dl
5. Fibrinolytic activity	10 - 22 %
6. Platelet count	250 - 500 x 10 ³ /cu.mm
7. Platelet aggregation with ADP	20 - 60 sec
8. Platelet factor 3. availability	30 - 110 sec



TEG of citrated blood

<i>r</i> time	70 - 270 sec
<i>k</i> time	45 - 300 sec
<i>me</i>	59 - 212

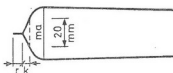


Fig. 1. The normal coagulation values are represented on the circumference of a circle. The values outside the circle indicate hypocoagulability, those inside the circle, hypercoagulability. The thromboelastogram prepared after recalcification of citrated rabbit blood, is different from that of human citrated blood, its *r* and *k* times being shorter and its maximal elasticity (*me*) being greater

Results

Table I sums up the normal means \pm S. E. for 11 parameters derived from 9 tests, and their changes. The data of the control groups treated with normal duck serum or physiological saline, are also represented. The changes were referred to the pretreatment values (Table I).

The parameters most affected by the nephrotoxic duck serum include the plasma fibrinogen level, the spontaneous fibrinolytic activity, the platelet count and the maximal thrombus elasticity. Reduction of platelet ADP aggregation time and platelet 3-factor availability also attained the level of significance, this was, however, the case in the controls as well. The false positive results being attributable to technical factors inherent in blood sampling, platelet function tests are regarded as unsuitable for the study of the question.

The nephrotoxic serum produced a shift of the plasma fibrinogen level, of the fibrinolytic activity and of the maximal elasticity of thrombus to the direction of hypercoagulability. A significant elevation of the fibrinogen values, together with a significant fall in fibrinolysis and an increase in maximal elasticity was found on the 4th day after treatment. These changes were still more marked at the onset of nephritis. On the other hand, the platelet count revealed a haemorrhagic tendency under the effect of the nephrotoxic serum: 30 min after its administration as well as at the onset of nephritis the platelet count displayed a statistically significant reduction. No such changes were demonstrable in the groups treated with normal duck serum or saline.

According to statistical analysis, the significant thrombocytopenia is a biphasic phenomenon, corresponding to the biphasic mechanism inherent in the delayed type Masugi nephritis.

The abnormalities demonstrable by statistical analysis obviously represent the mean values. The animals' responsiveness, particularly as concerns the platelet count, showed individual variations. In the majority (8 out of 11 animals) it was in the early stage that the platelet count fell significantly but in some cases (2 out of 11) this did not ensue until the onset of nephritis and exceptionally (1 out of 11 cases) the reaction was biphasic. The three types are illustrated by the following cases.

Case 1. Rabbit No. 20, 2600 g. As it can be seen in Figure 2, the platelet count fell to approximately 50% of the original values 30 min after administration of the nephrotoxic serum but during the pre-nephritic stage and at the onset of nephritis it was in the normal range. Plasma fibrinolytic activity showed a fall under the effect of the nephrotoxic serum; it declined further during the pre-nephritic stage and at the onset of nephritis a total absence of fibrinolysis was found. Parallel with the fall in fibrinolytic activity the plasma fibrinogen levels as well as the maximal amplitude of the thrombelastogram, in other words, the maximal elasticity of thrombus, increased (Fig. 2).

Case 2. Rabbit No. 3, 3000 g. As Figure 3 shows, no clinically significant thrombocytopenia was demonstrable until the onset of nephritis. Hyperfibrinogenaemia, absence of fibrinolysis and broadening of the thrombelastogram in the pre-nephritic phase and at the onset of nephritis were demonstrable in this animal, too (Fig. 3).

Table I
Coagulation parameters in delayed

	PTT sec	Recalcification time, sec	Heparin tolerance, sec	Fibrinogen, mg/dl	Fibrinolysis, per cent
Before treat- ment	x S.E.	x S.E.	x S.E.	x S.E.	x S.E.
Group I n = 11	78.4±6.2	78.7±3.8	120.8±5.7	238.2±11.5	14.5±0.8
Group II n = 6	74.1±6.2	87.3±5.9	121.0±5.2	268.3±18.3	15.5±0.9
Group III n = 7	80.7±6.0	79.6±6.6	135.2±15.7	257.1±17.9	13.6±0.8
Thirty min- utes after treatment	Δx S.E.	Δx S.E.	Δx S.E.	Δx S.E.	Δx S.E.
Group I n = 11	+1.6±8.9 p>0.05	+8.1±4.7 p>0.05	+0.6±9.1 p>0.05	+30.3±17.0 p>0.05	+1.0±4.5 p>0.8
Group II n = 6	-15.8±10.5 p>0.05	-2.0±11.8 p>0.05	-10.0±14.9 p>0.05	-8.3±38.4 p>0.05	+10.2±4.0 p>0.05
Group III n = 7	-11.5±7.2 p>0.05	-1.7±8.9 p>0.05	+7.0±6.0 p>0.05	+14.3±9.0 p>0.05	+0.7±1.7 p>0.6
Four days after treat- ment	Δx S.E.	Δx S.E.	Δx S.E.	Δx S.E.	Δx S.E.
Group I n = 11	+12.9±9.3 p>0.05	-2.1±5.7 p>0.05	+5.1±9.0 p>0.05	+88.2±24.6 p<0.01	-12.6±1.4 p<0.001
Group II n = 6	+6.3±14.6 p>0.05	-14.5±7.9 p>0.05	+0.6±19.6 p>0.05	+16.7±19.4 p>0.4	+0.8±5.0 p>0.8
Group III n = 7	+1.7±14.9 p>0.05	-3.2±11.0 p>0.05	+9.5±30.4 p>0.05	+2.9±11.0 p>0.8	-0.9±2.4 p>0.7
At onset of nephritis	Δx S.E.	Δx S.E.	Δx S.E.	Δx S.E.	Δx S.E.
Group I n = 11	+12.2±15.4 p>0.05	+3.1±5.4 p>0.05	+13.8±14.6 p>0.05	+175.5±21.0 p<0.001	-14.5±0.8 p<0.001
Group II n = 6	-21.3±9.8 p>0.05	-4.5±10.5 p>0.05	+6.0±11.1 p>0.05	+40.0±16.5 p>0.05	-3.5±3.0 p>0.05
Group III n = 7	-18.2±5.5 p>0.05	+2.2±7.8 p>0.05	-7.6±22.0 p>0.05	+22.9±19.7 p>0.05	+3.3±3.0 p>0.6

Group I: rabbits treated with nephrotoxic duck serum

Group II: rabbits treated with normal duck serum

Group III: rabbits treated with physiological saline

type Masugi nephritis

Platelet count $\times 10^9/\mu\text{l}$	Platelet aggregation, sec	Platelet factor-3, sec	Thrombelastogram		
			r time, sec	k time, sec	mc
x S.E.	x S.E.	x S.E.	x S.E.	x S.E.	x S.E.
364.0 \pm 12.4	35.6 \pm 1.8	55.1 \pm 6.0	160.1 \pm 14.0	95.2 \pm 13.9	147.8 \pm 8.7
357.5 \pm 24.1	40.9 \pm 4.6	55.1 \pm 5.9	182.5 \pm 26.1	66.2 \pm 5.6	178.9 \pm 10.1
351.4 \pm 14.0	36.4 \pm 2.1	55.3 \pm 4.7	167.7 \pm 18.3	90.0 \pm 19.9	147.9 \pm 12.3
Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.
-157.7 \pm 23.5 p<0.001	-3.3 \pm 4.0 p>0.05	-9.0 \pm 7.3 p>0.05	+13.6 \pm 18.6 p>0.05	+2.2 \pm 13.7 p>0.05	-10.2 \pm 13.6 p>0.4
-55.8 \pm 47.0 p>0.2	-12.2 \pm 6.5 p>0.05	-22.4 \pm 7.4 p<0.05	-34.2 \pm 22.6 p>0.05	+29.5 \pm 12.3 p>0.05	-21.5 \pm 24.9 p>0.4
+30.0 \pm 14.0 p>0.05	-7.7 \pm 3.1 p<0.05	-15.2 \pm 7.0 p>0.05	-17.5 \pm 22.4 p>0.05	+15.0 \pm 30.7 p>0.05	+13.4 \pm 17.4 p>0.4
Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.
-39.6 \pm 22.3 p>0.1	-13.3 \pm 1.5 p<0.001	-19.7 \pm 7.1 p<0.05	-9.6 \pm 19.9 p>0.05	-23.8 \pm 12.6 p>0.05	+57.5 \pm 16.2 p<0.01
-24.2 \pm 31.6 p>0.4	-7.2 \pm 5.6 p>0.05	-11.8 \pm 12.4 p>0.05	-29.0 \pm 16.0 p>0.05	+3.7 \pm 13.6 p>0.05	+8.5 \pm 16.6 p>0.6
+24.3 \pm 29.0 p>0.4	-5.1 \pm 1.7 p<0.05	-15.3 \pm 6.0 p<0.05	-51.0 \pm 24.8 p>0.05	-15.0 \pm 12.7 p>0.05	+34.9 \pm 14.9 p>0.05
Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.	Δ x S.E.
-78.6 \pm 28.8 p<0.05	-13.9 \pm 1.0 p<0.001	-17.1 \pm 7.3 p<0.05	-16.1 \pm 19.5 p>0.05	-31.5 \pm 16.7 p>0.05	+82.9 \pm 15.4 p<0.001
-10.8 \pm 38.0 p>0.7	-3.9 \pm 3.5 p>0.05	-19.5 \pm 7.3 p<0.05	+7.4 \pm 54.0 p>0.05	+36.2 \pm 16.7 p>0.05	-12.2 \pm 7.0 p>0.1
-5.7 \pm 25.0 p>0.8	-0.4 \pm 4.4 p>0.05	-19.7 \pm 7.8 p<0.05	+8.5 \pm 30.5 p>0.05	+10.7 \pm 12.2 p>0.05	+8.0 \pm 11.8 p>0.5

x: mean

S.E.: Standard error

 Δ x: Changes referred to initial values

Case 3. Rabbit No. 1, 2400 g. A significant fall in the platelet count was demonstrable 30 min after administration of the nephrotoxic serum as well as at the onset of nephritis. In this only case fibrinolysis was enhanced in the early stage and the TEG was also indicative of hypo-coagulability. Subsequently, however, fibrinolytic activity declined and ceased altogether, and a broadening of the TEG ensued (Fig. 4).

Microscopic study. In the rabbits with experimental nephritis the glomeruli revealed distinct proliferative changes and periglomerular lymphoid cell infiltrations (Fig. 5). In the affected glomeruli, fibrin deposits were revealed by Weigert staining (Fig. 6). The kidneys of the control animals remained unaffected.

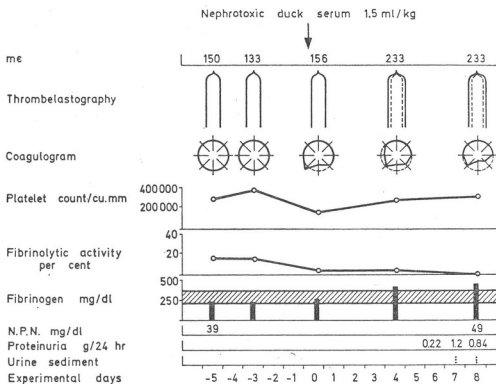


Fig. 2. Rabbit No. 20. Initial thrombocytopenia. During the prenephritic stage and at the onset of nephritis, the tests were indicative of hypercoagulability. The hatched area corresponds to the normal range. The normal thromboelastogram and coagulogram are represented by an interrupted line

Discussion

MASUGI [32] was the first to demonstrate intraglomerular fibrin thrombi in the kidneys of rats treated with nephrotoxic rabbit serum. HÁMORI [16, 17] and HÁMORI and TOMPA [21], on the evidence of Indian ink storage studies, confirmed indirectly the presence of hypercoagulability in rabbits after treatment with nephrotoxic duck serum. VASSALI and McCLUSKEY [45] pointed out to the role of fibrin deposition in the pathogenesis of endothelial proliferation.

We have confirmed the presence of intraglomerular fibrin deposits, and demonstrated that Masugi nephritis of the delayed type has a fall in the platelet count as its initial manifestation. The prenephritic stage and the onset of

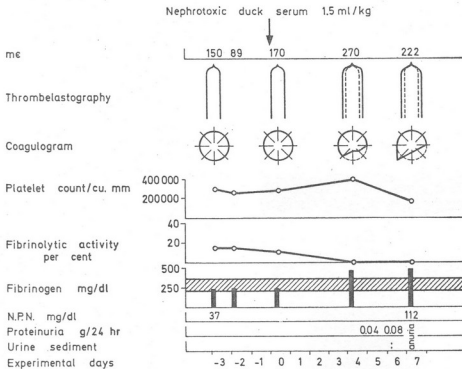


Fig. 3. Rabbit No. 3. Delayed thrombocytopenia

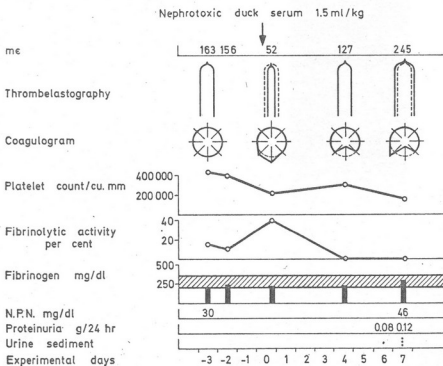


Fig. 4. Rabbit No. 1. Biphasic thrombocytopenia. Fibrinolysis was enhanced at the outset and declined later

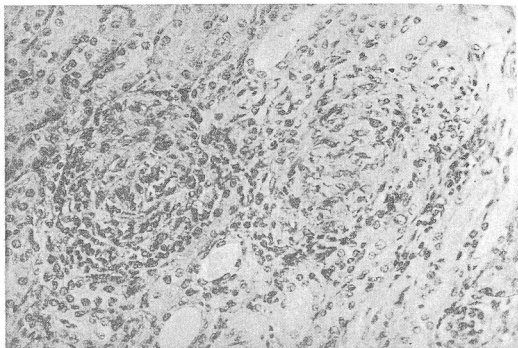


Fig. 5. Kidney of rabbit after treatment with nephrotoxic duck serum. Marked proliferative glomerular changes. Periglomerular lymphoid cell infiltrations. (HE) $\times 160$

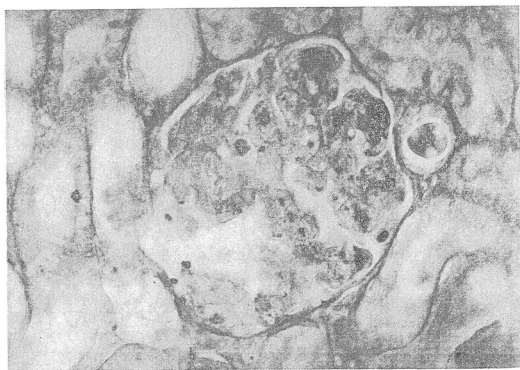


Fig. 6. Glomerulus of a rabbit after treatment with nephrotoxic duck serum. Weigert's stain reveals fibrin deposits. $\times 400$

nephritis are invariably associated with hypercoagulability, as reflected by the elevated plasma fibrinogen level, an increase in maximal thrombus elasticity and a loss of spontaneous fibrinolytic activity. The onset of nephritis is marked by the reappearance of thrombocytopenia.

The coagulation defects described in the foregoing are not confined to Masugi nephritis. Allergic processes have long been known to be associated with a fall in platelet count [43]. It has also been demonstrated that the antigen-antibody reaction increases the coagulability of blood [30, 46]. It was suggested by ROBBINS and STETSON [41] that the essential factor of hypercoagulability associated with the antigen-antibody reaction is provided by the platelets but experimental proof of the antigen-antibody reaction being accompanied by platelet aggregation and by a subsequent release of vasoactive factors has only been furnished in the last decade [10, 22, 23, 25, 35-37]. Today, it is known that the process resulting in the activation of the coagulation system and subsequent production of the blood clot is initiated by platelet changes of morphological and biological nature [2, 26, 38, 44].

The coagulation defects observed in Masugi nephritis may be interpreted as follows. The antigen-antibody reaction elicited by the nephrotoxic duck serum produces an intrarenal platelet aggregation resulting in a fall of the peripheral platelet count. The biphasic thrombocytopenia might be related to the biphasic mechanism proposed by KAY [27], this being alleged to account for the delayed type nephritis. On the evidence of the present findings the heterologous antigen-antibody reaction involves a greater platelet consumption than does the autologous phase. The "release" reaction of the platelets induces an activation of the coagulation system, thus resulting in intraglomerular fibrin production. The hypercoagulability observed *in vitro* is presumably the result of a feedback mechanism consecutive to intravascular coagulation in the kidney.

The present results suggest that experimental glomerulonephritis, similarly to the human disease, has hypercoagulability as one of its cardinal features. Our findings are consistent with the data according to which anticoagulant therapy has a beneficial influence on experimental as well as on clinical nephritis [9, 13, 19, 20, 24, 28, 29, 33, 42, 45].

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