Heparin $^{35}$S Removal in Anephric Patients Undergoing Regular Dialysis

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In spite of the fact that heparin is used as an anticoagulant in nearly every dialysis unit in the world, no studies of heparin turnover during the procedure seem to have been undertaken. Normally, 20% of injected heparin is excreted unchanged in the urine within 24 hours. The anephric state might reasonably be expected to modify this. In addition, patients on regular dialysis treatment receive 80,000 to 100,000 units of heparin weekly for many years and this might modify heparin disposal.

MATERIALS AND METHODS

Commercial heparin obtained from Weddell laboratories and labelled with $^{35}$S at the sulphate group, was kindly made available by Dr Ogle of the Radiochemical Centre, Amersham. It was supplied as an 18 mg/ml solution with a specific activity of 5.1 $\mu$Ci/g.

The $^{35}$S in dialysate and urine was assayed in a liquid scintillation counter without deproteinising, as 0.5 ml samples in 12 ml Bray's solution (naphthalene-PPO-POPPOP), together with 2 ml 1.5 m hyamine 10X. To compensate for variable quenching of counts, all samples were recounted with an internal standard of $^{35}$S and corrected. In three patients $^{35}$S radioactivity was also assessed after drying 0.2 ml of plasma on planchettes by counting in a low background counter. The results agreed excellently with the liquid scintillation method and the results given here are those of the liquid scintillation method.

The biological activity of the heparin was assayed (1) in vitro, and (2) after injection into two patients by the thrombin time, and (3) in three patients using a protamine titration, expressing the results as a heparin equivalent.

Only ten experiments were performed, three in vitro and seven in vivo. One of the in vitro experiments and two of the in vivo gave unsatisfactory results owing to misjudgment of dose.
RESULTS

In vitro experiments In two experiments a standard dialysis set-up using PVC tubing and a Kill dialyser was mounted, with a 3 l volume representing the patient. After injection of heparin $^{35}$S into the 3 l circulating volume, the kidney was placed in the circuit, then, after a further 40 minutes, the dialysate was switched on. This procedure was adopted to test whether the heparin was being absorbed either onto the dialysis tubing or onto the cellulose membrane of the dialyser, or whether it was being removed by dialysis.

![Graph showing dialysis experiments]

In the first experiment (Figure 1) an aqueous saline solution was employed. There was no detectable adsorption in 30 minutes to the dialysis tubing, nor in a further 40 minutes to the dialyser membrane. However, after the dialysate flow was started at 500 ml/min, the amount of $^{35}$S counts halved in 40 minutes and remained relatively constant thereafter. In the second experiment (Figure 1) a plasma protein solution at 5 g/100 ml was employed. The biological activity of the material, as judged by thrombin times, again showed no loss before the dialysate was started. Thereafter, the activity declined mono-exponentially with a half life of 62 minutes.

In vivo experiments Two normal subjects and three patients on regular dialysis who had had nephrectomies gave satisfactory results (Figures 2 and 3). Two of the anephric patients were studied during dialysis and one between dialyses, which were performed in these patients at the dialysis centre twice
Figure 2. Biological heparin activity following injection of $^{35}$S heparin

Figure 3. $^{35}$S counts in plasma following injection of $^{35}$S heparin
weekly. Kil dialysers were wet built with PT 300 cellulose membranes; a
dialysate flow rate of 500 ml/min and a mean blood flow rate of 160 ml/min
were used. The results are shown in Table I and Figures 2 and 3.

Table I

<table>
<thead>
<tr>
<th></th>
<th>Dose of heparin injected (units)</th>
<th>t(½) 20-80 min (min)</th>
<th>Duration of RDT (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>35S</td>
<td>Biological (method)**</td>
</tr>
<tr>
<td>Normals</td>
<td>DF</td>
<td>4500</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>PC</td>
<td>8000</td>
<td>34</td>
</tr>
<tr>
<td>Anephrics</td>
<td>HN</td>
<td>4500</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>BB*</td>
<td>4500</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>TB*</td>
<td>5000</td>
<td>94</td>
</tr>
</tbody>
</table>

* on dialysis during study
**TT thrombin time
pr.t protamine titration

These results show that two of the three patients who had been on dialy-
sis for considerable periods before study were capable of eliminating the
biological activity in a relatively normal manner. There is some suggestion
from this small amount of data that the 35S counts did not behave identically
with the biological activity of the material. During the first 20 minutes of
each study there is a more rapid elimination of counts followed by a period
from 20-80 minutes during which counts are removed more slowly than bio-
logical activity. These findings would suggest the initial elimination of counts
not attached to active heparin molecules, followed by the slow accumulation
in the plasma of 35S, possibly from metabolised heparin molecules or involved
with circulating lipids and again not biologically active.

CONCLUSIONS

i) An insignificant amount of heparin is adsorbed by PVC dialysis tubing
and cellulose membrane in the extracorporeal circuit of the haemo-
dialyser.

ii) A small amount of heparin activity is removed by dialysis; in view of
the size of the heparin molecule this probably does not represent the
whole molecule.

iii) Anephric patients were capable of removing injected heparin in a
manner similar to the normal subjects.

iv) Biological methods are satisfactory for the study of heparin turnover;
total 35S counts on unfractionated plasma give results which require
complex interpretation.