Biochemical changes after spontaneous subarachnoid haemorrhage

Part III

Coagulation and lysis with special reference to recurrent haemorrhage

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The coagulation and lysis systems of the blood are being examined as part of our investigation of the biochemical economy of subarachnoid haemorrhage. Alterations in the balance of these related mechanisms might influence the recurrence of haemorrhage or extension of infarction. The introduction of the fibrinolytic inhibitor epsilon amino caproic acid (EACA) in the management of subarachnoid haemorrhage adds further interest to this work.

CASE MATERIAL

Fifty-two cases were investigated, all referred from general hospitals, the presence of blood in the cerebrospinal fluid having been confirmed by lumbar puncture. Twenty-five patients (10 men and 15 women) had suffered an initial period of coma and the remaining 27 (nine men and 18 women) had each sustained a non-coma-producing subarachnoid haemorrhage.

Investigation was started on the third day of the illness in 16 cases, eight patients were first studied on day 2, eight on day 1, and in one case observations began on the day of her haemorrhage, that is, on day 0. Six patients were first treated on day 4, five on day 5 and five on day 6. The remaining six were admitted seven or more days after their haemorrhage. In 27 cases a second sample was obtained, usually 48 hours after the first. Where repeated haemorrhage was suspected investigation was more frequent. At the time of the first sample, 33 patients were fully alert, 15 were drowsy and six remained in coma. Blood samples were taken soon after admission, either before or a minimum of 20 hours after angiography.

Bilateral carotid angiography according to the scheme of McKissock, Richardson, and Walsh (1960) was carried out in 49 cases. Where carotid angiography was negative, vertebral or subclavian angiography was performed after an interval of two days, with the exception of one

woman, aged 64 years, not subjected to this procedure because of her age and benign hypertension. Two of the three cases not examined angiographically died before investigation and the diagnosis was established at necropsy. The third was a 33-year-old man in whom angiography was contraindicated by the presence of malignant hypertension to which his non-coma-producing subarachnoid haemorrhage was attributed.

LABORATORY METHODS

COAGULATION Thromboplastin generation test (Biggs and Douglas, 1953, modified using platelet substitute, Bell and Alton, 1954), recalcification time (Biggs and Macfarlane, 1962), one stage prothrombin time (Douglas, 1962), platelet count (Dacie, 1956). Antihaemophilic globulin (Factor VIII, AHG) and Christmas factor (Factor IX) by a method dependent on the recalcification times of factor VIII and factor IX deficient plasma when mixed in varying dilutions with the test plasma. The system is maximally contact-activated with kaolin and platelet substitute added to the substrate (Douglas, 1965).

FIBRINOLYSIS Dilute whole blood clot lysis time (Fearnley, Balmforth, and Fearnley, 1957), euglobulin lysis time (Nilsson and Olow, 1962), plasminogen assay (Remmert and Cohen, 1949, as described by McNicol and Douglas, 1964), fibrinogen (Ratnoff and Menzie, 1951, as described by McNicol and Douglas, 1964).

LIVER FUNCTION Bilirubin, thymol turbidity, alkaline phosphatase and transaminases (aspartate and alanine amino-transferase) were estimated by standard methods.

RESULTS

Blood samples were obtained from the 52 patients on a total of 93 occasions. With very few exceptions, the full range of tests were made on all specimens.

LIVER FUNCTION, ELECTROLYTES Nothing to account for the coagulation abnormalities was found in the

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routine blood chemistry. All liver function tests were normal. Four patients showed evidence of haemoconcentration: three more had an isolated raised blood urea. In six cases (four with anterior communicating artery aneurysms, one with a middle cerebral artery aneurysm and one case of primary intracerebral haemorrhage) there was a mild to moderate (124-132 m-equiv sodium/l.) degree of hyponatraemia. Five cases were slightly hypokalaemic (2.5-3.0 m-equiv potassium/l.).

THROMBOPLASTIN GENERATION TEST (TGT) Results are expressed in clotting times of substrate plasma at 6 minutes incubation in the test. Plasma and serum from 17 normal adults gave a range of 9-14 seconds with a mean time of 11.4 ± 1.7 seconds; clotting times greater than 14 seconds have therefore been taken to represent a pathological delay and/or deficiency of thromboplastin generation. Twelve of the 52 patients had an abnormal TGT. The results are shown in Fig. 1, where the longest time recorded for each case is plotted in relation to the normal range for the test, source of haemorrhage, and occurrence or not of coma at the time of haemorrhage. An abnormal TGT was more common among patients with a coma-producing haemorrhage, but by the time of sampling five of the eight

patients had returned to full consciousness, two were still markedly drowsy, and one remained in coma. In six women and three men with a prolonged TGT the bleeding came from an aneurysm (six anterior communicating, two posterior communicating, and one middle cerebral). In the remaining three patients, all men, no lesion was demonstrated on angiography, but in one case the presence of an interhemispheric haematoma and the distribution of spasm suggested that the haemorrhage might have come from an anterior communicating artery aneurysm.

In each of the abnormal cases the prolonged TGT was corrected to within normal limits by substitution of fresh normal plasma or serum for patients plasma or serum respectively in the test (Fig. 2). In one patient, with a TGT of 16 seconds on day 3, AHG and Christmas factor assays were performed. The level of AHG was 27% of that of a pool of 10 normal plasmas; Christmas factor was 100%.

Repeated estimations were made in 10 of the cases where the TGT was abnormal. Figure 3 shows these results in relation to the time since the initial haemorrhage and the occurrence of further bleeding; in some cases the TGT had returned to normal by the end of the first week while in others the abnormality was still present.

FIG. 1. Thromboplastin generation after spontaneous subarachnoid haemorrhage in relation to lesion and incidence of coma.

FIG. 2. Corrective effect of fresh normal plasma or serum fractions on the abnormal TGTs.

EUGLOBULIN LYSIS TIME Plasma fibrinolytic activator activity is proportional to the reciprocal of lysis time in this test; the normal range is 60 to 480 minutes (Sawyer, Fletcher, Alkjaersig, and Sherry, 1960). Lysis times of under 60 minutes were observed in six cases, five had prolonged TGTs. In four of these patients no lesion was found on angiography, in one there was a posterior communicating artery aneurysm, and the sixth was the severely affected patient with an anterior communicating artery aneurysm described in detail below. Six patients had euglobulin lysis times greater than 480 minutes and in all these the TGT was normal.

FEARNLEY DILUTE WHOLE BLOOD CLOT LYSIS TIME This test measures blood fibrinolytic activity in the presence of natural inhibitors; the normal range is 120 to 600 minutes (Fearnley *et al.*, 1957). Lysis times of less than 120 minutes were found in two cases, both of whom had prolonged TGTs and abnormally short euglobulin lysis times. In 24 cases the results were normal. Nine patients had lysis times between 600 minutes and 24 hours. Fearnley lysis times of more than 24 hours were found for 15 patients, including the six cases with long euglobulin lysis times.

OTHER RESULTS All recalcification times fell within the normal range of 90–250 seconds and in no instance did the prothrombin time differ from that of the day's control. All plasminogen assays except two were within the normal limits of 2–5 casein units. The abnormal plasminogen levels, of 5.5 and 6.1 units, were not accompanied by increased lytic activity or by changes in fibrinogen. No fibrinogen result lay below the lower limit of 150 mg/100 ml. Fibrinogen levels greater than the upper limit of 500 mg/100 ml. were found in five cases on at least one occasion; the increases, however, were small, the maximum being only 595 mg/100 ml. There were no low platelet counts and only one patient had a count above the normal range of 100,000 to 500,000/cmm.

RESULTS IN RELATION TO RECURRENT ANEURYSMAL HAEMORRHAGE Fourteen of the 33 patients with aneurysms suffered one or more further episodes of haemorrhage. Nine of them had a recurrence in the first week of the illness, four in the second week and one patient re-bled on day 20. Nine of the 12 deaths were associated with recurrent haemorrhage. Table I compares the results of the nine patients with recurrent haemorrhage during days 0–6 with those of the 16 patients who neither re-bled nor were subjected to any surgical treatment

FIG. 3. Serial observations of TGT in 10 of the patients with abnormal tests.

during the same period. Only results of samples taken from the time of admission up to and including day 6 are considered. Where multiple samples were obtained from a patient the longest TGT and recalcification time and the shortest lysis time has been used. Fearnley lysis tests were not observed beyond 24 hours; where the clot had not lysed in this period the time has been taken as 24 hours. Both the mean TGT and the mean recalcification time are longer for the group of cases who re-bled than for those who did not and the difference, by Student's *t* test, is statistically significant. For both the lysis tests, the mean time was shorter for the group who re-bled, but the scatter of results is very wide and the difference between the groups is not statistically significant.

Table II summarizes the results from the patient most severely affected—a woman of 49 admitted, conscious but mildly confused, on day 2 after a coma-producing subarachnoid haemorrhage. Her blood pressure was 160/90 mm Hg, there was neck stiffness and no abnormal neurological signs. Lumbar puncture at the referring hospital had produced evenly blood-stained cerebrospinal fluid at a pressure of 200 mm/H₂O. Next day she became more confused and disoriented and developed a 6th nerve palsy and increased neck stiffness and her aneurysm was thought to have bled again. Bilateral carotid angiograms showed a small berry aneurysm, pointing forwards and slightly upwards, arising

from the junction of the right anterior cerebral and anterior communicating arteries without any accompanying spasm or haematoma. Bilateral subclavian angiography, on day 6, was normal. On day 8 the aneurysm was exposed via a right frontal craniotomy and amputation of the tip of the frontal pole. The lesion was considered to be suitable for wrapping with muslin. The anterior aspect, particularly the area which appeared to have been the site of rupture, was thought to have been adequately enclosed and muslin was tucked behind the aneurysm quite satisfactorily, although it could not be determined with absolute certainty that all the posterior aspect had been covered. The patient made a good post-operative recovery and was returned to her referring hospital on the 14th post-operative day, still slightly drowsy but with no untoward neurological signs. On the 41st day after her initial haemorrhage she again collapsed with a coma-producing subarachnoid haemorrhage and was readmitted, conscious, but drowsy. Angiographically, the aneurysm now appeared to be larger and multilobular, with a small interhemispheric haematoma. On day 47 the patient's condition deteriorated and fresh blood was found in her CSF. She improved but 10 days later there was a similar incident, also confirmed by lumbar puncture, followed rapidly by further episodes two and three days later, the last being associated with her death at 60 days after her first subarachnoid haemorrhage.

TABLE I

COMPARISON OF COAGULATION/LYSIS STATUS IN FIRST WEEK OF ILLNESS IN PATIENTS WITH AND WITHOUT RECURRENT ANEURYSMAL HAEMORRHAGE

Test	Re-bled (9 cases) (mean $\pm$ S.D.)	No recurrence (16 cases) (mean $\pm$ S.D.)	Student's <i>t</i> test Significance (P)
TGT (sec)	14.9 $\pm$ 3.7	11.6 $\pm$ 2	0.001 < <i>P</i> < 0.01
Recalcification time (sec)	175 $\pm$ 40	131 $\pm$ 28	0.001 < <i>P</i> < 0.01
Euglobulin lysis (min)	238 $\pm$ 157	271 $\pm$ 133	not significant
Fearnley lysis (min)	732 $\pm$ 570	791 $\pm$ 454	not significant

TABLE II

COAGULATION AND LYSIS IN A WOMAN OF 49 WITH MULTIPLE HAEMORRHAGES FROM AN ANTERIOR COMMUNICATING ARTERY ANEURYSM

	(normal range)	TGT (9-14 sec)	Recalcification time (90-250 sec)	Euglobulin lysis (60-480 min)	Fearnley lysis (120-600 min)
Day 0	coma-producing SAH.				
3	? re-bled				
5		17	230	93	362
7		22	227	92	360
8	aneurysm wrapped				
12	(4 days post-op)	15	175	115	490
41	re-bled				
47	re-bled	23	185	53	180
49		14	129	36	60
57	re-bled	23	185	50	150
58		23	185	45	150
59	re-bled				
60	re-bled, died				

At necropsy, the aneurysm was found to be wrapped with adherent gauze except for an extension into the left frontal lobe. Further dissection of the fixed brain revealed the circle of Willis to be intact. There was no atheroma of the cerebral arteries. A biloculated aneurysm, 7 mm in diameter, arose by a 3 mm wide neck from the anterior communicating artery, with an apical rupture into the left frontal lobe through an unwrapped portion of the aneurysm which was buried in the brain. The ventricular system was full of blood. The ruptured locus was filled with recent thrombus.

During both admissions her coagulation mechanism showed some impairment and her lysis system was a little overactive. When first estimated (Table II), her TGT was prolonged, the recalcification time was just within the upper end of the normal range, and lysis times lay in the lower half of the normal range. Two days later the TGT was a little more prolonged and the other tests unchanged. After operation the TGT was less prolonged, recalcification time was shorter, and the lysis times were longer. When she was next tested, immediately after her fourth subarachnoid haemorrhage the TGT was more than twice the mean control time and the euglobulin lysis time was now below the lower limit of normal. Two days later the TGT was just within the normal range, the recalcification time was also a little shorter but both the lysis times were abnormally short. This temporary fall in the TGT could not be accounted for by any change in her clinical condition or its management. Another blood sample was taken eight days later, after she had once more re-bled, the TGT had risen again to 23 seconds, and the lysis times were very short. Similar results were obtained next day.

DISCUSSION

Recurrent haemorrhage is a major cause of death in patients surviving the initial rupture of an intracranial aneurysm. When such an event is survived the morbidity is often greater than that associated with a first subarachnoid haemorrhage. In a series of 173 patients who died after haemorrhage from an intracranial aneurysm, and in whom no surgery was undertaken, Crompton (1966) found post-mortem evidence of recurrent bleeding within six weeks in 38% of males and 62% of females. He noted that the site of the rupture was sealed by a laminated platelet and fibrin thrombus and that partial or even near total thrombosis did not guarantee against early recurrent haemorrhage. The results described above suggest that the quality of this clot and the balance between coagulation and lysis may be a factor in determining

whether an aneurysm will bleed again, though it must be remembered that there may also be purely local factors, not reflected in this investigation of the systemic blood.

Of the 52 patients with subarachnoid haemorrhage studied, 12 had a pathological delay and/or deficiency of thromboplastin generation. Nine of the 12 had a demonstrable aneurysm and in another there was a strong suspicion that an aneurysm was the source of his haemorrhage. Though it cannot be determined whether this coagulation disturbance preceded or followed the initial ictus, the patients with recurrent aneurysmal haemorrhage had a significantly longer TGT than those where the aneurysm, though not treated surgically, only bled once. A prolonged TGT, or a recalcification time near the upper limit of normal, would appear in some cases to be a warning of further trouble to come.

The defect of thromboplastic generation has not been fully defined. As it was corrected by normal plasma or by normal serum there could be minor disturbances in a number of clotting factors. Fibrinogen can be excluded with reasonable certainty as it was always found to be present in adequate amount. A quantitative platelet defect is excluded by the normal counts and a qualitative platelet abnormality is unlikely to have contributed to the impairment of thromboplastin generation since platelet substitute was used in all the tests. Raised serum glutamic-oxaloacetic transaminase levels had been found after subarachnoid haemorrhage in some patients (Buckell, Richardson, and Sarner, 1966), so it was thought possible that there might be decreased production of clotting factors due to disturbance of liver function. No such disturbance, however, could be demonstrated by the usual tests in these cases.

In one case an attempt was made to characterize the deficiency more precisely by assay of AHG and of Christmas factor. The latter was present in full amount while the AHG content of this patient's plasma was only 27% of that of the normal pool used for comparison. There is evidence that the central nervous system may influence the plasma levels of AHG. Gunn and Hampton (1967) report that electrical activation of the posterior hypothalamic areas and associated mammillary structures of the dog's brain produced a fall in plasma levels of AHG. Hypothalamic damage is a common post-mortem finding in cases of ruptured aneurysm (Crompton, 1963).

On the lysis side of the haemostatic balance sheet the results were not so clear cut. Though there was a tendency for the patients with aneurysms that re-bled to have shorter lysis times, the scatter of

results was very wide and the difference between the groups was not significant. A prolonged TGT was usually accompanied by euglobulin and Fearnley lysis times in the lower half of the normal range. Four patients having accelerated lysis times were noted to be particularly agitated during venepuncture.

If confirmed, these results could have important clinical applications. The combination of a mild coagulation defect with a tendency towards increased lytic activity in some patients with ruptured aneurysms may predispose them to recurrent bleeding so that these are the cases who might benefit from early surgery or from measures designed to protect what clot they have. It is well known that stress causes a rise in fibrinolytic activity (Macfarlane and Biggs, 1946) and, though the changes found did not constitute a pathological fibrinolytic state, they were coupled with poor quality clotting and in this situation it would seem wise to try and minimize the disturbing influence of headache and the anxiety of being in a neurosurgical ward so that natural lysis is not increased.

Antifibrinolytic therapy for intracranial aneurysms has recently been described. Mullan, Raimondi, Dobben, Vailati, and Hekmatpanah (1965) used epsilon amino caproic acid (EACA) clinically in an attempt to prolong the duration of clot in three of a series of 12 aneurysms subjected to electrical thrombosis. No definite conclusion was drawn as to the effect of EACA on the procedure. In one patient, after 12 g daily for five days, EACA was discontinued because of increasing dysphasia and hemiparesis. The signs improved but reappeared with resumption of the drug, which was then finally discontinued. No laboratory data are recorded. Gibbs and O'Gorman (1967) investigated fibrinolysis, by Fearnley's method, and the effect of EACA, in a series of patients with subarachnoid haemorrhage. They conclude, firstly, that there was not sufficient evidence for using lysis times as a prognostic test, though recurrent bleeding might be more likely where lysis times approached two hours, and, secondly, that, though their trial provided no evidence that the administration of EACA altered the prognosis in subarachnoid haemorrhage, recurrence may prove to be less likely if the whole-blood-clot lysis times can be consistently raised to the level of 40 hours. Mullan and Dawley (1968) treated 35 cases of recent subarachnoid haemorrhage with 24 g per day of EACA for periods of several days to six weeks. Of the 30 patients with demonstrable aneurysms 14 were treated by surgery and two of the others had a second haemorrhage while receiving EACA.

SUMMARY

Thromboplastin generation, recalcification time, euglobulin lysis time, Fearnley lysis time, plasminogen level, platelet count, liver function, and electrolytes were investigated in 52 patients with spontaneous subarachnoid haemorrhage from various causes.

The principal abnormality was a delay and/or deficiency of thromboplastin generation which occurred in 12 cases on repeated occasions.

A statistically significant difference was found between the TGTs of patients with and without recurrent aneurysmal haemorrhage. Recalcification times of the two groups also differed significantly. Lysis times for the patients with recurrent haemorrhage tended to be shorter, but the difference was not significant.

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