EFFECT OF ETHYLENEDIAMINETETRAACETIC ACID ON DEPOSITION AND EXCRETION OF CERTAIN RARE EARTH ELEMENTS*

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This paper deals with a small facet of the problem of understanding and predicting the behavior of chelating agents in the body; namely, on the relationship between the effect of a chelating agent introduced into the body and stability constants as determined in the test tube.

Methods

The study was carried out by determining the effect of calcium ethylenediaminetetraacetate on the excretion and distribution in rats of a number of rare earths and yttrium, and correlating the magnitude of these effects with the stability constants of the chelates formed with some of the rare earths. These rare earths were selected because they form a long series of closely related elements with similar chemical and physical properties and because the stability constants of the chelates formed with ethylenediaminetetraacetic acid (EDTA) have been determined (1). Yttrium was studied because of its close relationship to the rare earths.

The following isotopes1 were used: Ce144, Pm147, Tb160, Y91, and Tm170. The isotopes were administered, either essentially carrier-free or with trace amounts of carrier, intravenously in 0.5 per cent sodium citrate solution at pH 5 to male Sprague-Dawley rats weighing 300 gm. Ten animals were injected with each isotope. Half of the animals were given 200 mg. of Ca EDTA intraperitoneally per kilo once daily for 4 days. Urine and feces were collected and assayed daily. At the end of 4 days, all of the animals were killed, and liver, spleen, and long bones (humeri, radii, ulnae, femorae, and tibiae, including all epiphyses) were removed. The remainder of the carcass was assayed as a unit. In preparation for counting, the tissues were dry ashed and the ash was dissolved in 6 N HNO3. Cerium, yttrium, terbium, and thulium were counted in a scintillation

* This work was carried out under the auspices of the United States Atomic Energy Commission.

1 The Ce, Pm, and Y were obtained from the Oak Ridge National Laboratory. The Tb and Tm were obtained through the courtesy of Dr. P. W. Durbin at the Crocker Laboratory in Berkeley, California.
counter with a well type sodium iodide crystal. The Pm samples were plated on stainless steel disks and β-counted in a proportional flow counter. Only the bone samples produced sufficient mass on the counting plates to require correction for self-absorption.

Results

The results of the study, including recovery values and ranges of variation, are presented in Table I. The pattern of distribution and excretion of the various rare earths in the control animals is fairly similar to that obtained by Durbin et al. (2). The few differences in the results of the two studies, such as lower values of Ce and Pm in the liver and lower skeletal value of Tm in our study, might well be due to the difference in mode of administration, intravenously in our experiments as compared to intramuscularly in theirs. Spleen values in the Ce study were high, suggesting the presence of some colloid in the injection solution. For the other isotopes, however, the spleen values are low, as in the study of Durbin et al., indicating that the rare earths were present in the blood stream in ionic form rather than as colloids.

An index to the relative binding affinities of the chelating agent for the rare earths under physiological conditions, which in this study are manifest by the effectiveness of the chelating agent in altering distribution and excretion of the injected elements, was obtained by calculating the ratios of the amount of rare earth in the tissues and excretion of the control animals to the amount of rare earth in the tissues and excretion of the treated animals. The ratios are calculated from data expressed in terms of per cent of recovered dose rather than the data presented in Table I, which are given in per cent of injected dose. Ratios were calculated for total excretion, whole body content, and for certain specific tissues; namely, liver and long bones.

In Fig. 1 these ratios are plotted against the log of the stability constant for the chelates formed by EDTA and the respective rare earths. The values for the stability constants are those determined by Wheelwright et al. (1), with the exception of Pm. Since the Pm value was not determined by these authors, it was necessary to estimate the value from their data. This was done by assuming that the Pm value would follow the straight line relationship illustrated and by merely selecting the assumed Pm value from its position on the curve. The ratios for whole body content, bone, and liver range from 1 to 2.4 and are plotted along the scale on the left of the graph. The ratios for excretion range from 2 to 3.4 and are plotted along the scale to the right of the graph. Excretion ratios are calculated as the reciprocal of the distribution ratios; namely, the values for the treated animals divided by the values for the controls. Calculation of the ratio in this fashion was made to rotate the slope of the plot by
TABLE I

Effect of Ca EDTA on Distribution and Excretion of Intravenously Administered Rare Earths and Yttrium

<table>
<thead>
<tr>
<th></th>
<th>Ce</th>
<th>Pm</th>
<th>Tb</th>
<th>Y</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca EDTA-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine........</td>
<td>22.82*</td>
<td>18.93 ± 1.26</td>
<td>47.94 ± 5.29</td>
<td>51.34 ± 3.20</td>
<td>53.15 ± 0.83</td>
</tr>
<tr>
<td>±0.96†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces........</td>
<td>7.09 ± 0.99</td>
<td>6.01 ± 0.09</td>
<td>3.95 ± 0.41</td>
<td>3.89 ± 1.10</td>
<td>5.59 ± 0.60</td>
</tr>
<tr>
<td>Liver........</td>
<td>24.58 ± 0.70</td>
<td>18.69 ± 2.28</td>
<td>2.00 ± 0.03</td>
<td>1.36 ± 0.80</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td>Spleen.......</td>
<td>2.08 ± 0.00</td>
<td>0.43 ± 0.01</td>
<td>0.11 ± 0.00</td>
<td>0.64 ± 0.13</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Long bones...</td>
<td>5.73 ± 0.40</td>
<td>8.03 ± 0.10</td>
<td>8.11 ± 0.06</td>
<td>5.36 ± 0.41</td>
<td>1.79 ± 0.13</td>
</tr>
<tr>
<td>Carcass‡.....</td>
<td>31.46 ± 0.91</td>
<td>36.51 ± 5.61</td>
<td>38.43 ± 0.78</td>
<td>31.37 ± 0.50</td>
<td>29.18 ± 2.36</td>
</tr>
<tr>
<td>Total........</td>
<td>93.76 ± 2.8</td>
<td>88.60 ± 2.90</td>
<td>100.54 ± 4.2</td>
<td>98.90 ± 2.7</td>
<td>90.58 ± 2.11</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine........</td>
<td>3.24 ± 0.12</td>
<td>8.57 ± 2.0</td>
<td>15.62 ± 0.49</td>
<td>18.11 ± 1.01</td>
<td>9.71 ± 1.08</td>
</tr>
<tr>
<td>Feces........</td>
<td>11.52 ± 1.66</td>
<td>4.73 ± 0.4</td>
<td>4.99 ± 0.87</td>
<td>2.85 ± 0.04</td>
<td>7.03 ± 0.60</td>
</tr>
<tr>
<td>Liver........</td>
<td>32.08 ± 0.70</td>
<td>28.85 ± 5.99</td>
<td>5.94 ± 0.35</td>
<td>2.81 ± 0.46</td>
<td>1.83 ± 0.03</td>
</tr>
<tr>
<td>Spleen.......</td>
<td>3.24 ± 0.02</td>
<td>0.88 ± 0.08</td>
<td>0.24 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Long bones...</td>
<td>4.73 ± 0.13</td>
<td>8.37 ± 0.02</td>
<td>13.84 ± 0.23</td>
<td>11.84 ± 0.38</td>
<td>4.39 ± 0.90</td>
</tr>
<tr>
<td>Carcass‡.....</td>
<td>29.86 ± 0.58</td>
<td>42.02 ± 3.83</td>
<td>63.37 ± 1.48</td>
<td>60.68 ± 1.35</td>
<td>63.58 ± 4.2</td>
</tr>
<tr>
<td>Total........</td>
<td>84.67 ± 1.7</td>
<td>91.42 ± 3.65</td>
<td>103.99 ± 3.7</td>
<td>96.44 ± 2.14</td>
<td>86.60 ± 4.6</td>
</tr>
</tbody>
</table>

* The results are expressed in terms of per cent of injected dose.
† Standard deviations are indicated.
‡ The carcass includes the remainder of the viscera, skeleton, and the skeletal muscle.

Fig. 1. The relationship of stability constants to chelating effectiveness in the body

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90° and thereby allow easier comparison of the excretion data with the distribution data. The apparent identity of the excretion data with the distribution data with respect to magnitude is fortuitous and comes about only because of the choice of the position of the ordinate scale on the right of Fig. 1.

DISCUSSION

Because of the variation inherent in a biological experiment of this sort, in which so few animals were used, the scatter of the data is such that it is not possible to obtain precise quantitative correlations. However, from the grouping of the data, a definite relationship is apparent. As seen in Fig. 1, the data tend to fall along a line to the region of Pm, where the curve flattens. Yttrium data deviate from the line, but this is not surprising. From its behavior on ion exchange columns, from which it is more readily stripped by EDTA than rare earths with about the same stability constants, one might have predicted that Y would lie above the line.

In view of the complicated series of reactions which occur when a chelating agent is introduced into the body, meaningful explanation of the shape of the curve is difficult and at best speculative. The chelating agent must compete with natural binding agents, protein, bone, the reticulo-endothelial system, and with the hydrolysis reactions of the rare earths. In addition, naturally occurring cations such as Ca compete with the rare earths for the chelating agent. Schubert (3) has devised an equation illustrating these relationships, which, for the purposes of comparing one rare earth with another, can be condensed to the following:

\[ R_{MV} = \frac{(K_{MV})^3(K_{M(OH)V})}{K_{MOH}K_{MP}} \times C \]

where \( R_{MV} \) = the ratio of EDTA complex-forming rare earth to ionic rare earth; \( K_{MV} \) = the stability constant of the EDTA rare earth chelate; \( K_{M(OH)V} \) = the stability constant of EDTA-hydroxylated rare earth chelate; \( K_{MOH} \) = the equilibrium constant for hydrolysis of the rare earth; \( K_{MP} \) = the stability constant of the rare earth natural binding agent complex; and \( C \) = a constant.

Of these factors, \( K_{MOH} \) has been shown by Wheelwright et al. (1) to vary very little from one rare earth to another. Therefore, it is not likely to play an important role in effecting the different affinities of EDTA for the various rare earths. The same probably holds true for \( K_{M(OH)V} \). The most important factors, then, which determine the effect of EDTA upon the distribution and excretion of the rare earths in the body are \( K_{MV} \), the affinity of EDTA for the various rare earths, and \( K_{MP} \), which is indicative...
of the net natural binding affinity. These two factors primarily influence the shape of the curve in Fig. 1.

The natural binding affinities are manifest most strongly in the region of the lower rare earths and decrease sharply with increase in atomic number, i.e., uptake of Ce by the liver of 32 per cent of dose as compared to 1.75 per cent of the dose of Tm held by the liver. The flattening of the curve probably results from the overwhelming effect of the natural binding affinities in the region of the lower rare earths. With increasing atomic number, binding with EDTA becomes the greater factor in influencing the distribution of the rare earths, and a direct relationship between EDTA stability constant and the chelating effect becomes apparent.

The site of the flattening of the curve can be varied by the conditions of the experiment. By varying the dose of the chelating agent, one can alter the magnitude of the effect of the agent and thereby shift the turnover point on the curve. However, at the high dose level used in this study, 200 mg. per kilo, the maximal effect of the chelating agent was obtained, and the change of slope on the curve at the region of Pm probably adequately represents the region in the series where the chelate binding overshadows the effect of natural binding forces.

SUMMARY

This study illustrates a relationship between the effect of a chelating agent, ethylenediaminetetraacetic acid, when introduced into the body and the stability constants of chelates formed by this agent and various members of the rare earth series.

BIBLIOGRAPHY

Effect of Ethylenediaminetetraacetic Acid on Deposition and Excretion of Certain Rare Earth Elements

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