**99mTc-Diethyl-HIDA**

**A Contribution to the Study of Its Structure**

Ugo Fonda* and Bente Pedersen
The Isotope-Pharmacy, 378 Frederikssundsvej, DK-2700 Brøndshøj, Denmark

**Abstract.** Sephadex column chromatography has shown that 99mTc-diethyl-HIDA consists of two clearly separable components. One of them seems to be an intermediate compound changing into the other, almost completely within two hours after reconstitution of the diethyl-HIDA (Solco HIDA) with pertechnetate. The two components could be the mono- and biscomplex of N-(2,6-diethyl-acetanilid)-iminodiacetic acid with one atom of technetium. Animal experiments show that there is no important difference in the biological distribution in mice between the two components.

**Introduction**

During routine quality control of 99mTc-diethyl-HIDA (Solco HIDA) at the Isotope-Pharmacy gel filtration on Sephadex G25f indicated that the labeled compound consists of two components, the amount of which varied from preparation to preparation. As separation on Sephadex G25f was not sufficient to allow calculation of the percentages, we decided to try other Sephadex-G types. Biological distribution of the two components was studied in mice.

**Materials and Methods**

Solco HIDA, lot 41208/4 [N-(2,6-diethyl-acetanilid)-iminodiacetic acid] (Wistow et al., 1977), was used in all the experiments. Each vial contained 40.2 mg, including 0.1 mg of tin. The kits were reconstituted in accordance with manufacturers’ directions, using 4 ml eluate (8–16 mCi) from 100 mCi Amersham generators. The preparations were studied by gel-chromatography-column-scanning using Sephadex G10, G15, G25 fine, and G25 superfine in glass columns (1.5 x 30 cm) (Persson, 1974). Four problems were examined:

1. Choice of Sephadex type to obtain separation of the two components.
2. Variation in the composition of the preparation in relation to time after reconstitution. 100 μl samples were applied and the columns eluted with 15–18 ml saline purged with nitrogen and scanned on a chromatographic scanner. The percentage of activity in each peak was calculated.
3. Properties of fractions eluted from G15 (1.5 x 17 cm) columns, determined by refiltration. To separate and study the two components in the preparation, a shorter G15 column was employed and the eluate was collected using a fraction collector. One of the fractions from each of the two eluted peaks was refiltered on G15 columns.
4. Biological distribution in mice. Three groups of mice (19–26 g) were injected in the tail vein with 100 μl of either 99mTc-diethyl-HIDA (2–4 min after reconstitution), 99mTc-diethyl-HIDA (15–20 min after reconstitution), or a fraction from the first eluted peak. The animals were killed 2 min after injection (Wistow et al., 1977). Liver, kidney, ileum (with stomach), and carcass were counted and activity in the organs were calculated as a percentage of total activity in the animal.

**Results**

The best separation of the two components in 99mTc-diethyl-HIDA was achieved with Sephadex G15. Figure 1 shows some characteristic chromatograms obtained with the four types of Sephadex. The chromatograms in Figure 2 show the influence of time elapsed since reconstitution of the kit on the ratio between the two components. Immediately after addition of pertechnetate, component I prevailed. With time, component I turned into component II, which had a higher mobility. The reaction also proceeded during elution, as indicated especially by Figures 2 (1 min) and 3a. Part of the activity was retained at the application point on all the columns. The percentage varied from 9 to 24% of the applied activity. The higher values were obtained when the sample was applied on the column 1 min after preparation. This fraction is probably due to exchange of 99mTc between the che-
counts

G 10

G 15

G 25-F

G 25-SF

Fig. 1. Characteristic chromatograms showing the degree of separation of the two components in Tc-99m-diethyl HIDA obtained with different types of Sephadex

Fig. 2. Characteristic chromatograms showing changes in the respective amounts of the two components in Tc-99m-diethyl-HIDA when applied on Sephadex G15 columns at different times after preparation

Fig. 3. a Activity in fractions (1 ml) eluted from a Sephadex G15 column. b Chromatograms of fraction 20 and 46 after refiltration on a Sephadex G15 column

Table 1. Biological distribution in mice. % of total activity in organ

<table>
<thead>
<tr>
<th></th>
<th>% in liver</th>
<th>% in kidney</th>
<th>% in liver and ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl-HIDA injected 2-4 min after reconstitution</td>
<td>$\bar{x}^*$</td>
<td>26.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Diethyl-HIDA injected 15-20 min after reconstitution</td>
<td>$\bar{x}^{**}$</td>
<td>31.0</td>
<td>1.9</td>
</tr>
<tr>
<td>'Fraction 20' injected after separation on Sephadex columns</td>
<td>$\bar{x}^{**}$</td>
<td>29.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

$\bar{x}^*$ mean of 5 values
$\bar{x}^{**}$ mean of 6 values