HEPARIN AND THE ADRENAL CORTEX*

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The occasional occurrence of thrombosis after operation has stimulated many attempts to demonstrate an increased coagulability of the blood in the post-operative period. Most of these attempts gave negative or equivocal results, probably because the methods employed were not sensitive enough to record the changes occurring in the complex process of blood coagulation. Nevertheless several investigators (Bergquist, 1945; Greig, 1949) reported a shortening of the whole blood clotting time soon after operation. Crafoord (1937), in the course of an investigation into the prevention of post-operative thrombosis by heparin, noted that a given dose of heparin had less effect in prolonging the clotting time in the first 48 hours after operation than in normal people, or in the later post-operative period. It was therefore considered that a new investigation of the coagulability of the blood after operation, as measured by a heparin clotting test, and an attempt to relate the changes observed to the increased activity of the adrenal cortex occurring as a result of the "stress" of operation (Selye, 1950), might be of interest.

Material and Methods

Twenty-nine surgical cases were investigated the day before operation and whenever possible immediately after operation, and on successive days thereafter into the second post-operative week. The test used was a slight modification of Soulier's *in vitro* heparin-resistance test (Soulier, 1951; Soulier and Le Bolloch, 1951). Five millilitres of venous blood, obtained by clean venepuncture with an oiled syringe, was added to a dry mixture of 4 mg. potassium and 6 mg. ammonium oxalate (Heller and Paul, 1934). The plasma was separated by centrifuging at 2,000 r.p.m. for five minutes. Three heparin and calcium-chloride solutions were prepared containing approximately 1.0, 0.7, and 0.3 units of heparin respectively in 0.5 ml. of M-40 CaCl_2 . The optimum amount of heparin in the first tube was determined by noting the clotting time at 37° with 0.25 ml. of normal plasma obtained and treated in the same way as the patient's plasma. If this was over 20 minutes or under nine

minutes the concentration was adjusted to bring it within this range. The other two heparin solutions were made by diluting this solution. In performing the test 0.25 ml. of M-40 CaCl_2 was placed in a small test-tube and 0.25 ml. of each of the dilutions of heparin and M-40 CaCl_2 in three other tubes all in a water-bath at 37° ; 0.25 ml. of plasma was added to each tube, mixed, and a stopwatch started as soon as the plasma had been added to the fourth tube. A control test, using normal plasma obtained and treated in the same way as the test and centrifuged at the same time, was performed simultaneously, using the same heparin and CaCl_2 solutions as used in the test. The results were expressed as "clotting delay"—that is, the difference in clotting time between the tube containing no heparin and that containing most. The intermediate tubes serve as checks only. This clotting delay is related to the normal by expressing it as a percentage of the normal control clotting delay, obtained simultaneously. In a series of 12 normals, the range was found to be $\pm 20\%$.

Eosinophil counts were carried out by the chamber technique of Randolph (1949).

Results

Only a summary of the results and graphs illustrating a few individual cases will be given here, details having been presented elsewhere (Garrett, 1953). Owing to difficulties in the collection of blood samples, in some of the 29 cases there are insufficient data for statistical analysis. Table I shows the figures for the clotting delay, expressed as a percentage of the control clotting delay, in the 20 cases with a pre-operative value and at least two values in the first three days after operation, including the day of operation itself. It will be seen that in 16 of the 20 cases the post-operative mean clotting delay value is lower than the pre-operative value. This pre-operative value shows considerable variation between cases. The variation in heparin resistance has been noted by others (Warren, Amdur, Belko, and Baker, 1950) and in this series may be due to the effects of the patient's illness itself. To overcome the error introduced by this variability an analysis of variance was performed on the results. This showed that when the variance between the

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TABLE I
RESULTS OF THE HEPARIN RESISTANCE TEST IN 20 CASES

Case Nos.	12		18		21		22		24		25		26		27		28		29	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
C.D.	53	38	100	120	122	72	54	92	107	92	93	108	220	100	110	46	72	50	50	43
Values		38		160		110		53		48		97		34		11		95		37
						125						123		43		43				35
Means	53	38	100	140	122	102	54	73	107	70	93	109	220	59	110	33	72	73	50	38

Case Nos.	30		32		33		34		35		36		38		39		40		41	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
C.D.	54	65	89	74	100	61	90	93	139	63	121	44	84	25	74	35	100	41	108	62
Values		57		30		88		55		83		115		96		45		21		44
										94		128		93		50		68		77
Means	54	61	89	52	100	75	90	74	139	80	121	96	84	71	74	43	100	43	108	61

A=pre-operation. B=post-operation. Clotting delay (C.D.) values expressed as percentage of control C.D. value obtained simultaneously.

pre- and post-operative groups within cases was tested against the variance within the groups the post-operative values were significantly lower than the pre-operative ($p < 0.01$). In other words, heparin had less effect in delaying the clotting time of plasma in the first two days after operation than it had before operation. In the later post-operative period the values for clotting delay returned to normal. The resistance to heparin after operation did not appear to be related to the occurrence of thrombosis or to the nature of the operation or anaesthetic, although in general those undergoing comparatively minor procedures, such as radical removal of hydrocele, showed less marked changes than those submitted to more severe operations, such as partial gastrectomy.

In every case the eosinophil count showed the post-operative fall described by others (Roche, Thorn, and Hills, 1950). The lowest levels were usually reached 24 to 48 hours after operation, whereas the shortest clotting delay values were often observed immediately after the operation. Otherwise the two observations correlated well, both returning to normal at about the same rate. Fig. 1 shows a typical response.

It has been noted that the heparin resistance test shows a shortened clotting time when the platelet count is high (Vaugh and Ruddick, 1944; Soulier and Le Bolloch, 1950; Carr and Fowler, 1949) and it might be argued that the increased resistance to heparin after operation was due to an increased platelet count. In fact the platelet count is often low immediately after operation and does not increase until the end of the second post-operative week (Dawbarn, Earlam, and Evans, 1928; Warren, Lauridsen, and Belko, 1953), when the heparin test is again normal. Fig. 2 shows

that the heparin resistance test correlates better with the eosinophil count than with the platelet count, although there was in this case an immediately post-operative slight rise in platelets, which is not significant when the error of the platelet count is considered (Biggs and Macmillan, 1948). This correlation appears to support the hypothesis that the increase in resistance to the effect of heparin is related to the increased amount of A.C.T.H. in the blood as a result of operation (Bornstein and Trehwella, 1950).

FIG. 1.—Partial gastrectomy in a man aged 44. Fall in eosinophils and heparin clotting delay after operation.

FIG. 2.—Radical mastectomy in a woman aged 86. Changes in heparin clotting delay, eosinophils, and platelets after operation.

Results in Cases Treated with Insulin and Electro-convulsions

In view of these results it was decided to investigate other kinds of "stress," and four cases receiving electro-convulsion therapy and six receiving insulin shock therapy for psychiatric disorders were investigated in the same way.

Fig. 3 shows the mean of the results of the heparin resistance tests and eosinophil counts of four acute schizophrenic or depressive patients treated with electro-convulsion therapy. It will be seen that there was a fall in the clotting delay and eosinophils from the initial level four hours after the shock, and a rise towards normal in both values 24 hours later. Owing to the small number of cases available and the variability of the clotting delay reading 28 hours after the shock the figures are not this time significant if all these values are considered. Nevertheless the difference between the pre-shock and four hours post-shock values taken alone are significant.

Six cases receiving insulin shock therapy showed similar changes in the clotting delay and eosinophil count. The mean clotting delay before the insulin was given was 100% of the control and four hours after the coma had been interrupted it was only 25%. This fall is highly significant ($t=5.6$; $p<0.001$).

Results of Administration of A.C.T.H.

The effect of the administration of A.C.T.H. on the heparin resistance and Lee and White whole blood clotting time of four patients under treatment for various ophthalmic disorders was investigated. Three of these showed a transient fall in the clotting delay after A.C.T.H. had been administered, but this returned to the previous level later although the A.C.T.H. therapy was continued. The Lee and White clotting time showed similar changes. Fig. 4 illustrates a typical case.

FIG. 3.—Mean values of heparin clotting delay and eosinophils in four cases before and after E.C.T.

FIG. 4.—Changes in heparin clotting delay and whole blood clotting time during A.C.T.H. therapy for chronic uveitis in a man aged 53.

From these results it might be expected that cases of Addison's disease would show a prolonged heparin clotting delay, and one such case, a woman aged 47, admitted in an Addisonian crisis, has been investigated. The clotting delay on admission was 260% of the control, the highest value found in this investigation, and fell to 84% four weeks later during treatment with "eucortone" and D.O.C.A.

Adrenalin is said to have an effect on the pituitary, stimulating it to release A.C.T.H. (Roche *et al.*, 1950) and in some cases causes a fall in the eosinophil count. Fig. 5 shows the effect on the clotting delay of a subcutaneous injection in a normal person of 1 mg. of adrenalin tartrate.

If adrenocortical hormones increase blood coagulability, then it might be expected that there would be a risk of thrombosis during therapy with A.C.T.H. and cortisone. Cosgriff (1951) and Coste, Galmiche, Piguet, Delbarre, and Laurent (1951) have described altogether 34 such cases and we have observed a similar one. This patient was a man aged 59, diagnosed four years before the present incident as a case of mycosis fungoides. He had found that the only treatment which kept him reasonably well was large doses of cortisone which he provided at his own expense. He had been receiving 250 mg. cortisone daily for 12 months when in December, 1952, he developed

thrombophlebitis in the veins of the right leg followed by a small pulmonary embolus. This incident was treated in a nursing-home, and records are not available. In March, 1953, the thrombophlebitis recurred and he was admitted to hospital for anticoagulant therapy. He continued to take 250 mg. cortisone daily, and excessively large doses of "tromexan" were required to depress the "prothrombin" efficiency level. An initial dose of 600 mg. lowered it on the following day to only 58% of the normal level and the dose was therefore raised to 900 mg. on the second, 1,200 mg. on the third, and 1,500 mg. on the fourth day. On the fifth day of therapy the "prothrombin" level was 27% and the dosage was continued at 1,200 mg. a day until the eighteenth day, when the "prothrombin" level fell suddenly to 5%. The "tromexan" dosage was then reduced.

This case indicates the resistance to "tromexan" therapy of patients receiving cortisone. Coste *et al.* (1951) recorded a similar case in which heparin treatment did not prolong the coagulation time.

Discussion

These results support the view that adrenocortical hormones cause an increase in the coagulability of the blood and are in agreement with those of Cosgriff, Diefenbach, and Vogt (1950). On the other hand, McGraw, Margulis, and Brush (1952) consider that A.C.T.H. tends to delay coagulation and that thrombosis occurs when it is suddenly withdrawn from patients who have been receiving it, unlike the case reported above. It has long been known, however, that "stress" and haemorrhage in the initial or "alarm reaction" stage cause a decreased clotting time. This was noted by William Hewson in 1771

FIG. 5.—Effect of injection of adrenalin on heparin clotting delay in a normal man aged 39.

and has been repeatedly confirmed since. Thus Macht (1952) records the shortening of clotting time in fright and anxiety, and Stoker (1952) proposes that this may account for the increased incidence of phlebothrombosis in patients treated in the unfamiliar environment of hospital compared with those treated at home. The occurrence of coronary thrombosis after operation and shock is also well known (Davis, Parlante, and Hallsted, 1951) and cerebral thrombosis is recognized as a complication of insulin shock therapy (Donnelly and Radley-Smith, 1950).

Interesting relationships between heparin, cortisone, and eosinophils have also been described by other authors. Thus De Takats and Marshall (1952), in a paper published while this work was in progress, noted a similar fall in heparin clotting time after operation and adrenalin injection as measured by a simplified *in vitro* technique. This fall in clotting time usually, but not invariably, occurred almost simultaneously with the fall in the eosinophil count. Godlowski (1951) stated that previous heparinization prevented the fall in the eosinophil count occurring when cortisone or A.C.T.H. was given *in vivo*, and Muehrcke, Staple, and Kark (1952) found that this effect could also be demonstrated with cortisone *in vitro*, although Godlowski (1952), using different dosages, was unable to confirm this.

Increased resistance of the clotting time to the effect of heparin has also been reported in two of the collagen diseases treatable with A.C.T.H. and cortisone. These are rheumatic fever (Abrahams, Glynn, and Loewi, 1951) and lupus erythematosus (Borrie, 1951). It is of interest to note that heparin has been reported to be of therapeutic value in rheumatic fever (Glazebrook and Wrigley, 1949; Donzelot and Kaufmann, 1949). These observations recall those of Macht (1943) on the effect of heparin in preventing experimental anaphylactic reactions and revive speculations that heparin may have functions other than those of an anticoagulant (Astrup, 1945; Magerl, 1942). In this connexion it is interesting to note that heparin is a powerful inhibitor of hyaluronidase (Glick and Sylvén, 1951). On the other hand, they may simply indicate an altered polysaccharide metabolism brought about by changes in the circulating adrenocortical hormones.

Summary

Statistically significant evidence is produced for a lessening of the effect of heparin on the plasma clotting time in the first 48 hours after operation.

It is shown that similar effects occur after insulin coma and electro-convulsion therapy.

These changes occur at approximately the same time as the fall in the eosinophil count and are assumed to be due to increased adrenocortical hormone production.

Patients receiving A.C.T.H. may show similar changes and there is a danger that A.C.T.H. and cortisone treatment may be complicated by thrombosis which is resistant to anticoagulant therapy.

The implications of these findings are discussed.

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REFERENCES

- Abrahams, D. G., Glynn, L. E., and Loewi, G. (1951). *Clin. Sci.*, **10**, 1.
 Astrup, P. (1945). *Nord. Med.*, **25**, 195.
 Bergquist, G. (1945). *Acta chir. scand.*, **92**, suppl. 101.
 Biggs, R., and Macmillan, R. L. (1948). *Journal of Clinical Pathology*, **1**, 269.
 Bornstein, J., and Trehwella, P. (1950). *Lancet*, **2**, 678.
 Borrie, P. (1951). *Brit. J. Derm.*, **63**, 21.
 Carr, T. L., and Fowler, W. M. (1949). *J. Lab. clin. Med.*, **34**, 122.
 Cosgriff, S. W. (1951). *J. Amer. med. Ass.*, **147**, 974.
 —, Diefenbach, A. F., and Vogt, W., Jr. (1950). *Amer. J. Med.*, **9**, 752.
 Coste, F., Galmiche, P., Piguët, B., Delbarre, F., and Laurent, F. (1951). *Rev. Rhum.*, **18**, 189.
 Crafoord, C. (1937). *Acta chir. scand.*, **79**, 407.
 Davis, H. A., Parlante, V. J., and Hallsted, A. M. (1951). *Arch. Surg., Chicago*, **62**, 698.
 Dawbarn, R. Y., Earlam, F., and Evans, W. H. (1928). *J. Path. Bact.*, **31**, 833.
 De Takats, G., and Marshall, M. H. (1952). *Surgery*, **31**, 13.
 Donnelly, J., and Radley-Smith, E. J. (1950). *Lancet*, **2**, 904.
 Donzelot, E., and Kaufmann, H. (1949). *Presse méd.*, **57**, 989.
 Garrett, J. V. (1953). D.M. Thesis, Oxford.
 Glazebrook, A. J., and Wrigley, F. (1949). *Brit. med. J.*, **2**, 789.
 Glick, D., and Sylvén, B. (1951). *Science*, **113**, 388.
 Godlowski, Z. Z. (1951). *Brit. med. J.*, **1**, 854.
 — (1952). *J. Endocrinol.*, **8**, 102.
 Greig, G. W. V. (1949). *Brit. med. J.*, **2**, 845.
 Heller, V. G., and Paul, H. (1934). *J. Lab. clin. Med.*, **19**, 777.
 Hewson, W. (1771). *An Experimental Inquiry into the Properties of the Blood with Remarks on Some of its Morbid Appearances*. London.
 Macht, D. I. (1943). *Ann. intern. Med.*, **18**, 772.
 — (1952). *J. Amer. med. Ass.*, **148**, 265.
 Magerl, J. F. (1942). *Z. Immunforsch.*, **101**, 122, 225.
 McGraw, A. B., Margulis, R. R., and Brush, B. E. (1952). *Arch. Surg., Chicago*, **65**, 81.
 Muehrcke, R. C., Staple, T. W., and Kark, R. M. (1952). *J. Lab. clin. Med.*, **40**, 169.
 Randolph, T. G. (1949). *Ibid.*, **34**, 1696.
 Roche, M., Thorn, G. W., and Hills, A. G. (1950). *New Engl. J. Med.*, **242**, 307.
 Selye, H. (1950). *Stress*. Acta, Montreal.
 Soulier, J. P. (1951). *Sem. Hôp., Paris*, **27**, 775.
 — and Le Bolloch, A. G. (1950). *Rev. Hémat.*, **5**, 148.
 — (1951). *Acta med. scand.*, **140**, 132.
 Stoker, S. B. (1952). *Lancet*, **2**, 709.
 Warren, R., Amdur, M. O., Belko, J., and Baker, D. V. (1950). *Arch. Surg., Chicago*, **61**, 419.
 —, Lauridsen, J., and Belko, J. S. (1953). *Circulation, N.Y.*, **7**, 481.
 Waugh, T. R., and Ruddick, D. W. (1944). *Canad. med. Ass. J.*, **51**, 11.