ABSORPTION AND METABOLISM OF CORTISONE-4-C\textsuperscript{14} ACETATE

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(Received for publication, September 2, 1954)

Previous work in this laboratory with steroids labeled with C\textsuperscript{14} (1-10) has been extended to include the present experiments on the absorption and disposition of the radioactive carbon of cortisone-4-C\textsuperscript{14} acetate\textsuperscript{1} in the rat. Since the conditions of these experiments, i.e. solvent vehicle, strain and type of animal studied, operative techniques, method of collection of specimens, and C\textsuperscript{14} assay, did not vary appreciably from past techniques, comparison of data obtained in the present studies on cortisone-4-C\textsuperscript{14} acetate with those of the other labeled steroids studied is facilitated.

EXPERIMENTAL

A solution of 0.2 mg. (0.24 mc.) of cortisone-4-C\textsuperscript{14} acetate in 1 ml. of 50 per cent ethanol or of 0.2 mg. in 0.2 ml. of dibutyl succinate was given intragastrically or intramuscularly, respectively, to adult male rats. In addition to normal adult rats, other animals studied were prepared either by cannulation of the bile duct, the intestinal lymphatic, or the thoracic duct, or by ligation of the bile duct. Polythene tubing with a beveled tip was used for all cannulations. Rats with intestinal lymphatic fistulas were given the compound intragastrically, while rats with cannulated thoracic ducts received an intramuscular injection 24 hours postoperatively. Cumulative samples of lymph were taken at the end of 4, 8, 12, 24, and 48 hours. Excreta and bile were obtained at regular intervals for 5 days in most cases and C\textsuperscript{14} was determined by the procedure already reported (2). Expired CO\textsubscript{2} was collected from representative animals in metabolism cages similar to those previously described (1).

RESULTS AND DISCUSSION

Examination of the expired air of normal rats failed to detect any C\textsuperscript{14} during the 5 day experimental period; this indicates that ring A of radio-cortisone is not completely oxidized by the rat to a significant extent.

\textsuperscript{1} Obtained through the kindness of the Radioactive Steroids Allocations Committee of the Endocrinology Study Section, National Institutes of Health, Bethesda 14, Maryland.

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Data on the absorption of cortisone-4-C\textsuperscript{14} acetate are presented in Table I. If this hormone were absorbed into the lymphatics from muscle, radioactivity in the lymph would have been removed from the animal by the thoracic duct fistula. The data show that absorption occurred in this experiment, but not by the lymphatics, since 42 per cent of the C\textsuperscript{14} was detected in the urine and no significant amount in the lymph over 48 hours. These data clearly indicate that absorption and transportation occur via the blood stream.

Similarly, a significant amount of C\textsuperscript{14} was not detected in intestinal lymph after intragastric administration, although 46 per cent was excreted by the kidney. Consequently, the portal blood must be the route of absorption of intragastrically administered radiocortisone. These data are analogous to those obtained in previous experiments with 17-methyl-C\textsuperscript{14}-estradiol-17\beta (2), 17a-methyl-C\textsuperscript{14}-testosterone (9), and carboxy-labeled C\textsuperscript{14}-deoxycholic acid (8).

The daily excretory pattern of C\textsuperscript{14} after both routes of injection is depicted in Fig. 1. In every case, the largest area representing total C\textsuperscript{14} excretion occurred during the first 24 hours, although, in the control animals of the intramuscular series, the excretion by the major pathway (fecal) reached its peak on the 2nd day.

Total excretion is listed in Table I. As in all similar experiments in these laboratories with steroids containing C\textsuperscript{14}, the principal route of excretion for C\textsuperscript{14} is dependent upon the type of the recipient animal. In

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
Type of rat & Lymph & Bile & Urine & Total \\
\hline
Thoracic duct fistula, i.m. & 0 & 42.0 & 45.9 \\
Intestinal lymph fistula, i.g. & 0 & 38.8 & 55.9 & 94.7 \\
Controls, i.g., i.m. & 77.0 & 41.5 & 9.1 & 101.1 \\
Bile fistula, i.g. & 73.1 & 20.7 & 11.4 & 98.5 \\
Ligated duct, i.g., i.m. & 88.0 & 98.5 & 0.5 & 88.5 \\
\hline
\end{tabular}
\caption{Average Excretion of C\textsuperscript{14} after Injection of 0.2 Mg. of Cortisone-4-C\textsuperscript{14} Acetate}
\end{table}

* Two rats were used in each of the types of lymph fistula experiments; three in each of the remaining experiments; i.g. = intragastric administration; i.m. = intramuscular administration.

† All values are totals for 5 days, except those of the lymph fistula series which are for 48 hours.
normal animals, the C\textsuperscript{14} was present in greatest amounts in fecal matter, this C\textsuperscript{14} reaching the gastrointestinal tract by biliary passage, since rats with cannulated bile ducts had greater amounts of C\textsuperscript{14} in the bile. Interruption of biliary excretion, as in the rats with ligated bile ducts, resulted in an amount of urinary radioactivity much greater than that present in urine of normal animals, this increase in renal excretion approaching the amount normally present in bile.

The rapidity with which C\textsuperscript{14} was excreted in these experiments is shown in Table II. Bile collected from rats in the intragastric and intramuscular series in the first 4 hours of the experiment contained 27.9 and 33.1 per cent of the administered C\textsuperscript{14}, respectively. During the first 12 hours, about 60 per cent was present in bile in both series, while approximately 10 per cent was excreted by the same route over the succeeding 12 hours. Only traces of C\textsuperscript{14} were present in bile after 24 hours and none after 36 hours, regardless of the route of administration.

Rapid excretion also occurred in the urine of duct-ligated animals in the first 12 hours (Table II). The average for the intragastric group was 53.8 per cent, in contrast to 31.6 per cent over the same interval by comparable animals in the intramuscular series. The rate of excretion of C\textsuperscript{14} after administration of cortisone-4-C\textsuperscript{14} acetate was significantly faster than that of other C\textsuperscript{14}-steroids studied in our laboratories under these conditions with the exception of 17-methyl-C\textsuperscript{14}-estradiol given intramuscularly (2).
It is interesting to note that the percentages of C\textsuperscript{14} in the urine of controls after administration of cortisone-4-C\textsuperscript{14} acetate (Table I) were higher than those after administration of physiological amounts of labeled estrogens (2, 7), progesterone (1, 3), androgens (4–6), and even after Compound A acetate (10) and hydrocortisone (11).

Fig. 1 shows differences in the rates of excretion of C\textsuperscript{14} between the two series for both the normal and duct-ligated animals. Since comparable amounts were present in the bile collected during the first 4 hours and the excretion was almost complete at the end of 36 hours, it appears that rates of absorption from the two sites do not differ significantly. Therefore, it seems more probable that the differences in rates of excretion may not be real differences, but only the result of irregularity in the elimination of urine and feces.

**Table II**

*Per Cent of Administered C\textsuperscript{14} in Bile and Urine after Intragastric and Intramuscular Administration*

<table>
<thead>
<tr>
<th>Hrs. after administration</th>
<th>0-4</th>
<th>4-8</th>
<th>8-12</th>
<th>12-24</th>
<th>24-36</th>
<th>36-48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile of bile fistulas, i.g.</td>
<td>27.9*</td>
<td>24.3</td>
<td>10.0</td>
<td>12.5</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; i.m.</td>
<td>33.1</td>
<td>21.1</td>
<td>9.7</td>
<td>8.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Urine of duct-ligated rats, i.g.</td>
<td>53.8†</td>
<td>26.2</td>
<td>8.9</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; i.m.</td>
<td>31.6</td>
<td>27.9</td>
<td>19.9</td>
<td>4.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each figure represents an average value for three rats.
† Cumulative samples collected from 0 to 12 hours.

Reabsorption of some biliary C\textsuperscript{14} from the intestine was indicated by differences in levels of C\textsuperscript{14} in the urine of normal rats, about 40 per cent in both series, and in urine of rats with cannulated bile ducts from 15 to 20 per cent. The former would include some of the recycled metabolites reaching the general circulation. In addition, total C\textsuperscript{14} in bile (77 and 73 per cent) of rats with bile fistulas differed from those in feces (56 and 59 per cent) of control rats, thus supporting the concept of some enterohepatic circulation of cortisone-4-C\textsuperscript{14} acetate or its metabolites. By similar techniques, Hyde and Williams (12) have suggested the same phenomenon for hydrocortisone in the rat.

Enterohepatic circulation has been directly demonstrated for the metabolites of testosterone-4-C\textsuperscript{14} (4), of 17a-methyl-C\textsuperscript{14}-testosterone (9), and of cholesterol-4-C\textsuperscript{14} (13) by refeeding bile to rats with bile fistulas. Bradlow et al. (14), in their cortisone-T studies in mice, suggest reabsorption of cortisone from the intestine since "much of the hormone or its metabolites rapidly reaches the gastrointestinal tract and yet so large a portion
is eventually excreted in urine." Apparently the degree of enterohepatic circulation is much more extensive for bile acids and cholesterol than for steroid hormones. Matschiner et al. (8) have shown that the C\textsuperscript{14} of carboxy-labeled C\textsuperscript{14}-deoxycholic acid is excreted approximately 10 times more rapidly in bile than in feces. Siperstein and Chaikoff (15) also observed a much slower fecal than biliary excretion of the C\textsuperscript{14} of cholesterol-4-C\textsuperscript{14}.

**SUMMARY**

The C\textsuperscript{14} of cortisone-4-C\textsuperscript{14} acetate is not absorbed by the intestinal lymphatic system, or by the peripheral lymphatics, after enteral or intramuscular administration into the rat under these experimental conditions. There is rapid appearance of radioactivity in bile after either intramuscular or intragastric injection of the compound, indicating rapid absorption from both sites. About 75 per cent of the administered C\textsuperscript{14} was present in the bile, about 60 per cent in the feces of normal rats, and 90 per cent in the urine of animals with ligated bile ducts. No significant amount of C\textsuperscript{14} was detected in feces of animals with biliary surgery after either method of administration or in the expired air of the control series.

**BIBLIOGRAPHY**

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