# FIBRINOLYSIS FOLLOWING ELECTRICALLY INDUCED CONVULSIONS

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The application in this hospital of alternating current for electro-convulsive therapy (E.C.T.) and electronarcosis in certain mental disturbances afforded the opportunity of investigating the effects of this form of therapy on some of the components of the blood.

Schütz (1942) observed *in vitro* a delayed coagulation of blood following application of a weak direct current.

Brecht and Kummer (1943) noted a leucocytosis immediately after electrical shock; there was no alteration of blood sugar or protein.

Spiegel-Adolf, Spiegel *et al.* (1945) reported that following the application of a 60 cycle current at 30 volts for 1·2 seconds there were changes in cerebrospinal fluid suggesting the breakdown of nuclear substances. This statement, however, has since been contradicted by M. H. Hack (1947). Huddleson (1946) observed haemorrhages in the central nervous system in rats following electrical shock. Trojaniello (1947) indicated that dogs subjected to electrical shock by alternating current at 120 volts for a fraction of a second, showed no changes in blood lipase and diastase, but that phosphatase activity decreased for about 12 hours. Delay and Soulairac (1943) observed, following E.C.T. in human subjects, a transient increase in blood albumin, and occasionally a slight increase in the globulin fraction. There was a concomitant increase of blood calcium and inorganic phosphate. Further, hyperglycemia, leucocytosis, and a decrease in the alkali reserve were demonstrable.

## EXPERIMENTAL.

Electrical convulsions were induced in suitable patients by alternating current (50 cycles) at 120 volts. Three to eight shocks, each of a duration of 0·1 seconds, were given. Blood samples were obtained by venepuncture before and after application of the current and mixed with one-ninth volume of 0·1 M sodium oxalate solution. The ratio of plasma: cell volume was determined in tubes of uniform size and shape and centrifugation was carried out under identical conditions, which should ensure comparable results, if not absolute values.

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TABLE 1.

*Influence of electrically induced shock on plasma cell ratio.*

(Figures are given for plasma volume per 100 ml. of whole blood, and are corrected for oxalate addition).

| | Before shock. | 1–3 mins. after shock. | Change. |
|---|---|---|---|
| 1 | 47·1 | 45·7 | —1·4 |
| 2 | 52·8 | 50·8 | —2·0 |
| 3 | 50·0 | 47·8 | —2·2 |
| 4 | 54·6 | 51·2 | —3·4 |
| 5 | 51·7 | 50·0 | —1·7 |
| 6 | 44·3 | 44·3 | 0·0 |
| 7 | 48·2 | 47·7 | —0·5 |
| 8 | 50·0 | 47·4 | —2·6 |
| 9 | 48·9 | 44·8 | —4·1 |
| 10 | 54·0 | 52·9 | —1·1 |

The results indicate that the convulsions are followed by a slight haemo-concentration.

*The Effect of E.C.T. on Plasma Proteins.*

The estimations of total protein in oxalated plasma were carried out by a biuret technique similar to that given by Fantl and Nance (1947). Fibrinogen was determined by using 1 ml. of plasma to which were added 25 ml. of 0·15 M sodium chloride and 1 ml. of 0·25 M calcium chloride solution. After standing for 2–3 hours at 20° C. the fibrin was centrifuged and washed with saline until free of soluble proteins, and the estimations carried out by the biuret reaction.

TABLE 2.

*Influence of electrical shock on concentration of plasma proteins.*

| No. | Before shock | After shock | P.c. change |
|---|---|---|---|
| 1 | 1,250 | 1,309 | +4·7 |
| 2 | — | 1,460 immediately after shock<br>1,351 40 min. after shock | +8·0 |
| 3 | 1,232 | 1,322 | +7·0 |
| 4 | 1,257 | 1,327 | +5·5 |
| 5 | 1,040 | 1,090 | +5·0 |
| 6 | 1,155 | 1,172 | +2·0 |
| 7 | 1,037 | 1,080 | +4·0 |
| 8 | 1,275 | 1,375 | +7·5 |
| 9 | 1,170 | 1,280 | +8·5 |
| 10 | 1,148 | 1,172 | +2·2 |

The figures represent mg. protein nitrogen per 100 ml. plasma, and are corrected for oxalate addition. They show a definite hyperproteinaemia following E.C.T.

Estimations of fibrinogen in the pre- and post-shock period did not show any variation greater than the limit of error of the technique, which is ± 3 p.c.

Prothrombin estimations carried out in whole plasma according to Quick's technique (1938) did not indicate any change in prothrombin activity when compared to the pre-shock level.

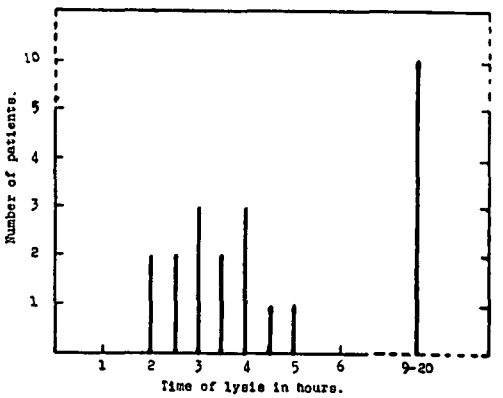


**Fig. 1.** Time of lysis of fibrin clot.

*Effect of E.C.T. on Fibrinolysis.*

The fibrinolytic activity of the plasma was determined by the following technique: to 0·1 ml. of oxalated plasma were added 2·4 ml. of 0·15 M sodium chloride containing 1 × 10–4 merthiolate, and 0·1 ml. of 0·25 M calcium chloride. The specimens were incubated at 37° C. and the time for complete disappearance of fibrin was determined. For the quantitative estimations 10 times greater volumes were used.

Dissolution of fibrin occurred in 24 out of 25 cases when blood was taken 1–3 minutes after convulsions. In the case in which no fibrinolysis was observed the patient obtained, 4 hours prior to E.C.T., 80 units of insulin. However, on repetition without pre-treatment with insulin, fibrinolysis in this patient occurred in 4 hours. Other patients obtaining insulin prior to E.C.T. showed fibrinolysis. As shown in Fig. 1, the time of fibrinolysis varied from 2 hours to approximately 19 hours. In some cases of extreme anxiety in the pre-shock state fibrinolysis occurred, and if it was of a minor degree it was enhanced following E.C.T. The phenomenon of fibrinolysis is only transient, since on several occasions in blood samples taken 40 minutes after application of the current no fibrinolysis could be observed whilst in blood samples taken 1 minute after the shock fibrinolysis occurred in 2 hours.

TABLE 4.

| No. of experiment | Fibrinolysis in hours | Fibrinogen N concentration in fresh plasma | Fibrinogen N concentration in plasma incubated at 37° C. |
|---|---|---|---|
| 1 | 3 | 44·7 | 43·0 |
| 2 | 18 | 49·0 | 47·0 |
| 3 | 18 | 67·2 | 68·5 |
| 4 | 18 | 60·3 | 66·0 |
| 5 | 3 | 54·1 | 50·0* |
| 6 | 2¼ | 56·7 | 55·0* |

Figures are given in mg. p.c. fibrinogen nitrogen, and are corrected for the addition of oxalate.

Experiments 5 and 6 were carried out on plasma from which prothrombin had been removed by alumina adsorption, and fibrin was produced by addition of thrombin.

In order to find out whether the proteolysis was confined to fibrin, oxalated plasma was incubated without calcium ions for 12 hours longer than was required for complete fibrinolysis in the presence of Ca ions. When to the former specimens calcium chloride was now added, fibrin formation took place, and an estimation indicated a complete recovery of fibrinogen, as can be seen from Table 4.

From these results it is evident that, in contrast to complete fibrin breakdown, fibrinogen was not affected.

The fibrinolytic enzyme is inactivated during prolonged incubation at 37° C. because fibrin produced by recalcification of oxalated plasma after 24 hours' incubation took considerably longer to disappear than it did when fresh plasma specimens were investigated.

In further experiments it could be shown that oxalate is not an inhibitor of this system, because following the addition of thrombin without calcium ions, fibrinolysis occurred in the same period as in the presence of calcium ions. In addition, it appears that calcium ions are not essential for fibrinolysis. Experiments were carried out in which oxalated plasma was rendered noncoagulable by treatment with Cγ alumina gel, and the filtrates incubated following the addition of calcium chloride for 24 hours. When thrombin was added fibrin was recovered in the expected amount (experiments 5 and 6 in Table 4). Incubation of this thrombin induced clot led to fibrinolysis.[2] However, the time for complete fibrin disappearance was longer in such specimens than in ones recalcified without alumina treatment because fibrinolysin was partly inactivated during the previous incubation period.

TABLE 5.

*Fibrinolysis in hours in active plasma diluted with normal plasma.*

| | Undiluted plasma | Diluted to 50 p.c. with normal plasma | Diluted to 20 p.c. with normal plasma | Diluted to 10 p.c. with normal plasma |
|---|---|---|---|---|
| 1 | 4 | 21 | | |
| 2 | 4½ | 21 | | |
| 3 | 21 | 21 | | |
| 4 | 3 | 18* | | |
| 5 | 18 | 18* | | |
| 6 | 2¼ | 3 | | |
| 7 | 4 | 21 | 21 | ∞ |
| 8 | 4 | 21 | 21 | ∞ |
| 9 | 3½ | 20 | 20 | 20 |
| 10 | <18 | 18 | | |
| 11 | 2½ | 3 | | |
| 12 | 5 | 22 | 26 | |

* Plasma diluted with rabbit's plasma.

Although from these experiments it appears that only fibrin is attacked by the enzyme produced during electrically induced convulsions, it can be shown that a plasma possessing active fibrinolysin is able to break down additional amounts of fibrin produced by addition of normal plasma. The breakdown is not confined to homologous fibrin, since fibrin produced from rabbit's plasma was also attacked.

2 The experiments have been carried out with human and commercial beef thrombin preparations; however, both occasionally contained fibrinolysin, whose absence was established prior to the experiment.

pH activity measurements carried out in veronal buffer (Michaelis) indicated dependence of pH optimum on the enzyme concentration. In an experiment in which lysis occurred in 2 hours optimal activity was found between pH 6·8–7·2, whilst in another case showing fibrinolytic activity after 9 hours, the optimal activity was between 6·6 and 7·0.

It should be pointed out that fibrinolysis could, in the majority of cases, be observed only in plasma diluted 25 times, whilst clots produced in whole blood did not show lysis when observed for much longer periods, indicating that the undiluted plasma contained inhibitors in amounts sufficient to prevent fibrinolysis. The presence of inhibitors could be demonstrated by comparing fibrinolysis in diluted plasma with that in the globulin fraction obtained by ammonium sulphate precipitation.

TABLE 6.

*Comparison of fibrinolysis in diluted plasma and the isolated globulin fraction.*

| Experiment | Lysis in diluted plasma in hours | | Lysis in globulin fraction in hours | |
|---|---|---|---|---|
| | Fibrin | Fibrinogen | Fibrin | Fibrinogen |
| 1 | 19 | no lysis in 20 hours | 3 | no lysis in 20 hours |
| 2 | 9 | no lysis in 20 hours | 3 | no lysis in 20 hours |
| 3 | 19 | no lysis in 20 hours | 4½ | no lysis in 20 hours |
| 4 | ∞ | no lysis in 20 hours | 24 | no lysis in 20 hours |
| 5 | ∞ | no lysis in 20 hours | ∞ | no lysis in 20 hours |

The globulin fraction was isolated by addition of 2 ml. of 50 p.c. saturated ammonium sulphate solution to 1 ml. of oxalated plasma, (Christensen, 1946). The precipitate obtained after centrifugation was dissolved in 1 ml. of 0·15 M sodium chloride containing 0·01 M sodium oxalate.

Experiments 1, 2 and 3 are specimens of plasma after E.C.T., 4 and 5 are normal specimens.

It is apparent from these results that even in the absence of inhibitor the process is confined to the breakdown of fibrin, since fibrinogen was present after a prolonged incubation period.

The most probable explanation for the observed phenomenon is that the electric current induces activation of an inactive precursor of the fibrinolytic enzyme.

Investigation of the mechanism of proteolysis indicated that fibrin was certainly not broken down beyond the stage of polypeptides, because protein estimations carried out on trichloracetic acid precipitates, and also on fractions obtained by ammonium sulphate precipitation before and after incubation of specimens which showed fibrinolysis, did not give any significantly different results.

The peculiar behaviour of the fibrinolytic enzyme produced following E.C.T. made a comparison with other proteolytic enzymes desirable. A commercial trypsin preparation and fibrinolysin made by chloroform treatment of human serum according to Loomis, George and Rider (1947) were used.

The trypsin experiments were carried out in the case of plasmas 1–4 with 0·1 ml. of a 1 p.c. solution of trypsin, 0·1 ml. of oxalated plasma, 2·4 ml. of veronal buffer pH 7·9. In the case of plasma 5, 0·2 ml. of 1·5 p.c. trypsin solution was used.

TABLE 7.

*Comparison between the action of trypsin and chloroform-fibrinolysin on human oxalated plasma.*

| Plasma specimen | Trypsin | | Plasma specimen | Chloroform-fibrinolysin | |
|---|---|---|---|---|---|
| | Fibrin | Fibrinogen | | Fibrin | Fibrinogen |
| 1 | 200 | <150 | 6 | 40 | >40 |
| 2 | 200 | <150 | 7 | 85 | >140 |
| 3 | 250 | 130 | 8 | 64 | 89 |
| 4 | 260 | 180 | 9 | 72 | 89 |
| 5 | <20 | 0·5 | | | |

Results indicate disappearance of substrates in minutes.

The fibrinolysin experiments were performed with 0·1 ml. of plasma, 1·0 ml. of the fibrinolysin preparation, 1·4 ml. veronal buffer. The pH of the reaction mixture was 6·7.

Experiments 6 and 7 were performed with a different fibrinolysin preparation to experiments 8 and 9.

Columns "Fibrin" indicate incubation at 37° C. with the addition of 0·1 ml. of 0·25 M calcium chloride. Columns "Fibrinogen" indicate incubation without calcium ions. Tests for the presence of fibrinogen were carried out with beef thrombin and calcium chloride.

From the results given in Table 7 it will be observed that trypsin attacked fibrinogen as well as fibrin; the former, however, was broken down at a faster rate than the latter. This phenomenon is in marked contrast to the E.C.T. induced fibrinolysis. Further, the fibrinolysin prepared by chloroform activation attacked fibrin at a slightly faster rate than fibrinogen.

The tryptic digestion of fibrin is also influenced by inhibitors present in plasma, since isolated fibrin is readily attacked by trypsin in a concentration which has no influence on fibrin produced by recalcification of whole plasma. From these results it is evident that the enzyme responsible for fibrinolysis in electrical shock is not identical with trypsin.

Convulsions were produced in four calves submitted to 3–8 shocks, each of a duration of 0·1 sec. at 180 volts. Fibrinolysis was not demonstrable in diluted plasma nor in the isolated globulin fraction. This negative result is unexpected since it has been reported by Loomis, George and Ryder (1947) that a globulin fraction from beef plasma can be converted by chloroform treatment into fibrinolysin, and Permin (1947) found that this activation can also be carried out by tissue fibrinokinase.

## DISCUSSION.

The phenomenon of fibrinolysis, that is, the disappearance of a visible fibrin clot, in whole blood or plasma, has been observed by a number of workers in a variety of pathological and experimental conditions.

Tagnon *et al.* (1946) found fibrinolysis in shock due to severe haemorrhages or burns. This was observed in whole blood or moderately diluted plasma. The plasma of dogs subjected to severe bleeding likewise developed marked fibrinolytic properties.

Macfarlane and Biggs (1946) found fibrinolysis immediately after operation in 50 p.c. of patients, and Macfarlane, Biggs and Pilling (1947), on investigation

of this phenomenon, showed that states of anxiety, or injection of adrenaline, produced fibrinolysis. From their work it appears that any condition which would stimulate the sympathetic system would lead to fibrinolysis.

According to Delay and Soulairac (1943) many of the effects of electrical convulsions are like those produced by excitation of the sympathetic nervous system.

Fibrinolysis following adrenaline injection occurs in 24 hours or longer, when diluted plasma is tested. Plasma from patients submitted to E.C.T. showed fibrinolysis in times varying from 2 hours to 19 hours. In common with Macfarlane's observations, the E.C.T. induced fibrinolysis was of a transient nature.

Extensive studies on the protease of human serum by Christensen and MacLeod (1945) indicated that it is present as a precursor called plasminogen which can be activated, among other means, by streptokinase or by treatment with chloroform to the enzyme plasmin. The factor thus obtained differs from trypsin, having a pH optimum of 7·2, and attacking different linkages than trypsin in the protein molecule. Permin (1947) finds the pH optimum 6·0–6·5 for fibrinolysin produced by activation with tissue fibrinokinase. Further, Kaplan (1946) produced evidence that the enzyme obtained from serum by streptokinase activation is not identical with trypsin, because the kinases required for the respective enzyme activities are different. Tagnon *et al.* (1942) and Christensen (1945) observed that fibrinogen and other proteins, as well as fibrin, are attacked with equal velocity and therefore Christensen abandons the previous term fibrinolysin in favour of plasmin.

From the experiments reported here it is, however, quite apparent that plasma obtained shortly after electrically induced convulsions shows only fibrinolytic activity. No action on fibrinogen could be observed. It should be pointed out that the potency of Christensen's preparation was very much greater than was ever obtained in the plasma specimens following the electrical seizure. Although the proteolytic effect is partially inhibited by plasma components, the active fraction can be isolated by ammonium sulphate precipitation free of inhibitor, and yet the preferential breakdown of fibrin still persists. In many respects fibrinogen is more labile than fibrin. It is therefore interesting to note that plasmin in a low concentration should react with the more stable fibrin. Whether the discrepancy between the plasmin activities is due to the different mode of plasminogen activation, or to the difference of enzyme concentration, needs further investigation.

That the enzyme appearing following electrical shock in the plasma is different from trypsin could be shown quite clearly. The latter attacks fibrinogen in preference to fibrin, which is in agreement with observations made by Ferguson *et al.* (1947).

It is very likely that fibrinolysis following electrically induced shock is due to the same stimulus as observed by Macfarlane and co-workers, although no

direct evidence is available at the moment that adrenaline is the factor eliciting plasminogen activation. The breakdown of fibrin by the plasmin concentration obtainable in E.C.T. does not go beyond the polypeptide stage, since no difference in trichloracetic acid precipitable protein could be observed in specimens before and after incubation. This is in line with observations made by Garner and Tillet (1934), who found that the breakdown of fibrin by fibrinolysin produced by streptokinase activation was not very profound, as the degradation products were precipitated by ammonium sulphate, a fact confirmed by Holmberg (1944). Likewise Seegers *et al.* (1945) found that fibrinogen isolated from bovine plasma loses its coagulability when kept under sterile conditions at room temperature, but the breakdown products show, apart from the non-coagulability by thrombin, little difference in other respects from native fibrinogen.

Tagnon *et al.* (1946) reported that severe haemorrhagic shock led, in addition to fibrinolysis, to reduction of prothrombin activity. No influence on the prothrombin level could be observed under our experimental conditions.

It would appear that the low concentration of plasmin produced by sympathetic stimulation is of physiological significance, and this process may serve to remove fibrin when intravascular coagulation takes place, without interfering with the haemostatic mechanism.

## SUMMARY.

Following convulsions induced by alternating current in humans, changes in the composition of blood are a slight haemo-concentration, a moderate increase in plasma proteins, and a transient fibrinolytic activity. The latter is confined to breakdown of fibrin, fibrinogen not being attacked. This phenomenon is in contrast to tryptic action, and is additional evidence that the plasma protease is different from trypsin.

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