INTRA-ARTERIAL BARBITURATES

A Study of some Factors leading to Intravascular Thrombosis

BY

S. STUART BROWN, S. MORRELL LYONS AND JOHN W. DUNDEE

SUMMARY

A study was made of the effects of mixing thiopentone and methohexitone with blood in the proportions likely to occur in accidental intra-arterial injection of the barbiturates. In addition to crystal formation, which had been previously reported, red blood cell haemolysis and platelet aggregation also occurred. Since these all lead to intravascular clotting the damaging effect of intra-arterial injection of barbiturates could be explained on this basis. On the basis of a further study of the crystal-forming properties of the commercially available barbiturates in aqueous solutions at differing dilutions, it was concluded that this was less likely to occur with low concentrations. Thus the more potent the drug the safer it is with respect to the sequelae of intra-arterial injection because it can be used in a more dilute solution.

The multiplicity of explanations for the damage resulting from intra-arterial thiopentone or other barbiturates suggests that there exists a lack of basic knowledge of the fundamental mechanisms involved (Cohen, 1948; Forrester and Saunders, 1955; Stone and Donnelly, 1961). The theories include:

1. Spasm of the vessel.
2. Intimal damage and thrombosis (Kimbrough and Shepherd, 1959).

Biopsy has revealed that, irrespective of the role played by various factors, thrombosis occurs eventually in all cases where there is permanent tissue damage. It is hard to see how thrombosis could result from spasm or noradrenaline release alone, but it could conceivably be caused by crystals in the blood stream. Waters' (1966) explanation, that crystals of insoluble thiopentone acid formed in the blood stream are swept along and block the small vessels of the hand, could account for the rapid onset of symptoms. This mechanical blockage might then bring about the release of noradrenaline, the resulting vascular spasm reinforcing the stagnation of the arterial blood flow. If persistent, this could lead to thrombosis in the arterioles, which might spread in a retrograde manner to the main artery. Waters' evidence of the formation of the crystals is indisputable, but his explanation of the connection between this and the thrombosis that occurs on the basis of a mechanical blockage, is difficult to accept. Mather (1966) questioned whether 50-100 mg of thiopentone could produce enough micro-emboli to be entirely responsible for the damage, while he pointed out that venous thrombosis can occur after thiopentone injection. Many other workers have shown that intravenous thiopentone can cause venous damage (Robertson and Williams, 1961; Hewitt et al., 1966).

Before discussing the topic further it would be appropriate to consider the process of intravascular clotting and the mechanisms which contribute to this (Roderique and Wynands, 1967). Blood clotting occurs after a series of enzymatic reactions which lead to the formation of insoluble fibrin from soluble fibrinogen. This requires thrombin which is formed from prothrombin under the action of thromboplastin. The latter may arise from an extrinsic tissue source or an intrinsic blood source. The earliest events in the intrinsic pathway involve the interaction of plasma clotting factor XII with a foreign surface.
This then reacts with plasma factor XI to form a new reactive product which in the presence of plasma factors V, VIII, IX and X, along with platelet phospholipids and calcium, leads to the thromboplastin formation.

The foreign surface for the action of factor XII may be an area of endothelial damage but might in the context of this paper be the surface of foreign matter present in the blood stream, namely thiopentone acid crystals. Also platelets will adhere directly to any area of endothelial damage and cause a nidus for thrombosis, or they might adhere to the crystals. Finally platelets adhere and aggregate under the influence of adenosine diphosphate (ADP) which is released both from the platelets themselves (from its precursor adenosine triphosphate) and also from damaged red blood cells.

Thus, clotting could be caused by:

1. A foreign solid material in the blood stream.
2. Intimal damage.
3. Release of ADP from damaged red blood cells or platelets.

It is conceivable that the crystals demonstrated by Waters (1966) in the blood stream could, by direct action, damage the formed elements in the blood, and the vessel intima. The present paper reports studies designed to investigate this possibility in greater detail. As a result of the findings further investigations were carried out to examine the crystal-forming properties of most clinically available intravenous barbiturates. The methodology and results of these two parts of the study will be presented independently and the final discussion will deal with the whole concept.

**STUDIES WITH WHOLE BLOOD**

Thiopentone in 2.5 and 5 per cent concentrations was added to varying volumes of fresh heparinized blood and, after mixing for 2 minutes, was centrifuged for a further 2 minutes at 4,000 r.p.m. Each specimen was then examined with the naked eye for the presence of crystals and haemolysis, and the pH was measured using a direct-reading EIL glass-electrode pH meter. Because an aqueous solution might produce haemolysis when added to blood, the experiment was repeated using isotonic saline as the solvent for thiopentone sodium. The procedure was also carried out using 2 and 5 per cent solutions of methohexitone in water and saline. The results are shown in tables I and II.

Mixtures containing 1 ml blood and 3 ml barbiturate result in marked haemolysis without detectable crystallization. Both crystallization and haemolysis occurred with equal volumes of blood and barbiturate and with mixtures containing more blood than barbiturate (V/V). With equal volumes and concentrations, haemolysis was more marked with thiopentone than with methohexitone. Whereas the extent of crystallization appeared to be similar with both drugs, the larger methohexitone crystals settled between the red cells and plasma after centrifuging, rather than on top of the plasma as occurred with thiopentone (figs. 2 and 3). Comparative sizes of the crystals can be seen in figures 4 and 5. The differences found between the effects of aqueous and saline solutions of either drug were negligible.

Since crystallization was only found in the presence of a marked fall in alkalinity, an attempt was made to prevent this fall by using thiopentone dissolved in an isotonic phosphate buffer, pH 11.6. However, the buffering power of whole blood was so great that this failed to prevent the fall in pH which was almost as great as when saline or water were the solvents. Equal volumes of 5 per cent aqueous solutions of commercially available thiopentone and blood had a pH of 9.6, whereas equal volumes of buffered 5 per cent thiopentone solution and blood had a pH of 9.8.

Normal arterial forearm blood flow is in the range of 0.5–1 ml/sec (Dittmer and Grebe, 1959). With the rate of injection of barbiturates normally employed in clinical practice, it will be seen that the proportions of barbiturate solution and blood studied in tables I and II more than cover the range likely to be encountered in the event of accidental intra-arterial injection. Thus, this study shows that, in such an event, intra-vascular haemolysis and crystal formation will occur.

**STUDIES WITH PLATELET-RICH PLASMA**

Fresh heparinized blood was centrifuged at 1200 r.p.m. for 10 minutes and the platelet-rich plasma separated, the heparin content of the blood
### TABLE I

*Observations made following mixing of whole blood with varying amounts of thiopentone solution.*

<table>
<thead>
<tr>
<th>Thioptone %</th>
<th>Volume of whole blood (ml)</th>
<th>Volume of solution added (ml)</th>
<th>Concentration* mg/ml</th>
<th>Crystal formation</th>
<th>Haemolysis</th>
<th>pH</th>
<th>In water</th>
<th>Crystal formation</th>
<th>Haemolysis</th>
<th>pH</th>
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<td>+</td>
<td>7.5</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Concentration of thiopentone is calculated in mg/ml by subtracting the red cell volume from the total volume of the specimen.

The extent of crystal formation was graded as follows:

- **+** definite crystal formation;
- **++** heavy deposit of crystals adhering to side of test tube.

The degree of haemolysis is illustrated in figure 1, **++** being very marked and **+** indicating definite but less obvious haemolysis.

### TABLE II

*Observations made following mixing of whole blood with varying amounts of methohexitone solution.*

<table>
<thead>
<tr>
<th>Methohexitone %</th>
<th>Volume of whole blood (ml)</th>
<th>Volume of solution added (ml)</th>
<th>Concentration* mg/ml</th>
<th>Crystal formation</th>
<th>Haemolysis</th>
<th>pH</th>
<th>In water</th>
<th>Crystal formation</th>
<th>Haemolysis</th>
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</table>

* Concentration of methohexitone is calculated in mg/ml by subtracting the red cell volume from the total volume of the specimen.

The extent of the crystal formation was graded as follows:

- **+** definite crystal formation;
- **++** heavy deposit of crystals adhering to side of test tube.
Platelet counts, expressed as $10^9$/ml, found with mixtures of plasma and barbiturate or barbiturate crystals as described in text.

<table>
<thead>
<tr>
<th>Barbiturate</th>
<th>Time (min)</th>
<th>Plasma plus</th>
<th>Plasma plus</th>
<th>Plasma plus</th>
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<tr>
<td></td>
<td></td>
<td>Saline</td>
<td>Aqueous</td>
<td>Saline and</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>barbiturate</td>
<td>crystals</td>
</tr>
<tr>
<td>Thiopentone</td>
<td>0</td>
<td>235</td>
<td>214</td>
<td>158</td>
</tr>
<tr>
<td>5%</td>
<td>5</td>
<td>235</td>
<td>182</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>235</td>
<td>139</td>
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<tr>
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<td>205</td>
<td>220</td>
</tr>
<tr>
<td>5%</td>
<td>5</td>
<td>224</td>
<td>183</td>
<td>207</td>
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<tr>
<td></td>
<td>10</td>
<td>224</td>
<td>190</td>
<td>175</td>
</tr>
<tr>
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<td>272</td>
<td>258</td>
<td>142</td>
</tr>
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<td>5</td>
<td>272</td>
<td>180</td>
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<td>10</td>
<td>272</td>
<td>180</td>
<td>121</td>
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</table>

Platelet aggregation, seen as a fall in platelet count, is produced by all three barbiturates, the effect being most marked with thiopentone. Crystals produced a greater fall in platelet count than the parent barbiturate solution, this again being more marked with thiopentone.

Thus, three factors which could contribute to intravascular thrombosis have been demonstrated. Whilst the size of the crystals (figs. 4 and 5) would inevitably lead to intimal damage in small blood vessels, the degree of platelet aggregation which they cause (table III) suggests that they may be of even greater significance. For this reason the ability of a large number of barbiturates to produce crystals was studied in detail.

Studies with aqueous solutions

Ten barbiturates, all of which have been used clinically, and which are drawn from different chemical groups (table IV) were selected for study. Each was made up in aqueous solution at concentrations varying between 0.5 and 100 mg/ml. The alkalinity of these was measured using the glass-electrode pH meter. A mixture of 5 per cent carbon dioxide in oxygen was then bubbled through each solution to reduce alkalinity without altering the volume (Waters 1966), until precipitation was first visible to the naked eye, at which point the pH was again measured.

<table>
<thead>
<tr>
<th>Barbiturates studied, grouped according to the classification of Dundee (1961).</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=2 — Side chain</td>
</tr>
<tr>
<td>O</td>
</tr>
<tr>
<td>S</td>
</tr>
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</table>

Generally speaking, the constituents of each of the four chemical groups behaved similarly, both with respect to the fall in pH on dilution and the pH at which precipitation became evident. A typical result from each of the groups is shown in figure 6. Table V lists the pH at which precipitation occurs with varying concentrations of the currently commercially available drugs.
Fig. 1
Haemolysis following mixing thiopentone and blood and centrifuging for 2 minutes (−, 0, + and ++). From left to right:
(a) control solution (equal volumes blood and saline);
(b) 0.75 per cent thiopentone added to equal volumes of blood (degree of haemolysis graded as +);
(c) 5.0 per cent thiopentone added to equal volume of blood (degree of haemolysis graded as ++).

Fig. 2
Same solutions as in fig. 1, photographed obliquely to show crystals on top of plasma.

Fig. 3
Mixture of methohexitone and blood showing crystals at red cell/plasma interface.

Left: 7.5 per cent methohexitone added to an equal volume of blood (no crystals seen).
Right: 5.0 per cent methohexitone added to an equal volume of blood (crystals seen).

Fig. 4
Microphotograph showing size and shape of thiopentone acid crystals relative to blood cells.

Fig. 5
Microphotograph showing size and shape of methohexitone crystals relative to blood cells.
Table V and figure 6 shows that dilution, and consequent change in pH, is a major factor in determining the liability of different drugs to precipitate free acid crystals. However, oxybarbiturates, as compared with thiobarbiturates, appear less likely to cause crystal formation, whereas methylation was not found to be an important factor.

DISCUSSION

With normal forearm blood flow and the usual clinical rates of administration, the injection of thiopentone into the brachial artery will result in a 0.3–3.0:1 mixture of barbiturate and blood. The current findings show that, using 5 per cent thiopentone, the resulting range of blood concentrations would invariably result in crystal formation, red blood cell haemolysis and platelet aggregation. Any of these alone could initiate intravascular thrombosis and with all three present some degree of clotting is likely to occur. Although other mechanisms may be involved these factors could account for the undesirable sequelae of the intra-arterial injection of thiopentone.

**TABLE V**

*pH at which precipitate occurs when 5 per cent carbon dioxide in oxygen is bubbled through commercially available barbiturates.*

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Thiopentone (Intraval; Pentothal)</th>
<th>Thiobutabarbitone (Inactin)</th>
<th>Methohexitone (Brietal; Brevital)</th>
<th>Hexobarbitone (Cyclonal; Evipan)</th>
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<tr>
<td>0.5</td>
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</tbody>
</table>

* No precipitate visible.
This study throws no light on treatment once the damage has been done, but reinforces the views already expressed by many writers that the use of dilute solutions constitutes the most reliable safety factor. This is particularly important with the thiobarbiturates (thiopentone, thialbarbitone) which are commonly used in concentrations which will cause extensive intravascular crystal formation and haemolysis. Methohexitone is safer than thiopentone in this respect, as it is rarely used clinically in concentrations exceeding 2 per cent. There may also be an additional safety factor because of the absence of the sulphur atom.

In the search for new intravenous barbiturates, this study would suggest that the safety of these drugs, with respect to accidental intra-arterial injection, is directly related to their potency. The more dilute the solution which can be used clinically, the less is the likelihood of intravascular clotting. Other factors being equal, an oxybarbiturate should be favoured over a thiobarbiturate.

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REFERENCES