A STUDY OF INTRAVASCULAR COAGULATION IN IMMUNE COMPLEX GLOMERULONEPHRITIS BY USE OF $^{131}$I-LABELLED FIBRINOGEN AND $^{125}$I-LABELLED ANTIGEN

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SUMMARY

1. Intravascular coagulation has been assessed by following the catabolism of $^{131}$I-fibrinogen in relation to $^{125}$I-labelled antigen disposal during anaphylaxis and acute and chronic serum sickness glomerulonephritis in rabbits.

2. As a result of antigen challenge early in the bovine serum albumin (BSA) immunization schedule, in vivo formation of immune complexes caused platelet damage, intravascular coagulation shown as 'rebound defibrination' (that is a transient fall of labelled fibrinogen), and also activation of kinins and of fibrinolysis.

3. 'Rebound defibrination' was associated with precipitating antibody to BSA, with rapid removal of antigen, and with the appearance of fibrin microthrombi in the renal glomeruli. It also appeared to be related to the development of proliferative glomerular histology. Further, inhibition of fibrinolysis before antigen challenge may lead to patchy renal cortical infarction.

4. Florid 'rebound defibrination' occurred in animals with anaphylaxis and was accompanied by obstruction of the vasculature of lungs and kidneys by fibrin. These animals had high levels of precipitating antibody.

5. Animals which developed chronic proteinuria late in immunization had relatively more non-precipitating antibody to BSA and showed slower removal of antigen, no rebound effect and thus normal fibrinogen catabolism. Glomerular histology showed little proliferative response.


Key words: fibrinogen, anaphylaxis, glomerulonephritis, immune complexes, antibody.

In a variety of types of human glomerular disease fibrin may be demonstrated by immuno-
fluorescent techniques. Examples are acute post-streptococcal and subacute and chronic glomerulonephritis (Koffler & Paranetto, 1965; Vassalli & McCluskey, 1965) and systemic lupus nephritis (McCluskey, Vassalli, Gallo & Baldwin, 1966). Fibrin may be found in the kidneys both when antibody is produced against basement membrane, as in experimental Masugi nephritis (Vassalli & McCluskey, 1964a) and when antigen–antibody complexes lodge in the renal filters (Dixon, 1968). The use of anticoagulants has been shown to modify the course of these diseases (Vassalli & McCluskey, 1964b; Halpern, Milliez, Lagruè, Fray & Morand, 1965) and, conversely, when fibrinolysis is inhibited or there is blockade of the reticuloendothelial system (RES), renal cortical necrosis may result (Lee, 1963). Moreover the thrombocytopenia which is a feature of anaphylaxis is a harbinger in some species of intravascular coagulation (Salmon, Lambert & Hiernaux, 1968; Eagle, Johnston & Raudin, 1937; Gans & Krivit, 1961) partly because immune complexes damage platelets but also because large immune precipitates may block the renal and pulmonary circulation (McKinnon, Andrews, Heptinstall & Germuth, 1957; Walters, Frank & Irwin, 1961) before they can be cleared from the circulation by the RES. Soluble complexes have been shown in vitro to induce intravascular coagulation (Robbins & Stetson, 1959; Siqueira & Nelson, 1961) and no doubt do so during serum sickness (Dixon, Vazquez, Weigle & Cochrane, 1958) when during the phase of immune elimination of antigen, fibrin may appear in the glomeruli in a proportion of animals (McCluskey & Vassalli, 1969; Rich, 1956; Germuth, Flanagan & Montenegro, 1957).

This study was undertaken to see how the catabolism of radioactive fibrinogen is altered during the phase of acute bovine serum albumin (BSA) sickness, in which acute glomerulonephritis occurs, and during the phase of chronic immunization to antigen when some animals have a chronic glomerulonephritis; the effect of inhibition of fibrinolysis was also observed. It was also of interest to determine whether intravascular coagulation can be triggered by soluble complexes formed in antigen excess as well as by insoluble complexes produced by antibody excess, and whether the coagulation process can be dissociated from the primary effect on the platelet.

METHODS

General plan

$^{131}$I-labelled fibrinogen catabolism studies were made in rabbits. They were immunized by a single large dose of 300 mg of bovine serum albumin, or 10 ml of Burroughs Wellcome No. 2 horse serum, followed by a challenging dose of BSA (5 mg/kg) or 2 ml of horse serum per kg on the seventh day, which was also arranged to be the fourth day of the fibrinogen study. These were designated ‘acute serum sickness’ animals. Twelve animals with BSA serum sickness and six with horse serum sickness were studied. In addition six animals of each type were maintained on long-term immunization for 16 weeks by intravenous BSA (50 mg/kg) thrice weekly or horse serum (5 ml) thrice weekly. Similar challenging doses were used for serum sickness animals, and they were designated ‘long term immunization’ animals. All of these animals were studied on at least two separate occasions. Two drugs with platelet inhibitory potential were also studied for their modifying effect on anaphylaxis: these were salicylaldoxime at a dose of 25 mg/kg and ‘Persantin’ (dipyridamole) at 5 mg/kg.
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Fibrinogen catabolism

For the study of fibrinogen catabolism rabbits in metabolic cages were injected with 10 mg of rabbit fibrinogen (Baxter Cohn Fraction I), labelled with 30 \( \mu \)Ci of \(^{131}\)I by the iodine monochloride technique. Quantitative urine collections were made and plasma samples were taken for counting of isolated radioactive fibrinogen and free serum iodide so that the decline of intravascular protein bound radioactivity could be plotted and the fibrinogen catabolic rate determined (McFarlane, 1963). The rabbits drank 0.45% saline containing 50 mg of NaI before and during the study. Plasma samples were taken at least twice per day, but after antigen challenge at 10, 20, 60 and 120 min. Fibrinogen was also fractioned into the fraction of low solubility in 8% ethanol (LSF) and the high soluble (HSF) fraction (Sherman, Fletcher & Sherry, 1969) and the total plasma fibrinogen of each sample measured by the method of Ratnoff & Menzie (1951).

The activity of the plasma sample 15 min after the injection of a known amount of labelled fibrinogen was used to calculate the plasma volume. Then, knowing the body weight, plasma fibrinogen and plasma volume, the intravascular fibrinogen pool could first be calculated. The fractional catabolic rate (FCR) of the labelled fibrinogen, which is the percentage of the intravascular fibrinogen pool broken down each day, was calculated by overall resolution of the plasma decay curve into its two exponential components according to the method of Matthews (1957), and on a day-to-day basis by dividing the 24 h urinary radioactivity by the mean plasma radioactivity for that day, as described by Pearson, Veall & Vetter (1958). The absolute catabolic rate for fibrinogen (mg kg body weight\(^{-1}\) day\(^{-1}\)) was derived as the product of the plasma fibrinogen pool and the fractional catabolic rate.

In most experiments the antigen also was labelled with \(^{125}\)I so as to follow the time course of its disappearance, as three exponential components, and also its participation in immune complex formation. Separation of complexes from free unbound antigen was effected by precipitation with 50% ammonium sulphate by the method of Farr (1958). Since the amount of antigen injected was known, it was also possible to quantitate later the organ distribution of antigen.

Combination of appropriate molar concentrations of antigen and antibody will at physico-chemical equivalence produce a maximal precipitate, leaving no unreacting antigen or antibody in solution. On either side of this equivalence zone, soluble antigen-antibody complexes occur. Those soluble complexes formed in antigen excess are those that initiate serum sickness. In contrast those complexes formed in antibody excess are larger, like a branched chain polymer, and therefore are cleared by the RES of the liver and spleen before they can provoke serum sickness (Haurowitz, 1968).

Fibrin degradation products in serum and \( \beta \) 1-C complement after injection of antigen were measured quantitatively by radial diffusion in agar against specific antisera (Biocult Laboratories Ltd) and fibrin monomer by the protamine sulphate turbidity method of Lipinski, Worowski, Jeljaszewicz, Niewiarowski & Rejniack (1968). In additional experiments after injection of antigen the production of kallikrein and kallikrein inhibitor was measured by the method of Colman, Mason & Sherry (1969) and of serum lysozyme by a modification of the method of Harrison, Lunt, Scott & Blainey (1968).

The organ distribution of both \(^{125}\)I-labelled antigen and of \(^{131}\)I-labelled fibrin was studied during the final experiment on each animal. Whole organ slices were counted and the results were expressed as the ‘distribution ratio’ of counts per unit wet weight of an organ compared
with those of standard tissue which was heart muscle. Normal values were found to be less than 2.0 units in kidney and liver and less than 3.0 units in lung. It was also possible to calculate in absolute terms the amount of fibrin in mg/g organ weight and also the amount of antigen in an organ either in μg or as the percentage of the challenging dose. Comparison of these quantitative values was made with histological sections which were stained with Haematoxylin and Eosin and for detection of fibrin with Martius-scarlet blue stain (Lendrum, Fraser, Slidders & Henderson, 1962).

**Coagulation studies**

Some further coagulation studies were also performed as a result of the findings. First, the difference on a modified Stypven time (Brøns & Gormsen, 1968) of adding antigen to antibody-containing platelet-poor plasma as compared with platelet-rich plasma was determined. In this system the coagulation time in seconds was recorded for a mixture of 0.1 ml of saline, 0.1 ml of plasma, 0.1 ml of antigen, 0.1 ml of Stypven (1:20,000) and finally 0.1 ml of 0.05 m-CaCl₂ added successively. The final result depends on the amount of platelet factor 3 activity offered. Platelet factor 3 is a procoagulant phospholipid which is not available in intact circulating platelets but which becomes exposed when platelet aggregation occurs, as a result of a change affecting the platelet surface membrane. Secondly the effect of adding preformed immune complexes on Stypven and plasma recalcification times were determined. Stypven times are used to measure accelerated clotting times in particular platelet factor 3 activities by the addition of a thromboplastin (Russell’s viper venom). Plasma recalcification times are used to indicate the overall time of contact activation of a coagulation system. Thirdly assays were made of in vivo production of platelet factor 4 in plasma after antigen challenge, using the heparinized thromboplastin clotting time described by Farbiszewski, Niewiarowski, Worowski & Lipinski (1968). This measures the heparin-neutralizing factor in platelets known as platelet factor 4. Fourthly, before and after in vivo antigen challenge plasma recalcification times were measured, assay of Hageman factor XII by the method of Mueller-Berghaus & Lasch (1969) and of whole clot fibrinolysis time by the method of Gallimore, Tyler & Shaw (1971).

**Antibody studies**

Sera were also collected before each injection of antigen from each animal for a period of 12 weeks and stored at -20°C. They were later analysed for their antibody content.

Precipitin antibody titres were measured at equivalence using the techniques of Vincent, Harris & Yaverbaum (1970). Protein estimations were made with Folin–Ciocalteau reagent using standards of bovine serum albumin.

Relative proportions of precipitating and non-precipitating antibody were also determined according to the method used by Feinberg (1958) utilizing the principle of Heidelberger & Kendall (1935). 131I-labelled BSA was produced by Chloramine-T iodination and the free iodide separated by dialysis. To 12.5 μg of 131I-labelled BSA in 0.5 ml of borate buffer, pH 8.4, was added 0.2 ml amounts of the antisera. Incubation was carried out for 2 h at 37°C, followed by 2 days at 4°C. The precipitates were then centrifuged, washed with cold phosphate buffered saline and finally dissolved in 0.2 m-NaOH for counting in a Nuclear Chicago gamma counter. To the supernatants sheep anti-rabbit serum gamma globulin was added and the non-precipitating antibody likewise separated for counting. The amount of free BSA remaining was less than 10%.
The antigen binding capacity in this system could also be determined as the percentage of 12.5 μg of labelled BSA bound in the precipitate compared with that using 100 μg of BSA using the Farr technique (1971), as described also by Wei & Stavitsky (1967). In a few experiments (e.g. Fig. 2) BSA was labelled with $^{125}$I rather than $^{131}$I.

RESULTS

Table 1 shows the results that were obtained for the fractional catabolic rate of fibrinogen, i.e. the percentage turnover of the intravascular pool per day, and of the absolute catabolic rate, a value which allows for the body fibrinogen pool, for animals in the phase of acute immune nephritis.

<table>
<thead>
<tr>
<th></th>
<th>Fractional catabolic rate ( % per day)</th>
<th>Absolute catabolic rate (mg kg$^{-1}$ day$^{-1}$)</th>
</tr>
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<tbody>
<tr>
<td>Normal (20)</td>
<td>45±1± 6.1</td>
<td>52±3± 8.8</td>
</tr>
<tr>
<td>BSA short term (5)</td>
<td>49±3± 4.7</td>
<td>86±3± 7.15</td>
</tr>
<tr>
<td>BSA short term (7)</td>
<td>88±4± 28.3</td>
<td>144±3± 63.2</td>
</tr>
<tr>
<td>BSA long term (6)</td>
<td>50±8± 5.6</td>
<td>97±7± 15.1</td>
</tr>
<tr>
<td>BSA long term (6)</td>
<td>83±5± 8.4</td>
<td>178±0± 30.8</td>
</tr>
<tr>
<td>Horse serum short term (6)</td>
<td>62±7± 2.2</td>
<td>104±9± 16.7</td>
</tr>
<tr>
<td>Horse serum long term (4)</td>
<td>48±0± 7.5</td>
<td>69±6± 23.6</td>
</tr>
<tr>
<td>Horse serum long term (4)</td>
<td>76±4± 11.6</td>
<td>173±7± 95.0</td>
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</tbody>
</table>

Fig. 1. The fall of intravascular labelled fibrinogen (●) after antigen challenge, as a percentage reduction of the original value. Animals pre-treated with $\varepsilon$-aminocaproic acid (EACA) are indicated by the open circles.
serum sickness and during long-term immunization with bovine serum albumin and horse serum. The normal half-life of the rabbit fibrinogen was $43.5 \pm 3.6$ h corresponding to a fractional catabolic rate of $45.1 \pm 6.1$ of the intravascular pool per day, and normal absolute catabolic rates of $52.3 \pm 8.8$ mg kg$^{-1}$ day$^{-1}$. The mean fractional and absolute catabolic rates were increased in half of the animals in each of the experimental groups. However, it should be noted that half of the animals in each of the long-term immunization groups had fractional catabolic rates that were within the normal range.

Fig. 1 shows the percentage fall of the intravascular fibrinogen which occurred 10–20 min after antigen challenge, referred to as 'rebound defibrination', a process which is depicted in Fig. 2. This occurred frequently when antigen challenge was made during the short-term serum sickness phase but also after injection of preformed immune complexes made in either antibody or antigen excess. It was also seen in half of the BSA animals during long-term immunization.

![Graph showing the percentage fall of fibrinogen](image)

**Fig. 2.** Decay of plasma labelled fibrinogen before and during two successive episodes of 'rebound defibrination' following antigen challenge, and accompanying changes in other variables. The curved interrupted line represents non-quantitatively the period over which immune complexes are being formed after challenge with $^{125}$I-labelled BSA.

The phenomenon was seen both with immunization to BSA and to horse serum during the phase of antigen challenge, and was associated with a fall of the low soluble fraction of fibrinogen (Fig. 2). On some occasions this fall was greater than the total fall of intravascular fibrinogen, indicating, therefore an overall transformation of fibrinogen to the HSF form. Coincident with 'rebound defibrination' was a fall of the platelet count, and a rise of platelet factor 4 could be shown in the plasma, and also a small and transient fall of complement. Following such an episode the fibrin monomer in the plasma rose and there was thereafter a rise of both fibrinogen and fibrin monomer over several days (Fig. 2).

Even when there was no rebound defibrination, thrombocytopenia and release of platelet
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factor 4 were demonstrable after injection of antigen. Neither dipyrimadole at a dose of 5 mg/kg nor salicylaldoxime at a dose of 25 mg/kg prevented rebound defibrination but neither did they prevent the fall of platelets. Injection of antigen generated release of kallikrein esterase and a lesser rise of kallikrein inhibitor and also a rise of serum lysozyme, even when no effect on coagulation was demonstrable. Typical results are given in Table 2.

<table>
<thead>
<tr>
<th>TABLE 2. Typical kallikrein and lysozyme response to antigen injection</th>
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<tbody>
<tr>
<td>Kallikrein*</td>
</tr>
<tr>
<td>Basal</td>
</tr>
<tr>
<td>10 min</td>
</tr>
<tr>
<td>45 min</td>
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<tr>
<td>90 min</td>
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</tbody>
</table>

* As µ-mol of tosyl arginine methyl ester hydrolysed ml of plasma⁻¹ h⁻¹.

Results of the coagulation studies using immune complexes are shown in Table 3. The points to note are that antigen shortened the Stypven time of rabbit platelet rich plasma containing antibody but not of platelet poor plasma so that a primary effect of immune complexes on the platelet seems essential to the triggering of coagulation. Preformed immune complexes shortened both the Stypven time and the plasma calcification time. The same shortening of the plasma calcification time indicating 'hypercoagulability' was demonstrable in vivo after antigen challenge, together with release of platelet factor 4 causing heparin resistance and activation of fibrinolysis (Table 4).

One relationship that appeared from the in vivo studies in twelve animals immunized long-

<table>
<thead>
<tr>
<th>TABLE 3. Coagulation tests using rabbit plasma. Results are expressed as percentage deviation from control times.</th>
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<tbody>
<tr>
<td>Platelet factor 4 release in vivo as heparin thrombin clotting time</td>
</tr>
<tr>
<td>-------------------------------------------------------------------</td>
</tr>
<tr>
<td>Platelet-poor plasma</td>
</tr>
<tr>
<td>Platelet-poor plasma</td>
</tr>
<tr>
<td>Plasma recalcification time</td>
</tr>
<tr>
<td>-16·0</td>
</tr>
<tr>
<td>-29·5</td>
</tr>
<tr>
<td>-27·0</td>
</tr>
<tr>
<td>-21·0</td>
</tr>
<tr>
<td>-12·0</td>
</tr>
<tr>
<td>Shortened</td>
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</tbody>
</table>
term with BSA was that the half-life of free labelled antigen bore a relation to the half-life of radioactive-fibrinogen (Fig. 3). The clearance of antigen was rapid in those animals with accelerated fibrinogen catabolism and these same animals were those that exhibited ‘rebound defibrination’. They are in fact those animals on long-term BSA immunization whose overall fractional catabolic rate for fibrinogen was increased. The second component half-life of labelled antigen clearance was of the order of less than 2·0 h in animals exhibiting rebound defibrination but in animals with normal fibrinogen catabolism it was 2·5–8·0 h. The clearance of antigen could in fact be resolved into two or three exponential components.

<table>
<thead>
<tr>
<th>Plasma recalcification time (min)</th>
<th>Hageman factor assay (%)</th>
<th>Blood clot lysis time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>4·5</td>
<td>100</td>
</tr>
<tr>
<td>2 min</td>
<td>4·5</td>
<td>50</td>
</tr>
<tr>
<td>5 min</td>
<td>4·0</td>
<td>50</td>
</tr>
<tr>
<td>10 min</td>
<td>4·0</td>
<td>70</td>
</tr>
<tr>
<td>20 min</td>
<td>4·0</td>
<td>50</td>
</tr>
</tbody>
</table>

‘Rebound defibrination’ was the usual result of antigen challenge during short-term acute serum sickness (Fig. 1). The kidneys of these animals showed a moderately intense proliferative glomerular response. Most BSA animals (seven of twelve) at this time showed an increase of the fractional catabolic rate of fibrinogen, i.e. during the period 8–14 days after immunization. At the stage of long-term immunization with antigen the overall fractional catabolic rate of fibrinogen was still increased in half the animals, and, as shown in Fig. 1, these same animals showed rebound defibrination. Kidneys examined at the late stage of 3 months showed only minimal proliferative change of the renal glomeruli of BSA animals although some degree of interstitial nephritis was present, but in the animals immunized with horse serum there was vasculitis with fibrinoid changes in vessels. In fact, at this stage half of the latter animals (four of eight) showed increased fibrinogen catabolism and, as shown in Fig. 4, an advanced stage of vasculitis was associated with greater acceleration of fibrinogen catabolism. This picture is the counterpart of human polyarteritis.

In animals that died of anaphylaxis, histology showed that there was abundant fibrin in the glomerular capillaries and pulmonary vasculature but under ordinary rebound conditions there was very little persisting glomerular fibrin, unless animals were pretreated with EACA at the time of antigen challenge. There was a suggestion of accentuation of rebound defibrination in animals treated with EACA but the treatment very often made little difference to the urine output over the next 24 h. Most animals killed within that time had cleared the fibrin from their glomeruli except for the rare occasion in which inhibition of fibrinolysis produced patchy cortical infarction. This happened in two of seven occasions of fibrinolytic inhibition.

Typical results of counting the organ distribution of labelled fibrin and immune complexes
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FIG. 3. Showing how the relation between labelled fibrinogen half-life and the half-life of clearance of antigen segregates two groups of animals (● and ○).

FIG. 4. The effect on fibrinogen catabolism of antigen challenge (‖) during the second (-----) and seventh (-- – –) weeks of immunization to horse serum: vasculitis was present by the seventh week.
are depicted in Fig. 5. In one animal complexes formed in antibody excess localized in both kidney and lung with an appreciable deposition of fibrin in the lungs. On the other hand, injection of antigen to form complexes in antigen excess in the six long-term immunization animals caused increased localization in the kidney in four of the animals and the kidney was also a site for excess fibrin deposition (three of six cases), although it could also occur in liver or lung. Fig. 5 shows that the animal with rebound defibrination was the animal with the greatest deposition of fibrin in the kidneys. Absolute quantitation of the localization of antigen revealed that an average of 0.18% of the injected dose was found in the kidneys whereas some 31.0% was located in the liver.

Complete sera over a 3 month period were available for antibody analysis on only four animals, one of which had repeated attacks of anaphylaxis to antigen challenge and the other three were from animals which showed no reactions but had chronic proteinuria. Not only were anti-BSA titres higher in animals which early in immunization developed anaphylaxis (30–150 μg anti-BSA/ml) but also, as shown in Fig. 6 and Table 4, this type of animal had a preponderance of precipitating antibody. Fig. 7 shows, on the other hand, results from an animal with poor antibody titre (mainly of the order of 20 μg/ml) which had relatively more non-precipitating antibody. Sporadic sera were also available (Fig. 6 and Table 4) on another eight animals, two of which showed anaphylaxis, and it is of interest that these were two of three animals which had more precipitating than non-precipitating antibody. It is evident that anaphylaxis was associated with the presence of precipitating antibody but that relatively
more non-precipitating antibody appeared in those with long-term proteinuria. In turn the interpretation of Fig. 3 seems to be that the rebound phenomenon and overall acceleration of fibrinogen catabolism was the feature of those animals that can clear antigen rapidly because of precipitating antibody.

**TABLE 5.** Quantitative antibody results (µg/ml) for long-term BSA animals (the same data is presented in a different way in Fig. 6)

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
<th>2</th>
<th>3*</th>
<th>4</th>
<th>5*</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitating antibody</td>
<td>15.4</td>
<td>1.8</td>
<td>24.0</td>
<td>8.0</td>
<td>24.0</td>
<td>25.0</td>
<td>21.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Non-precipitating antibody</td>
<td>24.0</td>
<td>28.0</td>
<td>12.0</td>
<td>11.0</td>
<td>10.5</td>
<td>9.5</td>
<td>8.5</td>
<td>15.0</td>
</tr>
<tr>
<td>Total</td>
<td>39.4</td>
<td>29.8</td>
<td>36.0</td>
<td>19.0</td>
<td>34.5</td>
<td>34.5</td>
<td>29.5</td>
<td>17.5</td>
</tr>
</tbody>
</table>

* Animals with anaphylaxis.

**DISCUSSION**

The results show that platelet damage resulting in thrombocytopenia and release of platelet factor 4, and very often a procoagulant action causing 'rebound defibrination' with conversion of low soluble fibrinogen (LSF) to high soluble fibrinogen (HSF), is initiated by immune complexes produced *in vivo* by injection of antigen into the immunized animal. The action of immune complexes is not only on platelets but also via Hageman factor activation of coagulation. It is noteworthy that a febrile episode *per se* does not cause any alteration of the fibrinogen
catabolic rate (Regoeczi, Herley, Holloway & McFarlane, 1963). Whether or not there is
defibrination, there is also evidence of activation of kinins, and a rise of serum lysozyme which
suggests the involvement of polymorph leucocytes. The findings also indicate that neither
dipyridamole nor salicylaldoxime prevent this action of immune complexes on platelets. Co-
incident with 'rebound defibrination', fibrin appears in the renal glomeruli and these functional

![Graph](image)

FIG. 7. Precipitating (hatched) and non-precipitating antibody to BSA over 14 weeks in one animal,
which after the eighth week had chronic proteinuria.

studies explain the morphological description of fibrin in acute serum sickness glomerulo-
nephritis by Salmon et al. (1968). They also explain findings in thromboelastographic studies

This defibrination in glomerulonephritis must be seen as a sequel to a chain of events in
which immune complexes initiate platelet clumping (Henson & Cochrane, 1969; Mustard,
Evans, Packham & Nishizowa, 1969) with release of vasoactive amines, followed by local
fixation of the complexes with complement and attraction of polymorphonuclear leucocytes
(Cochrane & Dixon, 1968). The consistent appearance of fibrin in the rabbit is in part a
reflection of that animals low plasminogen activator and high antiplasmin levels, but unless
fibrinolysis is further inhibited the deposition appears to be transient and does not impede urine
output. Fibrin deposition is mainly a secondary process and may only be expected to make a
significant contribution to oliguria when there is already gross immunological damage to the
glomerulus, or when the circulating complexes have a particular propensity for inducing
coaugulation, as could be the case with streptococcal immune complexes (Humair, Potter &
Kwaan, 1969). That fibrinolytic inhibition could produce patchy renal cortical infarction in
two of seven animals is, however, highly relevant to the understanding of the acute renal
failure syndrome of rapidly progressive glomerulonephritis. Amounts of fibrin in the kidney
short of that needed to induce anuria are frequently found and do contribute to the picture of
glomerulonephritis, as phagocytosis of fibrin induces proliferation of endothelial cells. At the
same time there is an increase of fibrinolytic activity in the glomeruli (Humair et al., 1969), as
indeed of whole body blood clot fibrinolysis, and this explains why fibrin deposition is only
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Localization of preformed large complexes formed in antibody excess was principally in the lung, accompanied by fibrin deposition, and the combination usually resulted in death from obstruction of the pulmonary vasculature. On the other hand, soluble complexes were concentrated in the kidney. When there were high levels of serum antibody, death often occurred by anaphylaxis and when this happened in the rabbit much fibrin was seen in the lungs and kidney. It is known that inhibition of fibrinolysis by EACA does not affect the lethal outcome of anaphylaxis (Gans & Krivit, 1961) which is principally a pharmacological effect. Some of the thrombi found in anaphylaxis are primary antigen–antibody precipitates (Dixon, 1953; Walters et al., 1961) and it seems that fibrin deposition was probably secondary to obstruction of the vasculature.

The main point to be emphasized here is that precipitating antibody to BSA resulted in the formation of complexes that were rapidly cleared from the circulation and at the same time there was intravascular coagulation. The slower clearance of antigen which occurred when the animal had non-precipitating antibody was apparently not associated with accelerated fibrinogen catabolism (Fig. 3).

This finding may well be relevant to the interpretation of the histology. Acute serum sickness due to BSA produced a good proliferative response (Germuth, 1953) and with horse serum a vasculitis (Rich & Gregory, 1943). However, long-term immunization with BSA produced only mild glomerular changes, although there was tubulo-interstitial nephritis. A similar finding was indeed noted by Boyns & Hardwicke (1968) in this same experimental situation in which repeated injection of antigen was designed to maintain the perpetuation of complexes in the circulation. There is a possibility that the presence of too much antigen could result in dissolution of complexes from the glomeruli (Valdes, Sterterfit, Pollack & Germuth, 1969). Dixon, Feldman & Vaquez (1961) in their classical studies found that very poor and good antibody producers do not develop progressive renal disease and those animals that do develop such disease produce moderate amounts of precipitating antibody. Pincus, Haberkern & Christian (1968) clarified the situation by showing that production of non-precipitating antibody correlated with the development of chronic serum sickness, but Dixon has stressed that some precipitating antibody, forming complexes of greater than 19S size, is essential for the development of proteinuria (Wilson & Dixon, 1971). It has also been shown that non-precipitating antibody can be identified by the fact that, whereas precipitating antibody will clear labelled antigen from the circulation with great rapidity, non-precipitating antibody clears labelled BSA much more slowly (Christian, 1969). It was with this principle in mind that labelled antigen was used for the in vivo interpretations of these studies. Herein lies the probable explanation of the data of Fig. 3, namely that precipitating antibody cleared antigen rapidly from the circulation and that such complexes caused rebound defibrination. It seems, however, that coagulation was in part secondary to the formation of immune precipitates in the microcirculation, because the in vitro tests show that all types of complexes exert some procoagulant effect via a primary action on the platelet. On the other hand, slower immune elimination by non-precipitating antibody soluble complexes was not associated with accelerated fibrinogen catabolism. This means that the quality of the antibody is also a determinant of intravascular
coagulation. Indeed this is confirmed by the antibody studies which show that the type of animal which had rebound defibrination and even anaphylaxis was one with large amounts of precipitating antibody in the circulation. Conversely, the long-term immunization animals, were animals which had less precipitating antibody but greater amounts of non-precipitating antibody. Proliferation in the glomeruli of these animals was minimal. The indications are that non-precipitating antibody results from a limited recognition of antigenic determinants on complex antigens (Christian, 1970). How this relates to antibody avidity or affinity (Siskind & Benacerraf, 1969) is a challenge for future investigation.

The finding that it is precipitating antibody that is associated with manifest intravascular coagulation adds another facet to knowledge of the biological behaviour of complexes. It has recently been shown that complexes that are rapidly cleared by precipitating antibody are also those that fix complement (Marnik, Arend, Hall & Gilliland, 1971). Since it is known that fibrin deposition in the glomeruli is a stimulus to the proliferative response, the data suggest that this in turn depends on the relative amounts of precipitating and non-precipitating antibody.

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Coagulation in immune nephritis


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