FORMAZAN PRODUCTION: TEST FOR TISSUE VIABILITY PRIOR TO TRANSPLANTATION

SUMMARY

Formazan production by tissue slices has been proposed as a rapid, prospective method of assessing organ viability prior to transplantation. In these experiments, formazan production by biopsies of canine liver and kidney has been measured by two methods. The biopsies were obtained from the excised organs during storage at 37°C for 00-90 min. Neither method demonstrated a significant change in formazan production during storage under these conditions.

A simple, rapid, prospective test of the viability of an organ would be of great value in renal and hepatic transplantation. The preliminary results of a proposed test have been reported recently (4, 5). The principle of the test is to incubate small pieces of the tissue with a tetrazolium salt, which is reduced in the presence of functioning dehydrogenase enzyme systems to coloured water-insoluble formazan (1). The time elapsed until the appearance of the first perceptible colour change is measured, and this period is stated to be related to the function of the dehydrogenase enzyme systems and, thus, to the viability of the organ (4).

We have measured formazan production by biopsies of canine liver and kidney in two ways. In the first experiments, a liver lobe and a kidney were removed from a dog and stored at 37°C. Small biopsies (1 mm³ approximately) were cut from the periphery of the organ at 1/2-hr intervals. The time until the appearance of a colour change (after incubation with tetrazolium bromide) was measured using the method described by Terawaki et al. (4) and Maginn (4). The measurements were performed by at least two independent observers.

The results of one set of experiments are shown in Table I. The time for the appearance of a colour change did not alter significantly for kidney or liver with storage up to 1½ hr at 37°C. Similar results were obtained in two further experiments.

These results suggested that either our application of the method was at fault or the method did not detect changes in formazan production during the relatively short periods of storage.

We, therefore, decided to measure the production of formazan directly using a method similar to that of Black et al. (1). A canine liver lobe was stored at 37°C, and slices (1 mm thick and 1 cm square) were cut at intervals. The slices were incubated at 37°C for 1 hr in a flask containing 3 parts of 1% aqueous tetrazolium chloride (TTC) and 1 part of 0.9% sodium chloride (total volume 5 ml) buffered to pH 7.2 with phosphate. At the end of the hour, the supernatant was decanted and the reaction was stopped by the addition of 10% formalin in saline. The formazan was extracted from the tissue with four successive acetone washings; formazan is readily soluble in acetone, and acetone has no effect on the reduction of tetrazolium (unpublished observations). The acetone washings were pooled and made up to a final volume of 5 ml. The optical density was read at 470 mµ, this wavelength being the peak of the emission spectrum for formazan. The number of µg of TTC/mg of tissue reduced was then determined from a standard curve obtained as follows. Standard amounts of TTC were re-

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TABLE 1. Time required for first perceptible darkening of TTC during storage of canine liver and kidney

<table>
<thead>
<tr>
<th>Storage time at 37 °C (min)</th>
<th>Mean time for colour change (sec) ± S.E.</th>
<th>Liver*</th>
<th>Kidney*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.7 ± 0.3 (2)</td>
<td>7.3 ± 0.7 (7)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>9.8 ± 0.4 (6)</td>
<td>8.0 ± 0.4 (6)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>9.2 ± 0.4 (14)</td>
<td>6.7 ± 0.4 (6)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>9.0 ± 0.6 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The figure in parentheses indicates the number of biopsies tested.

account such factors as the variation in the penetration of the tetrazolium salt into the tissue (3), the effect of meso- 
thelial barriers on the penetration (3), and the varying amount of endogenous 
substrate for the enzyme systems (3). It 
seems likely that our results could be ex-
plained in terms of these, and perhaps 
other, factors. It might be possible to alter 
the methods to allow for such variables. 
However, the essence of the tests, namely, simplicity and speed, would then be lost.

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