THE ANTI-HAEMORRHAGIC ACTIVITY OF ETHAMSYLATE (DICYNENE*)
An Experimental Study
BY
A. R. de C. Deacock and Doreen M. Birley

SUMMARY
The haemostatic effect of ethamsylate was examined by means of a controlled, "blind" trial in which forty-three experiments were performed on a series of twenty-two pigs. The experimental preparation consisted of a standard wound produced by means of an electric dermatome and designed to cause capillary haemorrhage. Blood loss was estimated by weighing swabs. Statistical evaluation of the results indicates that ethamsylate is effective in reducing bleeding and that the magnitude of the reduction is directly proportional to the severity of the unmodified bleeding. No effect of the drug on pulse rate, blood pressure or platelet count was observed.

Surgery is inevitably accompanied by haemorrhage, so familiar a phenomenon that it is easy to lose sight of its implications, both medical and economic. Much of the resources of the operating theatre and the efforts of those who work therein are devoted to dealing with haemorrhage and the consequences of haemorrhage.

Apart from the direct methods of the surgeon, various other techniques have been employed in order to diminish blood loss. These include appropriate positioning of the patient, arteriotomy, blockade of the sympathetic nervous system by various means and reduction of cardiac output by means of drugs, all measures designed to reduce blood pressure. These techniques are, in general, neither simple nor without danger for the patient. As an alternative, the systemic administration of a drug specifically to reduce bleeding would have obvious advantages, were such an agent available.

Two substances for which this action has been claimed are adrenochrome monosemicarbazone (Adrenoxyl) (Beal and Gondaert, 1947; Huygebaert, 1949) and, more recently, ethamsylate (Dicynene). The former has been available for some twenty years, but it has not become established as a clinically effective agent. Ethamsylate, however, has been generally available only since 1963 and, as yet, it is not clear to what extent it may be of value in the control of haemorrhage.

Ethamsylate is diethylammonium 1,4-dihydroxy 3-benzenesulphonate and the structural formula is shown in figure 1. It is a white, crystalline substance presented for clinical use in tablet form for oral administration and in ampoules containing a solution for parenteral use. Both preparations contain 250 mg of the drug. If administered orally, ethamsylate is usually given on the evening prior to surgery. After intravenous injection some 30 minutes is required for the development of the full haemostatic effect of the drug. Any combination of oral, intramuscular or intravenous administration may be adopted and some regimes make use of all three routes (Hachen, 1967, personal communication). Dosage is usually in the range of 5–20 mg/kg and in clinical use the drug appears to be virtually non-toxic with no known contraindications.

Detailed consideration of the mode of action of ethamsylate is beyond the scope of this paper, but

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it appears to be connected with blood platelet activity and, in particular, to thromboplastin formation and platelet agglutination (Hachen, 1964; Raby and Coupier, 1964; Esteve and Laporte, 1965; Wayoff and Jeannin, 1965). Copley (1963) and Johnson and others (1966) have stated that capillary permeability is related to platelet activity and there have been several reports concerning the effect of ethamsylate on capillary wall resistance (Raby and Coupier, 1964; Hachen, 1965a; Plisnier and Annaert, 1965).

Evaluation of ethamsylate as an anti-haemorrhagic agent for use during surgery presents some difficulty. Certain clinical studies have been based upon estimations of the bleeding-time (Muller 1962; Cernea and Mazza, 1965; Hachen, 1965b; Alavoine, Lefebvre and Delpy, 1966; Louis and Paulus, 1966; Ribuot, 1966; Benoit, Michelet and Gironet, 1967), but this is not necessarily closely related to surgical haemorrhage. There have been a number of reports of clinical trials on patients undergoing surgery (Gray and Noble, 1966; Hypher and Carpenter, 1968), but in work of this type it is difficult to arrange adequately controlled conditions because of the many variable factors affecting bleeding and, in addition, accurate measurement of blood loss is not easy. Possibly because of these difficulties, the published work does not always agree as to the value of ethamsylate as a haemostatic and judgements based upon “clinical impressions”—notoriously unreliable—are similarly at variance. The work described in the present report was undertaken in order to determine the effect of ethamsylate on surgical haemorrhage under closely controlled conditions.

METHOD

Animal experiment was selected as the basis for this investigation since the required standardization of technique would not have been possible in the human subject. In order to produce valid results it was considered necessary to employ a “blind”, placebo-controlled method. There was a requirement, therefore, for an animal preparation in which to compare the effects of trial drug and placebo on haemorrhage from a “standard wound”. In accordance with its mode of action, outlined above, ethamsylate is said to be most effective in the control of capillary haemorrhage. For this reason, it was desirable that the standard wound should be such as to produce haemorrhage of this type in order to reveal any haemostatic activity to the best advantage. After several preliminary trials using different animal preparations the following experimental method was adopted.

Experimental technique.

The preparation consisted of the intact, anaesthetized pig and the standard wound was produced by means of an electric dermatome of the type used clinically for the preparation of Tiersch (split skin) grafts. The skin of the pig is similar to that of man and it was found that application of the dermatome produced capillary haemorrhage similar to that seen in the human subject. The animals were all young females weighing between 20 and 35 kg when first used as subjects for the trial and between 28 and 44 kg at the time of completion of the work. They were housed (in indoor sties) and fed in accordance with normal farming practice.

Each animal was the subject of two experiments, receiving on the one occasion ethamsylate and on the other placebo (normal saline), both by the intravenous route. In this way each animal acted as its own control. For each animal ampoules of placebo and of the trial drug were made available, the latter containing 1 g of ethamsylate in 8 ml solution. All ampoules were of similar type and were identified only by a code number, bearing one figure corresponding to that of the pig for which they were intended and another to indicate at which of the two experiments on that animal they should be given. On any one day only two pigs were the subjects of experiments and the preparation of the ampoules was so arranged that one animal received the trial drug and the other the placebo. This resulted in an even “spread” of ethamsylate and placebo throughout the series and also ensured that half of the animals received the trial drug at the first experiment and placebo at the second whilst for the remainder this sequence was reversed. At the same time the random element was retained in the allocation of trial drug or placebo to each pair of pigs and, of course, the person conducting the experiment had no knowledge as to which substance had been administered.

Animals were admitted to the experimental unit for an acclimatization period of two weeks prior
to use in the trial. On the eve of each experiment the site of operation was shaved and washed.

A standardized anaesthetic technique was employed, induction being by means of nitrous oxide and halothane, with oxygen, a cuffed endotracheal tube being inserted as soon as conditions permitted. Thereafter, anaesthesia was maintained with nitrous oxide 5 l./min, oxygen 2 l./min, and halothane 2 per cent (delivered from a Fluotec vaporizer).

After a stabilization period of 10 minutes the contents of the appropriate coded ampoule were given by intravenous injection into an ear vein. Forty minutes later the dermatome was applied to the lateral aspect of the thigh. The instrument was set to cut at a depth of 0.05 inch (1.27 mm) and a strip of partial thickness of skin as nearly as possible 3 inches (7.6 cm) in length was removed. The width of the strip was governed by the size of the cutting blade which was approximately 3 inches (7.6 cm) also. Ten minutes later the blood which had collected on the wound was removed by wiping quickly with a large surgical swab. Any blood which trickled beyond the area of the wound prior to wiping was also absorbed on the swab, although this was necessary in only a few cases. Care was taken not to touch or otherwise disturb the wound itself during the 10-minute waiting period. As soon as the swab had been used it was resealed into a nylon-film bag where it had been kept prior to use and in which it had previously been weighed to the nearest 0.001 g on an accurate balance. The piece of skin which had been excised was sealed into a second weighed nylon-film bag. Finally, the dimensions of the wound were measured. Following reweighing of the two nylon bags a process of simple subtraction gave the weight of blood lost and of skin removed, the latter serving as an indication of the depth of the wound, as mentioned below.

The pulse rate of the animals was monitored throughout by means of a pulse-meter and estimations of the blood pressure were made using an occlusion cuff in conjunction with the pulse-meter. The temperature within the operating theatre was thermostatically controlled. Measurements confirmed that a stable temperature was maintained throughout the series of experiments.

There was an interval of three weeks between the two experiments on each animal in order to allow full recovery from the effects of the first before undertaking the second. Opposite limbs were used on the two occasions and care was taken to position the dermatome in the same way throughout the series.

Venous blood samples were withdrawn from six animals on both occasions on which they were experimental subjects. This was done immediately prior to the application of the dermatome. Blood-platelet estimations were performed on these samples for comparison with results obtained using blood collected just before injection of the trial drug or placebo.

Twenty-two pigs were used in this work, forty-three experiments being performed (only one experiment was carried out on one animal—see below).

RESULTS

Table I presents a summary of the results obtained. Both experiments were successfully completed on each of the twenty-two pigs with the exception of No. 21 which became sick after the first trial and was destroyed. In the case of Nos. 1 and 2 it will be seen that the table is not complete. These animals were the subjects of the first two experiments, on the same day, and the blood loss was estimated using a balance measuring only to the nearest 0.5 g. It was considered that this level of accuracy was not acceptable and no results were recorded. For subsequent work a balance accurate to 0.001 g was used, as described above. For the reasons just given, pigs 1, 2 and 21 were omitted from all comparative analyses.

It was found that the "standard wound" was comparable in depth and size throughout the series. Variations in area (all within the range +11 to −18 per cent of the mean) were compensated for by assessing haemorrhage on the basis of blood loss per unit area.

The administration of ethamsylate produced no observable effect on pulse rate or blood pressure, these being comparable in the placebo and trial drug experiments on each individual and, in fact, throughout the series of animals as a whole. Platelet estimations on the venous blood samples from six of the pigs showed no significant variation between specimens taken before and after administration of either placebo or the trial drug.
Table I
Experimental results: tabulation of data.

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Column 1 = Size of wound in cm × cm.  
Column 2 = Area of wound in sq. cm.  
Column 3 = Weight of excised skin in g.  
Column 4 = Depth of wound expressed as g/sq.cm.  
Column 5 = Blood loss in g.  
Column 6 = Blood loss in g/sq.cm.  
Column A = Difference between blood loss under placebo and that under ethamsylate in g/sq.cm (i.e. placebo 6—trial drug 6).  
Column B = Ratio of blood loss under placebo to that under ethamsylate (i.e. placebo 6/trial drug 6).
**Statistical interpretation.**

Examination of the thickness of the excised skin by comparing the weight per sq.cm in the placebo and in the trial drug experiments shows that the difference is not significantly different from zero ($t=0.5306$, $P=0.6$). This suggests that variation in the depth of the wound may be excluded as a source of error.

Evaluation of the data obtained may be carried out in several ways. If the mean blood loss per sq.cm in the placebo experiments (0.0478 g/sq.cm) is compared with that in the trial drug experiments (0.0243 g/sq.cm) it is found that the difference is not significant. This is only to be expected in view of the large degree of between-subject variation and it was in anticipation of this that the investigation was so arranged that each animal could act as its own control.

A better method of evaluation is to examine the effect of the drug on each individual in turn and then to pool the data (see column A in table I). When tested against the null hypothesis that the drug is ineffective this method gives a value of $t=2.4266$, $P<0.05$. This is an acceptable level of significance.

A desirable property of a haemostatic drug is that it should control heavy bleeding at least as well as it controls light bleeding. From the results, it appears that the largest difference between the blood loss in the placebo experiment and that in the trial drug experiment occurred in those animals where the unmodified haemorrhage was greatest, i.e. ethamsylate appeared to be most effective in individuals having a tendency to bleed heavily. This apparent effect may be examined with the aid of the graph shown in figure 2 where

**FIG. 2**

Relationship between blood loss under placebo (ordinate) and difference between blood loss under placebo and under ethamsylate (abscissa) plotted for 19 animals concerned in the comparative analysis.
the unmodified (placebo) blood loss is plotted against the difference between that and the blood loss after ethamsylate for each of the nineteen individuals concerned in the comparative analyses. The graph indicates a direct relationship between the severity of the unmodified bleeding and the reduction in bleeding following the administration of ethamsylate. The associated regression coefficient is 0.5840 and the correlation coefficient is 0.9126, both very highly significant, P<0.001.

An additional inference from the high value of the correlation coefficient is that the experimental method adopted is satisfactory for the task in hand.

Confirmation of the belief that the suppression of bleeding is related to the bleeding tendency is obtained by examining the ratio of blood loss (in g/sq.cm) under placebo to that under ethamsylate. The values of this ratio in the nineteen animals concerned in the comparative analyses are shown in table I (column B). For these nineteen values of the ratio, the mean value of their logarithm is 0.2525 and its Standard Error is 0.0915. This gives a value of t=2.7600, P=0.007.

CONCLUSIONS
Under the conditions described:
(1) Ethamsylate is shown to be effective in reducing bleeding.
(2) The magnitude of the reduction is directly proportional to the severity of the unmodified bleeding.

ACKNOWLEDGEMENTS
We wish to express our thanks to Messrs. Baxter Laboratories Ltd., for the provision of supplies of ethamsylate and placebo; to Messrs. Laboratoires Om, Geneva, for very generous financial support; and to Mr. D. W. Clarke, for his invaluable and skilled assistance in the preparation and care of the animals and in the administration of anaesthesia.

REFERENCES

L’EFFET ANTIHÉMORRHAGIQUE DE L’ETHAMSYLATE (“DICYNENE”): UNE ETUDE PRÉLIMINAIRE

SOMMAIRE
L’effet hémostatique de l’éthamsylate a été étudié à l’aide d’un essai “aveugle” contrôlé, comprenant 43 expériences sur une série de 22 cochons. La préparation expérimentale était constituée par une blessure standard, faite avec un dermatome électrique et causant une hémorragie capillaire. La perte de sang a été estimée en pesant les compresses. L’évaluation statistique des résultats indique que l’éthamsylate réduit efficacement le saignement et que le degré de cette réduction est directement proportionnelle à la sévérité.
du saignement non-modifié. Aucun effet du médicamente n’a été observé sur le pouls, la pression sanguine ou le nombre de thrombocytes.

**DIE ANTIHÄMORRHAGISCHE AKTIVITÄT VON ATHAMSYLAT (“DICYNEN”): EINE EXPERIMENTELLE STUDIE**

*ZUSAMMENFASSUNG*


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**CORRESPONDENCE**

**THE INFLUENCE OF THE KALLIKREINTRYPSIN INACTIVATOR “TRASYLOL” ON THE SERUM CHOLINESTERASE**

Sir,—Thank you for the opportunity of replying to Dr. Doenicke’s letter.

It is true that in some cases, the serum esterase activity was higher than the initial values, 60 min after Trasylol administration. We have pointed out that the inhibitory activity of Trasylol on the serum cholinesterase lasted for 30 min approximately and that serum esterase activities returned to the initial values or surpassed them 60 min after Trasylol administration. The return to even higher values for a relatively short period of time, after a previous inhibition, is a well-known pharmacological phenomenon and this fact confirms in our opinion our findings, and should not arouse any doubts, as Dr. Doenicke thinks, about the method used.

The blood samples were centrifuged immediately after their withdrawal, then they were kept in the refrigerator (—2° to —4°C) and the determinations of serum cholinesterase were done as quickly as possible (in none of the cases was the time from the withdrawal of the blood until the determination was done, greater than 60 min).

None of the patients had an operation or was given an anaesthetic while the enzyme was determined. Most of them were in the medical wards, suffering from different pathological conditions (as can be seen from tables I and II) and others were in the surgical wards awaiting surgery, or they had had an operation a few days previously. Incidentally, case No. 4 was myself, and I had my serum cholinesterase activity determined too, before and after Trasylol administration.

No other drugs were administered to those patients between the time when the control sample was taken and the last sample of blood was withdrawn (except Trasylol) and there is no doubt that Trasylol caused the enzyme inhibition.

We did not claim that the prolonged apnoea observed in our three cases which received Trasylol under general anaesthesia with muscle relaxants (*Brit. J. Anaesth.* (1966), 38, 838) was due to the inhibition of cholinesterase by Trasylol, as two of those cases had been given tubocurarine as a relaxant. Certainly some other cause is to be blamed at least for the cases in which the patients received tubocurarine. Nevertheless, Trasylol temporarily inhibits the serum cholinesterase, and we postulated that this too could be a cause of prolonged apnoea, for a relatively short period of time, at least in some patients who have succinylcholine as a relaxant, and in whom their serum cholinesterase will be inhibited in a higher degree by Trasylol.

G. CHASAPAKIS

*Athens*

**CARDIOVASCULAR EFFECTS OF INTRAVENOUS ANAESTHETICS IN THE DOG**

Sir,—Drs. Conway, Ellis and King are to be congratulated on the excellence of their article published in the October issue of the Journal. We feel, however, that important results have been omitted. To quote: “Drugs were given through the catheter into the inferior vena cava at a constant speed of injection.”

It is not clear whether “constant speed” refers to total dose of drug or whether twice the time was taken to inject the double dose. The time to make the injection is also omitted; this is vital if their results are to be compared with similar work on either animals or man.

A. D. CLARKE

P. W. JACKSON

*Manchester*

**REFERENCE**


Sir,—The standard speed referred to in our paper was such that the mass of drugs at each dose level was given over 20 seconds.

C. M. CONWAY

D. B. ELLIS

N. W. KING

*London*