METABOLIC FATE OF DIETHYLSTILBESTROL
IMPLANTED IN THE EAR OF STEERS

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Summary

Four steers were implanted in the ear with approximately 28 mg of ¹⁴C-diethylstilbestrol (¹⁴C-DES) and were slaughtered 30, 60, 90 or 120 days later. All urine and feces were collected from time of implantation until slaughter. Total recoveries of ¹⁴C from excreta and tissues ranged from 82.7 to 90.9% of ¹⁴C implanted (avg 86.8%). The percentages of implanted ¹⁴C remaining in the ears were 65.9, 27.3, 30.0 and 4.2 at 30, 60, 90 and 120 days after implantation, respectively. Radioactivity was measurable in the plasma, urine and feces throughout the collection period for all steers.

Total excretions (urine and feces) were 34.7, 56.8, 52.5 and 84.9% of the ¹⁴C implanted for steers killed at 30, 60, 90 and 120 days, respectively. Approximately twice as much was excreted in feces as in urine. The concentrations of ¹⁴C were similar in the livers, kidneys, lungs and salivary glands of all steers. In the livers, 15 to 33% of the ¹⁴C present was characterized by isotopic dilution as ¹⁴C-DES or a conjugate of DES (.07 to .13 ppb DES equivalents).

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⁴Reference to a company or product name does not imply approval or recommendation of the product by the U.S.D.A. to the exclusion of others that may be suitable.

The major part of ¹⁴C in urine was present as either DES or a hydrolyzable conjugate of DES. The majority of the ¹⁴C in the feces was not present as DES or a DES conjugate.

Introduction

Interest in the metabolic fate of diethylstilbestrol (DES) implanted subcutaneously arose soon after animal scientists demonstrated that DES stimulated rate and efficiency of growth in ruminants. Stob (1956) reported increased estrogenic activity in feces of sheep implanted with 12 mg of DES. At 70 days after implantation (last sampling), the estrogenic activity had declined but was still evident. Estrogenic activity in muscle and liver from lambs 104 to 106 days after implantation with 12 mg of DES was reported by Ellis et al. (1954). Stob et al. (1954) found estrogenic activity in muscle from sheep 84 days after implantation with 12 mg of DES; however, these data were not entirely consistent. Later reports by these investigators indicated no estrogenic activity in muscle and liver from lambs 73 days after implantation with 12 mg of DES (Andrews et al., 1956) nor in liver and kidney from steers 140 days after implantation with 36 mg of DES (Stob et al., 1968).

The metabolic fate of oral DES in steers and sheep has been reported (Aschbacher, 1972; Aschbacher and Thacker, 1974; Hinds et al., 1965; Mitchell et al., 1959), and estrogenic activity has been found in excreta and tissues after implantation with DES, as indicated above. However, there are no studies in which the metabolic fate of implanted DES has been determined quantitatively. In the present study
we implanted $^{14}$C-DES in the ear of steers and measured (1) total excretion of $^{14}$C, (2) the distribution of $^{14}$C in tissues at various times after implantation and (3) the proportion of the $^{14}$C in liver and excreta present as $^{14}$C-DES or its conjugate.

**Experimental**

$^{14}$C-DES Pellets. Pellets containing $^{14}$C-DES with a specific activity of 49 mc/mmole were formulated as described by Rumsey et al. (1975b). The pellets weighed 15.7 to 16.6 mg and contained 88.2% DES. Two pellets were dissolved in benzene, one at the time the $^{14}$C-DES was implanted and the other after storage for approximately 1 year in the dark at 37 C. These solutions were used to determine the concentration of $^{14}$C in the pellets and radiopurity of the $^{14}$C-DES.

Experimental Conditions. White-faced steers were purchased at a local stockyard and acclimated to laboratory conditions for 6 weeks. They were fed free choice a 1:1 mixture of alfalfa, hay, s-c gnd pelleted, mn 15% protein IRN 1-00-121 and a pelleted grain mixture (34% oats, grain, grnd, IRN 4-08-471; 30% barley, grain, grnd, feed gr, IRN 4-08-343; 19% corn, yellow, grain, grnd, IRN 4-02-992; 15% soybean, seeds, solv-extd grnd, mx 7% fiber, IRN 5-04-604; 1% TM salt and 1% calcium phosphate, dibasic, commercial, IRN 6-01-080).

Four steers were each implanted in the base of one ear with two $^{14}$C-DES pellets by an individual experienced in the use of an implanting gun (Hess and Clark Automatic Implanter). The steers were placed in elevated metabolism stalls (Aschbacher, 1970) and all excreta were collected until slaughtered. Each steer was taken out of the stall three times a week for an exercise period and a thorough washing of the area of the abdomen covered by the large rubber urine-collecting funnel. A steer was slaughtered 30, 60, 90 and 120 days after implantation.

Four additional steers were implanted with commercial DES pellets (Hess and Clark) on the same day that the $^{14}$C-DES was implanted, and were maintained in conventional pens in an area removed from the $^{14}$C-DES-implanted steers. One was slaughtered 2 days before each of the $^{14}$C-DES-implanted steers was slaughtered; tissue samples from these additional steers were used to establish background values for $^{14}$C assays.

Sample Collection and Preparation. Blood samples were obtained by jugular puncture three times during the first 24 hr after implantation, daily for the next 3 days and twice weekly thereafter. Urine was collected, weighed and assayed for $^{14}$C daily. Feces were collected daily for the first 3 weeks, but thereafter were allowed to accumulate 2 or 3 days before weighing and sampling. Each feces collection was thoroughly mixed with a large food mixer before a subsample (approximately 100 g) was freeze-dried for $^{14}$C assay. Urine and feces held for later extraction were stored at -20 C.

Samples of the following materials were obtained at the time of slaughter: liver, kidney, lungs, adrenal, spleen, heart, gallbladder with bile, dermal fat, visceral fat, lean muscle (round), parotid salivary glands, rumenoreticular contents, omasal contents, small intestinal contents, large intestinal contents (including cecum), abomasum with contents, rumenoreticular tissue, omasal tissue, small intestinal tissue and large intestinal tissue.

With some sections of the gastrointestinal tract, tissues were separated from contents to facilitate sample preparation, but the $^{14}$C in the contents and tissue were combined to express $^{14}$C recovery from the gastrointestinal tract. The carcass was skinned and split, and one-half was ground after the leg bones and pelvic and pectoral girdles were removed. All other samples were ground if necessary and thoroughly mixed before an 80- to 250-g subsample was removed and freeze-dried for $^{14}$C assay.

The ear that contained the $^{14}$C-DES pellets was removed at slaughter, and the residual pellets were carefully dissected free from tissue. Each pellet was dissolved in benzene, and the resulting solutions were assayed for $^{14}$C and radiopurity of the $^{14}$C-DES. The tissue capsule surrounding the pellet was extracted three times with 10 ml of cold methanol, and the remainder of the ear was extracted with methanol in a Soxhlet apparatus. The $^{14}$C content of the extracted tissues was also assayed by combustion.
14C Assays. 14C was quantitated by liquid scintillation (Aschbacher and Thacker, 1974). The assay of freeze-dried tissue was modified to establish background levels with tissues from control animals. Control tissue samples (from a steer slaughtered 2 days before slaughtering a 14C-DES-implanted steer) were assayed just before samples from the 14C-DES-implanted steer were assayed. The t test was used to compare the counts/min (cpm) from a control tissue with the cpm from a similar tissue from a 14C-DES-implanted steer. When the value from an experimental animal was higher (P < .01), the net counts above background were converted to disintegrations/min (dpm) by applying the appropriate quench correction.

Isolation and Characterization of 14C Compounds. 14C from the dissolved pellets and 14C recovered from steers implanted with 14C-DES was examined by thin-layer chromatography (TLC). The TLC systems used silica gel (25-mm thickness on 5 x 20-cm glass plates, Brinkmann Instruments Inc.) and the following solvent mixtures: (1) hexane:diethylether:di-chloromethane, 4:3:2; (2) benzene:diethyl ether, 19:1; (3) chloroform:ethanol, 19:1; and (4) n-propanol:water, 8:1. 14C containing samples were spotted on TLC plates side by side with nonradioactive DES. Radioactive areas were located with a radiochromatogram strip scanner (Packard Instrument Co., Model 7200), and nonradioactive DES was visualized under ultraviolet light.

Radiopurity of 14C-DES was also examined by isotopic dilution procedures. For this purpose, tritiated DES (3H-DES) of approximately 2,000 dpm/mg was prepared by diluting high specific activity 3H-DES (Amersham-Searle Corp.) with nonradioactive DES and then recrystallizing from warm benzene. Approximately 400 mg of this 3H-DES was added to the 14C sample in question, and the mixture was dissolved in 30 to 40 ml of warm benzene. The 3H:14C ratio in the solution was determined, and then crystals were allowed to form at room temperature. The crystals were harvested on a fritted glass funnel, washed twice with 2 to 3 ml of cold benzene and redissolved in warm benzene. This procedure was repeated, and crystals from three successive crystallizations with the same 3H:14C ratio were regarded as evidence for the presence of 14C-DES. A comparison of this constant 3H:14C ratio with the ratio in the original solution was the basis for estimating the percentages of 14C (in the original solution) that was 14C-DES.

14C compounds were recovered from ground liver tissue by extracting once with acetone (2.5 ml/g wet tissue) and once with an acetone:water (8:2) mixture (3 ml/g). The extracts were combined and dried with a flash evaporator. The extract from approximately 500 g of fresh liver was dissolved in 250 ml of .05 M sodium acetate buffer (pH 4.75); β-glucuronidase-aryl sulfatase (Helix pomatia, Calbiochem B grade, GRD activity of approximately 7 IU/ml) was added (1 μl/ml); and the mixture was incubated overnight at 39 C. The incubation mixture was extracted twice with diethylether (1:1), the ether was removed with a flash evaporator, and the extract was dissolved in chloroform. The chloroform solution was partitioned against a NaHCO3 buffer (approximately .5 M adjusted to pH 10.5 with NaOH) and then against .05 M NaOH. After the NaOH was neutralized, it was extracted with chloroform, and the 14C in the chloroform was examined by isotopic dilution procedures. Figure 3 outlines the procedures described above.

Recovery of 14C compounds from feces was as described for liver, except that the hydrolysis step was omitted. The fecal extracts were examined by TLC and isotopic dilution procedures. In some instances, extracts were reacted with bis-trimethylsilyl trifluoroacetimide containing 1% trimethylchlorosilane (Regisil, Regis Chemical Co.), and the resulting trimethylsilyl derivatives were examined by gas-liquid chromatography (GLC) as described previously (Aschbacher, 1972).

14C compounds in urine were recovered with a Porapak Q column as described earlier (Aschbacher and Thacker, 1974), except that some of the nonradioactive material was eluted with a 30% methanol: water solution before 14C compounds were eluted with 100% methanol. The 14C compounds eluted with 100% methanol were hydrolyzed as described for liver extracts. A Porapak Q column was again used to recover the 14C compounds from the hydrolysate; the elution sequence was water, 30% methanol, 80% methanol and 100%
methanol. Remaining conjugates were expected to elute with 80% methanol and free DES with 100% methanol. The \( ^{14} \)C eluted with 100% methanol was characterized by TLC and isotopic dilution.

Two urinary and fecal samples from each \( ^{14} \)C-DES-implanted steer were examined; one collected during the first week after implantation and the other near the time of slaughter.

**Results and Discussion**

**Animal Performance.** The animals did not exhibit any digestive disturbances or other gross signs of pathological conditions. Steers slaughtered 30, 60, 90 and 120 days after implantation weighed 292, 322, 366 and 356 kg, respectively; and their gains from time of implantation to slaughter were .27, .85, .79 and .57 kg/day, respectively. The gains, although not outstanding, are acceptable for animals confined to metabolism stalls.

**Assay of Pellets and Radiopurity of \( ^{14} \)C-DES.** The dpm/mg in the two pellets assayed were 3.669 \( \times 10^{8} \) and 3.574 \( \times 10^{8} \) (avg = 3.621 \( \times 10^{8} \)). The pellets were expected to contain 3.564 \( \times 10^{8} \) dpm/mg, based on the formulation and the specific activity of the \( ^{14} \)C-DES indicated by the supplier. The radiopurity of the \( ^{14} \)C-DES in the pellet dissolved at the time the steers were implanted was 96.8% (± 1.2) as determined by isotopic dilution. TLC analysis (solvent system 1) revealed two major bands corresponding to cis and trans DES, which contained 95.9% of the \( ^{14} \)C that could be eluted from silica gel scraped from the TLC plate. There was a small radioactive band at the origin (2.8%) and a suggestion of a band in front of the DES. Results with solvent systems 2 and 3 were similar. Isotopic dilution studies indicated a radiopurity of 88.9% (± 1.4) for the \( ^{14} \)C-DES in the pellet that was stored for 1 year before it was dissolved in benzene. Recovery from bands corresponding to DES was 92.4% of the activity eluted from a TLC plate (solvent system 1). Again there was a radioactive band at the origin (4.6%) and one in front of the DES bands (2.0%). A decrease in radiopurity due to autodegradation was expected because the \( ^{14} \)C-DES was of very high specific activity and was stored as a solid with little dilution (Rochlin, 1965).

The radiopurities (isotopic dilution) of the pellets recovered from the ears at the time of slaughter were as follows: 30 days, 94.4% (± .9) and 92.6% (± .5); 60 days, 94.0% (±3.2); 90 days, 95.6% (±1.7) and 95.1% (±3.6); 120 days, 94.2% (±1.4). Radiopurity of the \( ^{14} \)C-DES in pellets recovered from the ears may have been slightly lower than the radiopurity observed for the pellet dissolved at the time of implanting; however, the mobilization of DES would still be adequately reflected by \( ^{14} \)C assays.

\( ^{14} \)C Levels in Plasma. \( ^{14} \)C was measurable in plasma of all blood samples taken 4 hr after implantation. Maximum values ranged from 218 to 1,188 dpm/ml and occurred 8 or 24 hr after implantation (two steers at each time). Figure 1 shows the \( ^{14} \)C levels in plasma from 2 to 120 days after implantation. The level at 120 days was approximately one-third that observed during the period from 5 to 20 days after implantation.

Excretion of \( ^{14} \)C. The weekly excretion (fecal plus urinary) of \( ^{14} \)C is shown in figure 2. As might be expected, the decline in the excretion rate with time was similar to the decline of plasma \( ^{14} \)C concentration. Fecal \( ^{14} \)C excretion was 2.5 to 3 times that in urine. Under what was thought to be similar conditions, Rumsey et al. (1975) observed fecal:urinary ratios of \( ^{14} \)C ranging from one to two. With orally administered \( ^{14} \)C-DES, fecal excretion of \( ^{14} \)C was approximately three
times that in urine (Aschbacher and Thacker, 1974).

Distribution and Recovery of $^{14}C$. Table 1 shows how $^{14}C$ was distributed at the time of slaughter. In the steer slaughtered 60 days post-implantation, only one pellet was found in the ear and several pieces of highly radioactive material were found near the implantation site. The pellet could have been crushed when implanted or later when the steer was in the metabolism stall. An increase in release rate probably resulted from the increased surface of the crushed pellet. Only one pellet was recovered from the steer slaughtered 120 days after implantation. The site of the other implant was apparent but less than .1% of the original $^{14}C$ remained at this site.

Large and small intestinal contents contained approximately 99% of the $^{14}C$ in the gastrointestinal tract of the steers. $^{14}C$ concentrations in the large intestinal contents were 1.5 to 2 times that in the small intestinal contents. Little radioactivity was observed in the abomasal contents, and levels in omasal and rumenoreticular contents were not above background.

$^{14}C$ in Tissues. Table 2 shows $^{14}C$ levels in various tissues of steers implanted with $^{14}C$-DES. As expected, bile-gallbladder, liver and kidney were among the tissues with the highest concentrations. Although the $^{14}C$ concentration in the parotid salivary gland indicated some preferential accumulation of $^{14}C$ in that gland, failure to detect $^{14}C$ in the rumen suggests that recycling via the saliva was not significant. The concentration of $^{14}C$ in the lungs was similar to that in the liver and kidney. This was somewhat unexpected, but Turner (1956) reported estrogenic activity in lung tissue of steers fed 10 mg of DES per day.

The data in table 2 do not suggest a decline in tissue $^{14}C$ concentrations with time after implantation. If these data are considered with results of similar experiments by Rumsey et al. (1975), considerable variation in tissue $^{14}C$

### Table 1. Distribution of $^{14}C$ after ear implantation with $^{14}C$-DES in steers

<table>
<thead>
<tr>
<th>Item</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
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<tr>
<td>Fecal excretion</td>
<td>17.2</td>
<td>39.8</td>
<td>40.1</td>
<td>60.5</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td>7.5</td>
<td>17.0</td>
<td>12.4</td>
<td>24.4</td>
</tr>
<tr>
<td>Earb</td>
<td>65.9</td>
<td>27.3</td>
<td>30.0</td>
<td>4.2</td>
</tr>
<tr>
<td>GI tract$^c$</td>
<td>.21</td>
<td>.18</td>
<td>.16</td>
<td>.10</td>
</tr>
<tr>
<td>Carcass and internal organs$^d$</td>
<td>.04</td>
<td>.03</td>
<td>.03</td>
<td>.05</td>
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<tr>
<td>Total recovery</td>
<td>90.9</td>
<td>84.3</td>
<td>82.7</td>
<td>89.3</td>
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</table>

$^a$Each steer was implanted with two pellets that contained a total of approximately 28 mg $^{14}C$-DES. Each value represents data from one steer.

$^b$Includes residual implants and ear tissue.

$^c$Includes tissue and contents of rumenoreticulum, omasum, abomasum, small intestine, gallbladder, large intestine, cecum and rectum.

$^d$Includes kidney, liver, lungs and carcass.

Figure 2. Weekly excretion (fecal and urinary) of $^{14}C$ after implantation with $^{14}C$-DES in the ear. The number of steers represented by the mean are shown in parentheses. Dots are means, and attached brackets are ranges.
FATE OF DES IMPLANTED IN THE EAR OF STEERS

TABLE 2. $^{14}\text{C}$ IN TISSUES FROM STEERS IMPLANTED WITH $^{14}\text{C}$-DES

<table>
<thead>
<tr>
<th>Tissues</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>dpm/g fresh tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile-gallbladder$^b$</td>
<td>5121 (359)</td>
<td>1360 (157)</td>
<td>1355 (47)</td>
<td>2075 (216)</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>335 (15)</td>
<td>519 (107)</td>
<td>221 (3.6)</td>
<td>409 (39)</td>
</tr>
<tr>
<td>Lungs</td>
<td>104 (1.9)</td>
<td>218 (11)</td>
<td>266 (3.5)</td>
<td>250 (8.1)</td>
</tr>
<tr>
<td>Liver</td>
<td>160 (8.6)</td>
<td>182 (5.8)</td>
<td>176 (1.5)</td>
<td>282 (4.3)</td>
</tr>
<tr>
<td>Kidney</td>
<td>135 (3.2)</td>
<td>148 (5.0)</td>
<td>142 (4.4)</td>
<td>219 (2.3)</td>
</tr>
<tr>
<td>Adrenals</td>
<td>77 (1.8)</td>
<td>65 (5.3)</td>
<td>111 (5.7)</td>
<td>68 (2.0)</td>
</tr>
<tr>
<td>Heart</td>
<td>32 (4.3)</td>
<td>20 (4.8)</td>
<td>20 (6.5)</td>
<td>28 (5.8)</td>
</tr>
<tr>
<td>Spleen</td>
<td>30 (2.0)</td>
<td>34 (1.0)</td>
<td>34 (.5)</td>
<td>62 (.7)</td>
</tr>
<tr>
<td>Dermal fat</td>
<td>85 (3.4)</td>
<td>62 (16)</td>
<td>25 (16)</td>
<td>46 (13)</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>73 (25)</td>
<td>34 (18)</td>
<td>ND$^c$</td>
<td>60 (35)</td>
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<tr>
<td>Carcass</td>
<td>23 (2.3)</td>
<td>15 (4.2)</td>
<td>13 (3.2)</td>
<td>20 (3.3)</td>
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<tr>
<td>Muscle</td>
<td>13 (1.1)</td>
<td>7 (1.7)</td>
<td>28 (8.7)</td>
<td>11 (2.7)</td>
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<td>Blood plasma$^d$</td>
<td>56 (2.7)</td>
<td>64 (1.6)</td>
<td>43 (.0)</td>
<td>42 (5.7)</td>
</tr>
</tbody>
</table>

$^a$Each value represents a sample from one steer. Numbers in parentheses are standard deviations of assays.

The specific activity of the $^{14}\text{C}$-DES was 404 dpm/ng.

$^b$Bile and gallbladder were homogenized together.

$^c$ND = not detected.

$^d$The blood sample was taken at time of slaughter or on the previous day. The concentration is expressed as dpm/ml of plasma.

Concentration among steers within the same time interval is evident. With only a small number of observations, this variation tends to mask trends in tissue concentrations of $^{14}\text{C}$ with respect to time from implantation.

Characterization of $^{14}\text{C}$ in Livers and Excreta. Table 3 and figure 3 summarize the data from liver extractions. A part of the $^{14}\text{C}$ in the livers was characterized as $^{14}\text{C}$-DES (equivalent to .07 to .13 ppb of DES based on the data in tables 2 and 3 and the specific activity of the $^{14}\text{C}$-DES implants). These analyses do not distinguish between the free $^{14}\text{C}$-DES and $^{14}\text{C}$-DES conjugates in the livers. Most of the extractable $^{14}\text{C}$ that did not partition as DES was found in the chloroform fraction after NaOH:chloroform partitioning (fraction 3, figure 3). Little can be said about the nature of this material except that it has obviously lost its phenolic character.

By omitting the hydrolysis step in the isolation procedure, we attempted to determine the amount of unconjugated $^{14}\text{C}$-DES present in one liver. Isotopic dilution procedures with the $^{14}\text{C}$ isolated in this fashion proved difficult because of the large experimental error associated with counting the small quantity of $^{14}\text{C}$ present after the first recrystallization. Only one sample (120-day steer) was analyzed in this manner, and approximately 7% of the $^{14}\text{C}$ in the liver was recovered as $^{14}\text{C}$-DES (.04 ppb DES).

When feces extracts were partitioned, 80 to 94% of the $^{14}\text{C}$ in feces behaved as free DES. Most of this $^{14}\text{C}$ migrated similarly to DES on TLC (solvent system 1); however, isotopic dilution confirmed less than 50% of the $^{14}\text{C}$ as $^{14}\text{C}$-DES. GLC analysis after silylation of the $^{14}\text{C}$ compounds from feces revealed a major radioactive component that eluted at a much lower temperature than the trimethylsilyl ethers of DES. The nature of the $^{14}\text{C}$ in feces collected during the first week after implantation did not differ from that in feces collected later.

These data show that $^{14}\text{C}$-DES was not the major $^{14}\text{C}$ compound in the feces of steers implanted with $^{14}\text{C}$-DES. Solubility data indicate that most of the fecal $^{14}\text{C}$ compounds retained a phenolic character similar to that of DES; however, GLC data suggest the presence of a radioactive molecule in the feces much smaller than DES.

No effort was made to distinguish free DES from DES conjugates during the characterization of $^{14}\text{C}$ in urine because the urine was not cooled during collection, and previous studies (Aschbacher and Thacker, 1974) indicate that...
<table>
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<tr>
<th>Days from implantation to slaughter</th>
<th>$^{14}$C extracted with acetone (Fraction 1)$^b$</th>
<th>$^{14}$C recovered in ether after hydrolysis of acetone extracts (Fraction 2)$^b$</th>
<th>$^{14}$C from hydrolysate that did not partition into NaOH from chloroform (Fraction 3)$^b$</th>
<th>$^{14}$C from hydrolysate that partitioned as DES (Fraction 4)$^b$</th>
<th>$^{14}$C in liver as DES (or DES conjugate)$^c$</th>
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<tr>
<td>30</td>
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<td>66.2</td>
<td>41.2</td>
<td>19.1</td>
<td>15.6</td>
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</table>

$^a$Each row of values represents data from one subsample. All values are expressed as a percentage of the $^{14}$C in the liver.

$^b$See figure 3.

$^c$These values represent the $^{14}$C in fraction 4, figure 3, that was shown by isotopic dilution to be $^{14}$C-DES.
conjugates of DES hydrolyze in urine at room temperature. After glucuronidase hydrolysis and column chromatography, 35 to 76% of the $^{14}$C in the urine was recovered as free DES. Of the eight samples analyzed, recovery as DES was less than 60% from three samples, and there was evidence of incomplete hydrolysis in two of the samples with low recovery. The third sample with low recovery of $^{14}$C as DES had an atypical elution pattern from Porapak Q (21% of the $^{14}$C eluted with 30% methanol). An additional sample of urine collected 1 week earlier from the same animal was analyzed; the elution pattern was typical, and approximately 60% of the $^{14}$C was recovered as DES. We concluded that most of the $^{14}$C in the urine was as DES (or as a DES conjugate), although there were indications of other $^{14}$C-compounds. There was no change in the nature of the urinary $^{14}$C with time after implantation.

**General Discussion.** The $^{14}$C-DES pellets used in this experiment were as similar to commercial DES pellets as possible, and the rate of disappearance of $^{14}$C from the ears of steers was comparable with disappearance of DES from commercial implants (Hale et al., 1959; Rumsey et al., 1974). Because the $^{14}$C-DES in the pellets was of high specific activity and was only slightly diluted, auto-

radiolysis caused a slight decrease in radiopurity with time. Since products of radiolysis could become tissue residues of $^{14}$C, caution should be used in interpreting relatively low levels of $^{14}$C in tissues when the $^{14}$C was not characterized. However, the $^{14}$C-DES in the pellets removed from the ears at the time of slaughter was of sufficient radiopurity that movement and excretion of $^{14}$C clearly indicated mobilization and excretion of DES (or its metabolites).

In this experiment, recoveries of $^{14}$C averaged 86.8% of the $^{14}$C implanted. Although our objective is to recover 100% of the $^{14}$C administered to an animal, we realize that this objective is unattainable. However, when recoveries fall below 90%, the experimental conditions are carefully examined to determine if losses could have occurred via a specific unmonitored route. Expired air was not monitored for $^{14}$C in these experiments, but a significant quantity of $^{14}$CO$_2$ was not expired by sheep given $^{14}$C-DES orally (Aschbacher, 1972). There are no other obvious unmonitored routes for loss of $^{14}$C.

Our data and that of Rumsey et al. (1975) indicate that absorption and excretion of DES continued throughout the 120-day observation period, but there was considerable individual variation in the rate of absorption. $^{14}$C was measurable in tissues of animals slaughtered at all intervals from implantation. A part of the $^{14}$C in the liver from each steer was characterized as $^{14}$C-DES (or a conjugate of DES); the concentrations of DES in the livers ranged from .03 to .36 ppb in these two studies. These levels are below the practical sensitivity of the GLC method for determining DES in tissue (Donoho et al., 1973).

More than 50% of the $^{14}$C in feces of $^{14}$C-DES implanted steers was not DES; this result is contrary to observations with oral DES (Aschbacher and Thacker, 1974). This difference should be interpreted cautiously until the stability of DES in feces is investigated. The major urinary excretion products (DES or its conjugates) were similar for implanted and oral DES.

**Literature Cited**