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THE EFFECT OF ADRENALINE INFUSION ON HUMAN BLOOD COAGULATION

By G. D. FORWELL and G. I. C. INGRAM*

From the Department of Surgery, University of Edinburgh

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In recent years, the study of a number of human clotting defects has led to a considerable extension of the theory of blood coagulation. It therefore seemed opportune to re-investigate in man the rapid clotting which has long been reported to follow the administration of adrenaline.

Although the problem has been examined several times previously, the evidence is still confused. In the first place, not all authors are agreed on the reality of this effect and secondly, because so many diverse drugs and procedures have been alleged to have a like effect, it is tempting to ascribe the shortening of the clotting time to some common, incidental cause.

In 1903, Vosburgh & Richards noticed a shorter clotting time in dog's blood after giving adrenaline. W. B. Cannon and his associates made the classical investigation in anaesthetized or decerebrate cats, hares and rabbits (Cannon & Gray, 1914; Cannon & Mendenhall, 1914*a*, *b*, *c*; Drinker & Drinker, 1914; Gray & Lunt, 1914; Mendenhall, 1915; Grabfield, 1916; Stern, 1916); an intravenous injection of 10^{-6} g/kg was characteristically followed by a shortening of the clotting time to $\frac{1}{2}$–$\frac{1}{3}$ of the control value, whereas a larger dose (*c.* 3×10^{-5} g/kg) often produced a lengthening of clotting time. Shortening of the clotting time also followed various procedures thought to lead to a liberation of endogenous adrenaline. The great majority of readings were obtained on arterial blood with a coagulometer (Cannon & Mendenhall, 1914*a*) which measured the increasing resistance offered to the repeated passage of a wire through clotting blood.

Many different drugs are reported to affect the clotting time when administered *in vivo*. In addition, more rapid clotting has been observed following various experimental procedures, including even repeated venepuncture, and in the face of pain and emotion. The literature is reviewed by Forwell (1955). It is apparent that some of these conditions might accidentally have operated

* External Staff, Medical Research Council.

during experiments in which the effects of drugs were being investigated, and might have caused the acceleration of clotting which was ascribed to the drugs.

METHODS

The investigation is in five parts:

(1) The main experiments of Cannon & Gray (1914) were repeated under anaesthesia in cats and in dogs.

(2) Because blood was to be obtained by venepuncture before and after administering adrenaline to conscious human subjects, observations were made to determine the effect of serial venepuncture upon the clotting time, in twenty-two untrained men.

(3) Twelve control and thirteen adrenaline infusions were administered to nine subjects well habituated to venepuncture, to distinguish changes in clotting time due to adrenaline from those incidental to the experimental procedures.

(4) In seven of the adrenaline infusions (in five subjects), more detailed clotting investigations were undertaken.

(5) Direct action upon the clotting mechanism was controlled by adding reagents to clotting tests *in vitro*.

Collection of blood. In animals, arterial blood was obtained from exposed vessels, either directly into the cannula described by Cannon & Mendenhall (1914*a*), or by needle and syringe. In man, blood was withdrawn by venepuncture. Needles and syringes were coated with silicone. When a pair of blood samples was obtained before and after an infusion of adrenaline they are referred to as 'control' and 'adrenaline' samples respectively.

A test for the beginning of thrombin generation was made by adding 0·5 ml. citrated plasma to each of four test-tubes containing 0·2 ml. normal platelet suspension and 0·5 ml. 0·025 M-$CaCl_2$, already warmed to 37° C. After 0, 1, 2 or 3 min incubation (and before clotting had occurred) 0·2 ml. 3·8 % (w/v) trisodium citrate was added to each tube and the contents mixed. (In the two-stage prothrombin time with brain suspension, a corresponding addition of citrate had been shown to arrest the generation of thrombin.) The tubes were then observed for the appearance of fibrin threads (a development assisted by freezing and thawing), which was taken to represent traces of thrombin which had been generated before the final addition of citrate, and probably, also, thrombin generated before the 'autocatalytic' phase since the yield of fibrin was small, and it appeared slowly. The test was thus thought to indicate the onset of an earlier stage in the clotting sequence than was represented by the thrombin generation test (Macfarlane & Biggs, 1953) in which a firm clot occurs. Paired plasma samples were tested together.

Glass contact for factor VII activity. Rapaport, Aas & Owren (1954, 1955) found that the one-stage prothrombin time obtained with brain reagent was shorter in plasma which had been shaken with crushed glass than in plasma handled only in siliconed tubes; they concluded that the effect was related to an increase in proconvertin activity, of threefold or more. Paired plasmas were therefore tested in this way for a difference in *glass contact effect*. In the analysis of variance of the results, the interaction between before/after adrenaline and silicone/glass was tested for significance.

Statistical conventions. Mean values with S.E. (and the number of contributory readings, n) are indicated thus: $20{\cdot}6 \pm 1{\cdot}3$ ($n = 8$) min. When standard errors are derived from an analysis of variance the n-value is replaced by the number of degrees of freedom for the error term (error d.f.). The number of d.f. in the t-test is shown thus: t_{22}. Confidence limits follow mean value thus: −3% (−12 to 11%). The correlation coefficient is denoted by r. 'Significance' refers to the 5% level. When a reading was lost from a series but was necessary for an analysis it was replaced by an estimated value (Snedecor, 1946).

RESULTS

Animal experiments

The effect on clotting time of the intravenous injection of synthetic adrenaline tartrate was tested in eight cats and four dogs under pentobarbitone anaesthesia. In two cats, a shortening of the clotting time was demonstrated with the Cannon-Mendenhall coagulometer (Forwell, 1955) following adrenaline injections of 2×10^{-6} and $1{\cdot}5 \times 10^{-6}$ g/kg in one, and of 2×10^{-7} g/kg in the other. A shortening of clotting time both in glass and silicone-coated tubes at 37° C (Lee & White, 1913) appeared to follow the injection of 2×10^{-6} g/kg

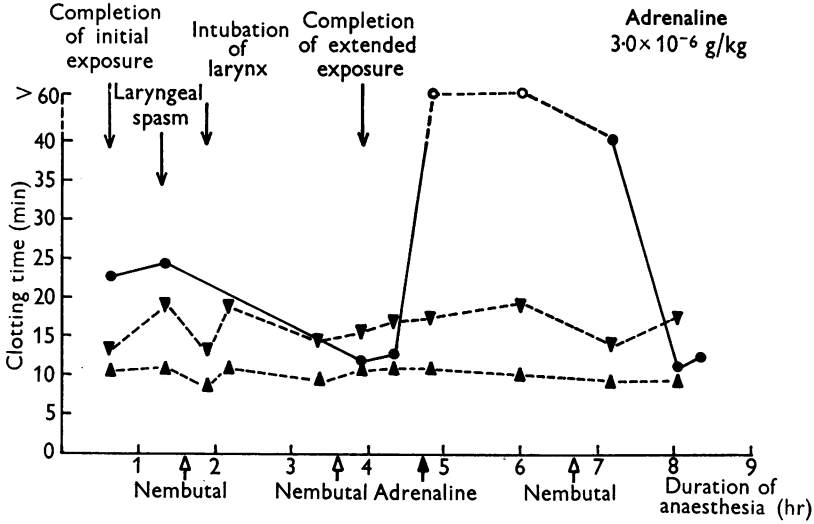

Fig. 1. Effects on clotting time of intravenous injections of synthetic adrenaline in anaesthetized dogs. ●—●, Cannon-Mendenhall coagulometer; ▲—▲, Lee-White clotting time in glass tubes; ▼—▼, Lee-White clotting time in silicone-coated tubes. The changes shown by the coagulometer agree with W. B. Cannon's findings, but are not reproduced by the Lee-White tests.

in one cat, but there was no effect in five others (one of which was splanchnicectomized at the beginning of the experiment) in which 10^{-6}, 2×10^{-6} and 10^{-5} g/kg were injected. One dog, tested only with Lee-White clotting times, showed no change following the injection of 10^{-6}, $2{\cdot}5 \times 10^{-6}$ or 10^{-5} g/kg. With the coagulometer, one dog showed a prolonged lengthening of clotting time following 3×10^{-6} g/kg (Fig. 1); in another, given 3×10^{-7} g/kg, the injection preceded a slow oscillation of the clotting time (Fig. 2); and in the last, in which the initial clotting time was unusually long, 3×10^{-8} g/kg was twice followed by a marked fall in clotting time (Fig. 3). Nevertheless, concurrent readings by the Lee-White methods did not show corresponding changes in these three animals.

In fact in these and other experiments in six dogs, 35 parallel readings of clotting time were obtained by all three methods, and the pooled correlations between the results from the six animals were: for glass and silicone-coated tubes, $r=0{\cdot}64$ ($t_{29}=4{\cdot}45$; $P<0{\cdot}01$); for glass tubes and the coagulometer,

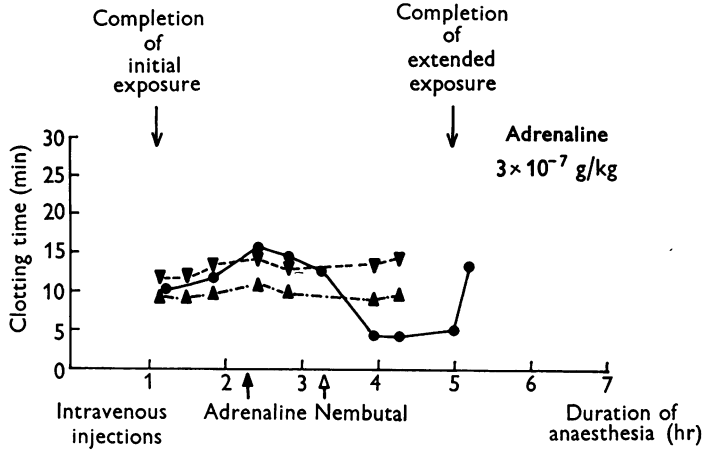

Fig. 2. Legend as in Fig. 1.

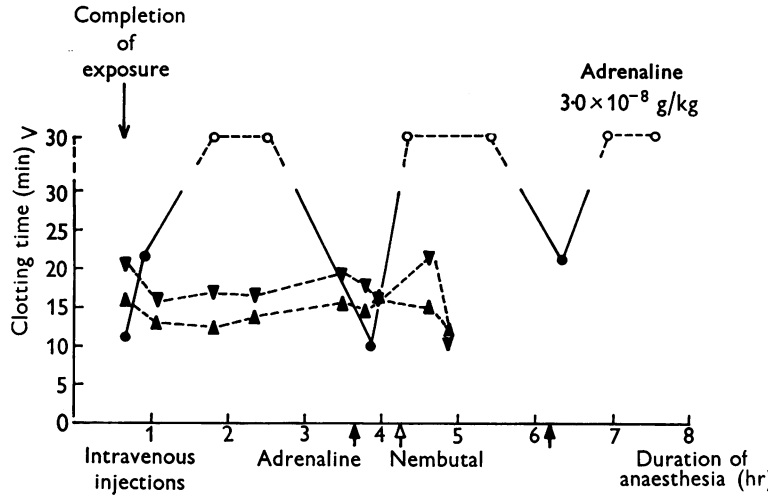

Fig. 3. Legend as Fig. 1.

$r=0{\cdot}29$ ($t_{29}=1{\cdot}61$; $P>0{\cdot}1$); and for siliconed tubes and the coagulometer, $r=0{\cdot}12$ ($t_{29}<1$). Thus, the results in glass and silicone-coated tubes correlated reasonably well, but the coagulometer readings did not correlate with them. Subsequent observations of whole blood clotting time are therefore restricted to glass and siliconed tubes, since there is no information on the relation of coagulometer readings to modern work on blood coagulation.

Serial venepuncture in man

Serial clotting times in siliconed tubes and direct eosinophil counts (Dunger, 1910) were obtained at half-hour intervals from eight fasting volunteers who drank a solution of 50 g fructose after the first venepuncture. Similar samples were obtained from fourteen other fasting subjects who received instead 50 g glucose. The subjects were not habituated to venepuncture but three in the second series were tested twice. Blood glucose (Hagedorn & Jensen, 1923*a*, *b*) and (in the first series) fructose (Stewart, Scarborough & Davidson, 1938) concentrations were also determined. The results from each patient were expressed as percentages of the corresponding readings obtained at the first venepunctures, and the mean changes in these percentages are shown in Figs. 4 and 5.

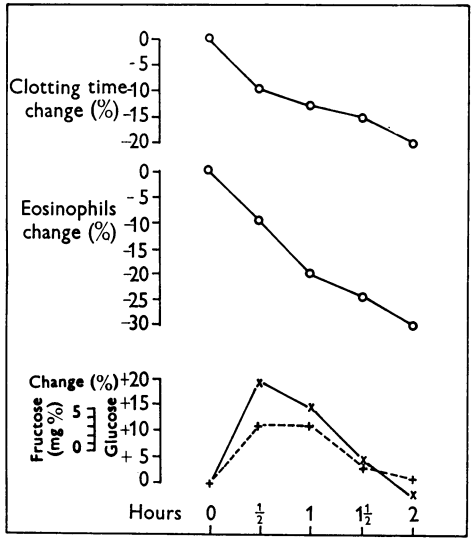

Fig. 4. Mean percentage changes in clotting time in silicone-coated tubes, eosinophil count and blood glucose (×) and fructose (+) concentrations in eight fasting volunteers receiving fructose orally after the first venepuncture.

Fructose was regarded as an inert control substance. In this series (Fig. 4) the mean initial siliconed tube clotting time and eosinophil count were $20{\cdot}6 \pm 1{\cdot}3$ ($n=8$) min and 158 ± 56 ($n=8$)/mm^3 respectively. Their average rates of fall, as given by the slopes of the least squares regression lines calculated from the percentage values, were respectively $-8{\cdot}8 \pm 2{\cdot}0$ (error d.f. 34) % of the initial clotting time/hr observed and $-14{\cdot}8 \pm 3{\cdot}8$ (error d.f. 28) % of the initial eosinophil count/hr observed, and were clearly significant. The two slopes did not differ significantly ($0{\cdot}1 < P < 0{\cdot}2$).

In the glucose series (Fig. 5), in which the clotting time and blood glucose concentration appear to have been negatively related, the mean initial siliconed

tube clotting time was $16{\cdot}6 \pm 1{\cdot}0$ ($n = 17$) min and the mean initial eosinophil count 182 ± 12 ($n = 13$)/mm^3. It is clear from Fig. 5 that neither variable showed the progressive fall seen in the previous figure, but in both Figs. 4 and 5 the siliconed tube clotting time and the eosinophil count tend to be correlated. The relation between clotting time and blood glucose concentration was further tested in the second series by fitting quadratic curvatures to the original clotting times and glucose concentrations: in fourteen of the seventeen tests the associated curvatures were of opposite sign. Parallel clotting times observed throughout in glass tubes did not show these effects.

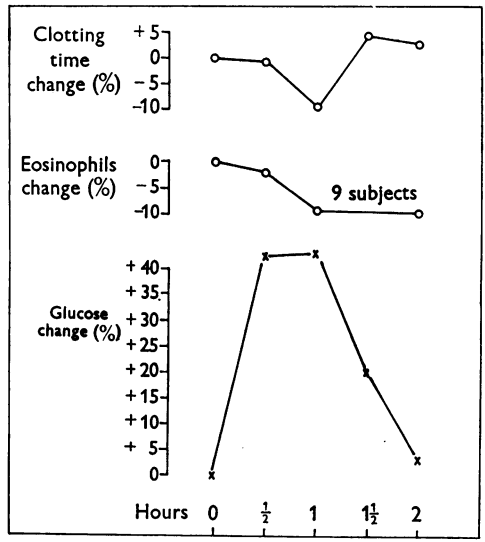

Fig. 5. Mean percentage changes in clotting time in silicone-coated tubes, eosinophil count and blood glucose concentration in fourteen fasting subjects receiving glucose orally after the first venepuncture.

Adrenaline infusions in man

In nine healthy young men (medical students and colleagues), well habituated to venepuncture, blood samples were obtained before and after thirteen infusions of adrenaline and at half-hour intervals during twelve control infusions. Synthetic adrenaline tartrate was infused at $2-8 \times 10^{-6}$ g/min, generally over about half an hour (i.e. *c.* $0{\cdot}03-0{\cdot}1 \times 10^{-6}$ g/kg/min, or $1-3 \times 10^{-6}$ g/kg), raising the pulse rate by *c.* 30% and the blood glucose concentration by *c.* 60%.

Clotting times were measured in glass and silicone-coated tubes, and the differences observed before and after adrenaline (13 pairs of blood samples) were compared with those found between consecutive control samples (24 pairs). Only in the results from the siliconed tubes were there even suggestive mean differences between control and adrenaline samples. These

figures showed large differences between the over-all mean clotting times on different occasions, but smaller differences between the proportional changes in clotting times in consecutive samples, so that the results were analysed in logs. A pooled error mean square was obtained from the differences within subjects and treatments, on 23 d.f.; and the mean changes in siliconed clotting time (from the antilogs. of the mean log. differences) were: control infusions, −3% (−12 to 11%); adrenaline infusions, −19% (−29 to −7%). The difference between the means was significant ($t_{23}=2{\cdot}22$; $0{\cdot}02<P<0{\cdot}05$). These findings suggest that the adrenaline infusions produced a real shortening of the clotting time in siliconè-coated tubes. A systematic fall in eosinophil count did not occur.

Detailed clotting tests

More detailed investigations of the clotting mechanism were carried out in seven of the adrenaline infusions. Comparison was made between the adrenaline and control samples from each infusion: it was assumed that any changes observed would represent the effects of adrenaline, on the evidence of the preceding section, and corresponding tests on control infusions were not made.

In the mean results of all the infusions, a shortening of clotting time had been observed in silicone-coated tubes but not in glass tubes; this suggested that the effect of adrenaline was mediated through the initial stages of clotting not later than the development of intrinsic thromboplastin (Biggs, Douglas & Macfarlane, 1953). The following evidence is therefore arranged to test this hypothesis.

Evidence of an acceleration of the early stages of clotting. Triplicate thrombin generation tests (Macfarlane & Biggs, 1953) were made in four infusions. Thrombin appeared earlier in the adrenaline than in the control samples in eight out of the twelve runs, and in no instance later. The test for the beginning of thrombin generation was made in two infusions. In both cases fibrin threads appeared in earlier tubes in the adrenaline series than in the control series.

Evidence of an acceleration of thrombin generation with brain reagent. There is evidence that when thrombin is generated from plasma by brain reagent (Biggs & Macfarlane, 1949) the brain reagent is first activated by factors V and VII (Hardisty, 1955). The rate of thrombin generation in the two-stage prothrombin time (Biggs & Douglas, 1953*a*) thus probably reflects the activity of factors V and/or VII or of the final product with brain. In six of the seven infusions the generation of thrombin in the two-stage system was more rapid in the adrenaline sample than in the control sample. After defibrination with thrombin (Warner, Brinkhous & Smith, 1936) there was no difference in four infusions tested. The loss of the adrenaline effect after defibrination suggested that this effect was mediated by a change in factor V, since this factor is inactivated by thrombin.

Evidence of an increase in factor V activity. In the thromboplastin generation test (Biggs & Douglas, 1953*b*), alumina-treated plasma (which contains factor V) from the adrenaline samples showed higher activity than that from the control samples in each of six infusions tested. In three of these, the factor V fractions (Biggs & Macfarlane, 1953) were separately examined, and in two the enhancement of activity was associated with this fraction.

Tests with brain reagent for factor V activity (Ware & Seegers, 1948; Wolf, 1953) were made in five infusions, testing either alumina-treated plasma or the factor V fraction, or both. In four in which plasma was tested the mean potency of the adrenaline samples was 124% of that of the controls; in four in which the factor V fraction was tested the corresponding value was 126%.

In one infusion, in which the one-stage prothrombin time was tested with Russell's Viper venom, the clotting time of the adrenaline sample was shorter than that of the control by *c.* 20%. By testing derivatives of the two plasmas, the heightened activity was shown to be associated with the factor V fraction of the adrenaline sample.

Tests for factor VII activity. In three infusions the glass contact effect was examined but in none was the effect significantly greater after the infusion than before. In two infusions alumina eluates (containing factor VII) were tested, but in neither did the paired samples differ. In two infusions whole plasma, defibrinated plasma and serum were each tested for their ability to shorten the prothrombin time of plasma from a patient under treatment with phenylindanedione but a consistent difference between the adrenaline and control samples was not found. It was concluded that adrenaline had not influenced the activity of factor VII.

Direct effect on the clotting mechanism

To eliminate direct effects upon the clotting mechanism, the reagents used, and certain other substances which might have been released *in vivo*, were added to clotting tests *in vitro*, in the concentrations which might have been obtained *in vivo*. No effect was noticed with glucose, fructose, noradrenaline, insulin or histamine.

With adrenaline, however, a significant shortening of whole blood clotting time was demonstrated in two experiments with silicone-coated tubes, and the results are shown in Table 1, together with comparable figures given by Waldron (1951) who used collodion tubes. It was shown that the slopes of the regressions of clotting time on adrenaline concentration did not differ significantly between the three series of data, and so a mean slope was derived from all the results ($0{\cdot}75 \pm 0{\cdot}22$ min fall in clotting time per tenfold rise in adrenaline concentration). Using this value, the regression of clotting time on adrenaline concentration of Expt. 1 (Table 1) was extrapolated forwards to determine the concentration of adrenaline required to produce the mean

degree of shortening of clotting time observed in the infusion experiments (−19%): a concentration of *c.* 6×10^{-1} g/l. would have been required. Similarly, the regression was extrapolated backwards to the mean saline control clotting

TABLE 1. Mean results in experiments in which fresh whole blood was added to adrenaline *in vitro* and the clotting times measured

i. Expt. 1. Silicone-coated tubes: twenty subjects tested

	Contents of tubes to which blood was added			
	Control		Adrenaline in 0·9% (w/v) NaCl (As final concentration after adding blood, g/l.) cf. data of Waldron (1951) *infra*	
	Empty tube	0·9% (w/v) NaCl	$1{\cdot}25 \times 10^{-3}$	$1{\cdot}25 \times 10^{-2}$
Mean clotting time (min), with % deviation from NaCl control reading	15·3 (+12)	13·7	13·2 (−4)	12·3 (−11)

Mean change in clotting time per tenfold increase in adrenaline concentration, $-0{\cdot}93 \pm 0{\cdot}37$ min

ii. Expt. 2. Silicone-coated tubes: eleven subjects tested

	Final concentrations of adrenaline ($\times 10^{-3}$, g/l.) after adding blood			
	0·9	2·2	5·2	12·5
Mean clotting time (min)	11·6	11·8	11·5	11·1

Mean change in clotting time per tenfold increase in adrenaline concentration $-0{\cdot}57 \pm 0{\cdot}41$ min

iii. Waldron (1951). Collodion tubes: twenty subjects tested at each adrenaline concentration

	Final concentration of adrenaline (g/l.) after adding blood (cf. data of Expt. 1, *supra*)			
	10^{-3}		10^{-2}	
	Control tube 0·9% (w/v) NaCl	Adrenaline tube	Control tube 0·9% (w/v) NaCl	Adrenaline tube
Mean clotting time (min), with % deviation from NaCl control reading	12·5	10·2 (−18)	12·3	9·3 (−24)

Mean change in clotting time per tenfold increase in adrenaline concentration, $-0{\cdot}72 \pm 0{\cdot}36$ min

In Expts. 1 and 2 blood from each subject was tested with each adrenaline concentration and clotting times were obtained from two replicate tubes in each category. Parallel tests in glass tubes showed similar but smaller effects.

In Waldron's (1951) experiment blood from a different subject was used for each pair of clotting times with 0·9% (w/v) NaCl and one or other concentration of adrenaline, so that the mean clotting times for the two concentrations are derived from different groups each of twenty subjects. In calculating the mean change in clotting time per tenfold increase in adrenaline concentration, an adjustment was made to allow for the differences in control clotting times.

The three estimates of mean change in clotting time do not differ significantly; the pooled value is $-0{\cdot}75 \pm 0{\cdot}22$ min per tenfold increase in adrenaline concentration.

time, which was reached at a concentration just above 10^{-4} g/l. It was therefore assumed that below this concentration there would be no appreciable direct effect of adrenaline upon the clotting mechanism.

Now, the resting concentration of adrenaline in the blood has been given as *c.* 10^{-6} g/l. (Outschoorn & Vogt, 1952; Weil-Malherbe & Bone, 1952). The levels attained by intravenous infusion in man are not known, but an estimate was obtained from the first six infusions of the present experiments by calculating the total quantity infused up to the time when the pulse rate began to fall after the initial rise, and then dividing this quantity by the estimated blood volume. This suggested that an infusion would raise the blood concentration of adrenaline to *c.* 10^{-5} g/l. If this estimate is even approximately correct, it is unlikely that the observed fall in clotting time of -19% could be ascribed to a direct effect of adrenaline upon the clotting mechanism.

DISCUSSION

Animal experiments

Cannon's observations with the coagulometer appeared to be confirmed by this small series of experiments, but the results could not be reproduced in animals with the Lee-White tests. It had been hoped that Lee-White tests made in animals in parallel with readings of the Cannon-Mendenhall coagulometer would throw light on Cannon's original observations, and would help to bridge the gap between his work and the modern study of coagulation. When it became apparent that Cannon's results were not reflected in the Lee-White tests, this line of investigation was discontinued. What function of clotting is measured by the coagulometer therefore remains unknown; and in particular, the prolongation of the clotting time by the larger doses of adrenaline is unexplained.

The effect of venepuncture in man

Menghini & Giunti (1948*a*, *b*), testing thirty-five fasting subjects, found that blood obtained by successive half-hourly venepunctures from different veins clotted progressively more quickly, and they suggested that this might be caused by an accumulation of tissue juice in the blood, the trauma of each venepuncture contributing a little more. In fact, their results showed a progressive fall in clotting time from initial values of 10–15 min (at room temperature) to a minimum value of 3–4 min over the first four punctures: but the final three punctures produced no further shortening. This pattern of results is difficult to explain on the hypothesis which they advance, since *in vitro* tissue juice will shorten the clotting time to as little as 10 sec, but rather suggests a response of the organism to the (presumably unpleasant) circumstances of the experiment. This is borne out by the fact that a comparable

shortening of clotting time has been reported to follow the administration of many different drugs, and also by the association between clotting time and eosinophil count in the present series of tests with fructose and glucose. It is clearly important, when comparing the clotting times of samples of blood obtained before and after a given procedure, to control the experiments for the effects of venepuncture alone.

Adrenaline infusion in man

In these tests, the effects of the procedures were controlled both by the control infusions, during which venepunctures were spaced at about the same intervals as those before and after the adrenaline infusions, and by showing that the eosinophil counts did not fall during the adrenaline infusions. It was therefore thought that the fall in clotting time observed in silicone-coated tubes after giving adrenaline was a real effect of the drug. It was hence not thought necessary to repeat the detailed clotting tests on control infusions.

The further tests made in seven adrenaline infusions indicated that after giving the drug the initial stages of spontaneous clotting were accelerated and that the plasma reacted more rapidly with brain reagent. The components believed to be common to these two reactions are clotting factors V and VII (Biggs *et al.* 1953; Hardisty, 1955); and the special tests for these factors indicated an increase in the activity of V but not of VII. This confirms an isolated observation of Perlick & Kalkoff (1955) in the dog.

These results suggest that an increase in factor V activity shortens the clotting time in silicone-coated tubes, but not in glass tubes. Stohlman, Harrington & Moloney (1951), studying a case of factor V deficiency, similarly found the clotting time normal in glass tubes but prolonged in silicone-coated tubes.

SUMMARY

1. The administration of adrenaline has been reported to shorten the clotting time of the blood.

2. The classical experiments of W. B. Cannon and his associates, using anaesthetized animals, were briefly confirmed with Cannon's technique but could not be reproduced with standard methods. It thus remains uncertain what function of clotting Cannon may have measured.

3. In men undergoing serial venepuncture the clotting time and the eosinophil count were associated. This suggested that the effect of repeated venepuncture was an important variable in experiments comparing the clotting of consecutive blood samples, and that the effect might be assessed by the behaviour of the eosinophil count.

4. In a group of nine men, the difference in clotting time between blood samples obtained before and after thirteen infusions of adrenaline was compared with the difference between clotting times obtained from consecutive

venepunctures spaced at about the same intervals during twelve control infusions. Only following adrenaline was there a significant fall in clotting time, demonstrable in silicone-coated tubes but not in glass tubes.

5. In seven of the adrenaline infusions special clotting investigations were carried out, and the results suggested an increase in the activity of clotting factor V.

We wish to thank the volunteer subjects on whom the experiments were made; Dr P. Armitage and Dr D. N. Lawley for their statistical help and advice; Dr C. P. Stewart and his staff for the fructose and glucose determinations and British Drug Houses, Ltd. for re-assaying the adrenaline solutions which were used. During the period of these investigations G. D. F. was in receipt of a Crichton Research Scholarship of the University of Edinburgh and of a personal grant from the Medical Research Council.

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