STUDIES WITH 4-\( ^{14} \)C-MESTRANOL IN LACTATING WOMEN

By

H. G. Wijmenga and H. J. van der Molen*

ABSTRACT

\( ^{14} \)C-Mestranol (5 \( \mu \)c) was administered orally in a Lyndiol\textsuperscript{®*}-tablet (= 5 mg lynestrenol\textsuperscript{**} + 150 \( \mu \)g mestranol) to four women using Lyndiol\textsuperscript{®} during the lactation period shortly after delivery. The concentration of radioactivity in the plasma and the excretion of radioactivity in the urine and milk were studied. The clearance rate of radioactivity from the blood was very low. A half-life in the order of 40–60 h was found for labelled mestranol and its metabolites. In three cases 31–36\% of the radioactivity was excreted into the urine within 5 days after oral administration of the labelled material; in the fourth patient this value was about 52\%.

During a collection period of 4 days after the oral administration of the \( ^{14} \)C-mestranol-containing tablet, 0.0002–0.013 per cent of the administered dose was excreted into the milk. These very low values were partly due to the low amounts of milk that could be collected. It was calculated that with the regular oral administration of one Lyndiol\textsuperscript{®}-tablet daily, with 150 \( \mu \)g mestranol per tablet, about 0.03–0.06 \( \mu \)g (0.02–0.04\% of the administered dose) of mestranol or its metabolites might be excreted per 100 ml milk. The significance of these amounts, in view of the transfer to infants during breast-feeding, is discussed.

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** Trade name N. V. Organon, Oss, The Netherlands.
*** The following trivial names have been used:
Lynestrenol: 17\( a \)-ethynyl-oestr-4-en-17\( \beta \)-ol
Mestranol: 17\( a \)-ethynyl-oestra-1,3,5(10)-triene-3,17\( \beta \)-diol 3-methyl ether
In a previous publication (Van der Molen et al. 1969) the fate of lynestrenol, the progestagen of the oral contraceptives Lyndiol-2.5® and Ovostat®** was studied after oral administration of 14C-lynestrenol to women taking Lyndiol®-tablets.

Since mestranol is the oestrogenic compound of the above mentioned products and is also used in many other oral contraceptives, it seemed of general interest to perform a similar study with 14C-mestranol. This communication presents the plasma levels of the radioactivity and the excretion of radioactivity in milk and urine after the oral administration of 4-14C-mestranol in Lyndiol®-tablets to four normal women during the early post-partum lactation period.

METHODS AND MATERIALS

14C-mestranol

4-14C-mestranol, with a specific activity of 34.2 µc/mg, was obtained from New England Nuclear Corp. (Boston, Massachusetts, U.S.A.). Checks on radiochemical purity were performed by thin layer chromatography on silica plates, with and without added unlabelled mestranol, using the following systems:

- benzene: ethyl acetate (9:1, v/v), cyclohexane: acetone (9:1, v/v), cyclohexane: ethyl acetate (9:1, v/v), and n-heptane: acetone (2:1, v/v).

In all cases, autoradiograms made from TLC-plates with Kodak No-Screen X-ray film, revealed for the radioactive compound only one spot, with the same mobility as non-radioactive mestranol. From the specific activities of 14C-mestranol, applied to the thin layer plates after dilution of the radioactive preparation with unlabelled mestranol, it could be concluded that the radiochemical purity of the preparation was at least 99.5%.

Preparation of 14C-mestranol-containing tablets

In previous investigations on the metabolism of 14C-lynestrenol (Van der Molen et al. 1969) it was observed that the pharmaceutical form of orally administered lynestrenol may greatly influence the absorption. Consequently Lyndiol®-tablets containing 14C-mestranol were used. As outlined by Cox et al. (1968), the preparation of small series of tablets with relatively low amounts of radioactive material needs special care. For our series of tablets containing 14C-mestranol a satisfactory procedure was essentially as follows:

1.997 g of a carefully powdered granulate, containing all the necessary ingredients for the preparation of Lyndiol®-tablets except mestranol, were mixed in a counting vial of 20 ml with 3 ml ethanol, containing 3 mg of a mixture of unlabelled mestranol and 100 µc of 14C-mestranol. The greater part of the ethanol was carefully removed in a slow stream of nitrogen at 50°C. Subsequently the walls of the vial were rinsed with ethanol and the ethanol was evaporated in the same way. To obtain a dry powder the remaining part of the ethanol was removed in a vacuum desiccator for 30 min and the resulting powder equilibrated with room-air. After carefully scraping any adhering powder from the glass wall, 6 glass beads of different sizes were added into the vial, after which the vial was closed and wrapped in polyethylene. Manual shaking for 30 min with tapping of the vial every 2–3 min to keep the glass walls free from adhering powder, yielded a fine homogenous powder. This powder, after removal of
the glass beads, was used for the preparation of the tablets. During tabletting the punchers of the tabletting machine were adjusted in such a way that the first 12 tablets weighed about 100 mg each, whereas the 8 remaining tablets weighed from 73 to 97 mg. These tablets were used for assessing the amount of radioactivity per tablet. Radioactivity was determined after dispersing a tablet of known weight in 1 ml of distilled water in a volumetric flask of 50 ml, and adding Bray's scintillator to 50 ml. Aliquots of 100 µl of the clear supernatant were counted in 16 ml of Bray's scintillator, using 1-14C-hexadecane as internal standard. The relationship between the amount of radioactivity per tablet, in µc/tablet (y), and the weight of the tablet in mg (x) was found to be: y = 0.0517·x, with a residual standard deviation of 0.032 (7 degrees of freedom).

From this relationship the radioactivity of the tablets used for the present study was calculated (Table 1). The equivalent mestranol weight as given in Table 1 was calculated from the amounts of mestranol used (carrier + radioactive) and the manufacturer's declaration for the specific activity of 14C-mestranol. A quantitative estimation of the mestranol content per 100 mg tablet weight was performed after thin layer chromatography of the extract, using a Kober reaction for the extract of the main spot. This estimation demonstrated the presence of 149.3 µg of mestranol per 100 mg instead of the calculated 150 µg, viz. 4.4% too low. In view of the reliability (precision) of this assay the calculated mestranol weight per tablet was accepted to be the true value.

**Lyndiol®-tablets**

Normal tablets, as commercially available, containing 5 mg lynestrenol and 150 µg mestranol, were used.

**Materials used for counting radioactivity**

All solvents used were of analytical grade. The scintillators were of »scintillation grade«. »Hyamine« refers to a one-molar solution of hydroxide of Hyamine 10X in methanol, as commercially available from Packard Instrument Company Inc.

**Radioactivity measurements**

Counting vials with plasma were prepared in triplicate, using the following pro

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**Table 1.**

Weight (µg) and µc of 14C-mestranol in labelled Lyndiol®-tablets.

Equivalent mestranol weight: calculated amount of mestranol per tablet.

<table>
<thead>
<tr>
<th>Tablet No.</th>
<th>Tablet weight (mg)</th>
<th>95% confidence interval for radioactivity (in µc)</th>
<th>Equivalent mestranol weight (µg)</th>
<th>Used for case</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100.9</td>
<td>5.22 ± 0.04</td>
<td>153</td>
<td>XI</td>
</tr>
<tr>
<td>4</td>
<td>98.8</td>
<td>5.11 ± 0.03</td>
<td>150</td>
<td>XII</td>
</tr>
<tr>
<td>2</td>
<td>96.6</td>
<td>5.00 ± 0.03</td>
<td>146</td>
<td>XIII</td>
</tr>
<tr>
<td>1</td>
<td>98.0</td>
<td>5.07 ± 0.03</td>
<td>148</td>
<td>XIV</td>
</tr>
</tbody>
</table>

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cEDURE: 0.5 ml of plasma was mixed in a counting vial with 3 ml Hyamine and the mixture shaken for 1–1.5 h at 55°C in a Dubnoff shaker. After cooling to room temperature, 12.5 ml of a scintillator mixture TCS (10 g PPO + 0.15 g POPOP + 1000 ml toluene + 250 ml methylcellosolve) was added and each vial was counted for at least two cycles of 30 min. In the present study the amounts of milk collected by mechanical means were too low for assaying the individual milk fractions. Consequently the volumes of the separate milk fractions were measured and the volumes collected at 1000, 1400, 1700, 2000, 2300 and 600 were quantitatively transferred into one glass vessel and the combined fractions were considered to represent the milk collected during one day. The daily milk fractions thus obtained were lyophilized and the dry residues extracted four times with methylcellosolve: ether (3:1, v/v). The first three extracts were combined and evaporated under a steam of nitrogen to about 0.1 ml. The precipitate was taken up in 9–10 ml of the same solvent mixture. From the clear supernatant two counting samples were prepared, each containing 4 ml of the extract and 12 ml of Bray scintillator. The fourth extract was treated separately in the same way to check the efficiency of the extraction. All milk samples were counted for at least $5 \times 30$ min per vial.

In a few instances, with milk samples containing a relatively high amount of radioactivity, the milk residue left after the fourth extraction was dried in vacuo and the dry residue used for the preparation of tablets of about 400 mg. These tablets were combusted with oxygen, as described by Kalberer & Rutschmann (1961). It was found that these residues contained negligible amounts of radioactivity, if any (the count rates obtained were hardly above background level).

Urine samples were prepared for counting in triplicate by mixing 1 ml of urine and 15 ml of Bray's scintillator (Bray 1960) in a counting vial. After centrifugation, the samples were counted for a period of 30 min each.

Measurement of radioactivity was performed in a TriCarb liquid scintillation spectrometer model 3375 and an automatic external standardization method was used for the determination of the counting efficiency. During the experimental period the standard error of the counting efficiency, as judged from standard samples with known radioactivity, was less than 1%.

Subjects and design of experiment

The four subjects XI–XIV were healthy lactating women who did not breastfeed their infants because of rhesus immunization. They were given Lyndiol®, one tablet daily, for a pretreatment period of 4 days, starting one day after delivery (Van der Molen et al. 1969). After this pretreatment period, one $^{14}$C-mestranol containing tablet was administered orally on the 5th day, and during the remainder of the experimental period normal Lyndiol®-tablets were again administered daily. All tablets were administered at 8 a.m. The 24-hours' period after the administration of the radioactive material is designated as: day 1. Subsequent 24-hours' periods are numbered: day 2, day 3 etc.

Collection of samples

Blood, milk and urine were collected as described previously (Van der Molen et al. 1969). Heparinized plasma was prepared from the blood in the usual way.
EXPERIMENTAL RESULTS

Plasma

The $^{14}$C-radioactivity measured in plasma at various time intervals after the oral administration of the $^{14}$C-mestranol-containing tablet is given in Table 2. The disappearance of radioactivity from the plasma is seen in Fig. 1 as the logarithm of the per cent of the administered dose of radioactivity per litre of plasma, plotted against the time elapsed between time of collection and time of oral administration. In three subjects a rapid increase in plasma radioactivity was found, with peak concentrations in the order of 1.65 % of the administered dose per litre plasma, observed 4 h after the time of administration. In subject XIII a lower peak concentration of about 1.1 % was found 8 h after the administration of the radioactive tablet. The final parts of the disappearance curves, after 24 h for subjects XI and XIV, and after 48 h for subjects XII and XIII, show an approximately straight line. Table 3 presents half-lives and »metabolic« clearance rates, calculated from the latter parts of these curves in the normal way (Tait & Burstein 1964; Van der Molen et al. 1969).

Milk

Table 4 summarizes the volumes of the collected milk and the results of the radioactivity measurements in the daily milk fractions. The total amount of both milk and radioactivity, excreted during 4 days, was extremely low in most

Table 2.

$^{14}$C-Radioactivity in plasma after oral administration of 5 µc $^{14}$C-mestranol.

<table>
<thead>
<tr>
<th>Plasma collected (hours after administration)</th>
<th>pc $^{14}$C per ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case XI</td>
</tr>
<tr>
<td>Mean S.E.M</td>
<td>Mean</td>
</tr>
<tr>
<td>1 h</td>
<td>6.4</td>
</tr>
<tr>
<td>2 h</td>
<td>29.0</td>
</tr>
<tr>
<td>4 h</td>
<td>84.4</td>
</tr>
<tr>
<td>8 h</td>
<td>58.1</td>
</tr>
<tr>
<td>24 h</td>
<td>31.8</td>
</tr>
<tr>
<td>48 h</td>
<td>23.8</td>
</tr>
<tr>
<td>72 h</td>
<td>18.2</td>
</tr>
<tr>
<td>96 h</td>
<td>...</td>
</tr>
<tr>
<td>120 h</td>
<td>...</td>
</tr>
</tbody>
</table>

S.E.M. = Standard error of mean.

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Radioactivity per litre plasma (in % oral dose)

Fig. 1.

$^{14}$C-radioactivity per litre plasma in per cent of the administered oral dose, as a function of time (in hours) after the time of oral administration of 5 µc $^{14}$C-mestranol (——). For comparison the results with 5 µc $^{14}$C-lynestrenol (-----), administered in the same way, are included (Van der Molen et al. 1969).

Table 3.

Half-lives, distribution volumes and »metabolic« clearance rates of $^{14}$C-radioactivity in plasma after oral administration of $^{14}$C-mestranol (data referring to the second part of the curves in Fig. 1).

<table>
<thead>
<tr>
<th></th>
<th>Half-life (hours)</th>
<th>$\gamma$ (hours$^{-1}$)</th>
<th>D' fraction of dose/l plasma</th>
<th>V litres</th>
<th>M. C. R. 1 plasma/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case XI</td>
<td>61</td>
<td>0.0114</td>
<td>0.0079</td>
<td>127</td>
<td>34.7</td>
</tr>
<tr>
<td>Case XII</td>
<td>61</td>
<td>0.0114</td>
<td>0.0072</td>
<td>139</td>
<td>38.0</td>
</tr>
<tr>
<td>Case XIII</td>
<td>40</td>
<td>0.0173</td>
<td>0.0055</td>
<td>182</td>
<td>75.6</td>
</tr>
<tr>
<td>Case XIV</td>
<td>53</td>
<td>0.0131</td>
<td>0.0120</td>
<td>83</td>
<td>26.1</td>
</tr>
</tbody>
</table>

cases. In case XI hardly any milk could be collected during the second day, and on the following days milk secretion stopped completely. The total amount of radioactivity recovered in the experimental period varied from 0.0002 to 0.0126 % of the administered dose.
Table 4.  
14C-Radioactivity in milk collected during 24 hours’ periods after oral administration of 14C-mestranol.

<table>
<thead>
<tr>
<th>Day</th>
<th>Case XI</th>
<th></th>
<th>Case XII</th>
<th></th>
<th>Case XIII</th>
<th></th>
<th>Case XIV</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk ml</td>
<td>Radioactivity in pc/day</td>
<td>Radioactivity in pc/ml</td>
<td>Milk ml</td>
<td>Radioactivity in pc/day</td>
<td>Radioactivity in pc/ml</td>
<td>Milk ml</td>
<td>Radioactivity in pc/day</td>
</tr>
<tr>
<td>1</td>
<td>12.1</td>
<td>8.8 ± 0.6</td>
<td>0.73</td>
<td>69.0</td>
<td>388.5</td>
<td>5.6</td>
<td>15.5</td>
<td>169.2</td>
</tr>
<tr>
<td>2</td>
<td>1.4</td>
<td>0</td>
<td>0</td>
<td>83.5</td>
<td>173.8</td>
<td>2.1</td>
<td>4.6</td>
<td>6.3</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>70.5</td>
<td>55.2</td>
<td>6.4</td>
<td>3.8</td>
<td>0.6</td>
<td>17.0</td>
<td>87.9</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>43.0</td>
<td>23.7</td>
<td>0.6</td>
<td>12.2</td>
<td>13.6</td>
<td>1.1</td>
<td>31.5</td>
</tr>
<tr>
<td>Total</td>
<td>13.5</td>
<td>8.8</td>
<td>266.0</td>
<td>641.2</td>
<td>38.7</td>
<td>192.9</td>
<td>95.2</td>
<td>440.0</td>
</tr>
</tbody>
</table>

Recovery in % dose: 0.0002, 0.013, 0.004, 0.009
<table>
<thead>
<tr>
<th>Day</th>
<th>Case XI</th>
<th>Case XII</th>
<th>Case XIII</th>
<th>Case XIV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine ml</td>
<td>Radioactivity in pc/ml</td>
<td>% oral dose/day</td>
<td>Urine ml</td>
</tr>
<tr>
<td>1</td>
<td>1400</td>
<td>778</td>
<td>20.9</td>
<td>1200</td>
</tr>
<tr>
<td>2</td>
<td>1380</td>
<td>536</td>
<td>14.2</td>
<td>1190</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>569</td>
<td>8.7</td>
<td>1100</td>
</tr>
<tr>
<td>4</td>
<td>900</td>
<td>325</td>
<td>5.6</td>
<td>1320</td>
</tr>
<tr>
<td>5</td>
<td>1060</td>
<td>146</td>
<td>3.0</td>
<td>1400</td>
</tr>
</tbody>
</table>

Total recovery 52.4% 36.3% 31.8% 34.8%
Urine

The urinary excretion of radioactivity is presented in Table 5. The overall recovery in subject XI (52.4%) is definitely higher than in the three other subjects (31.8–36.3%).

DISCUSSION

After oral administration of 14C-mestranol the total amounts of 14C-radioactivity were estimated in the plasma, milk and urine of women during the lactation period shortly after delivery. No attempt was made to isolate or identify any specific 14C-labelled chemical compound. Thus the conclusions from the 14C-estimations in the plasma, milk and urine may at best reflect the behaviour of »14C-labelled mestranol and metabolites«. When discussing the results of the present investigation with 14C-mestranol, it may be interesting to compare these results with those previously found after administration of 14C-lynestrenol under the same experimental conditions (Van der Molen et al. 1969). In both experiments the amount of radioactivity administered was of the same order (5 µc), but it should be stressed that the mass quantities for 14C-mestranol (150 µg) administered were far less than those for 14C-lynestrenol (5000 µg).

The peak concentration of radioactivity per litre of plasma after oral administrations of 14C-mestranol in Lyndiol®-tablets 4–8 h after administration is in the order of 1.5% of the administered dose (Fig. 1). As was found previously (Van der Molen et al. 1969) for 14C-lynestrenol which was administered in the same way, the highest concentrations of radioactivity in the plasma were in the order of 2–3% of the administered dose 4–6 h after administration (these results are included in Fig. 1).

The smaller peak concentrations of radioactivity in the plasma from mestranol could thus indicate that, on a percentage basis, either the resorption of mestranol from the Lyndiol®-tablet is less than that of lynestrenol, or that mestranol is more quickly removed from the circulation than lynestrenol. The total amount of radioactivity from mestranol that is recovered during 5 days in the urine is only in the order of 30–35% (Table 5, with the exception of case XI where the recovery was 52%) as compared to a recovery in the order of 54–60% during the same period after administration of 14C-lynestrenol (Van der Molen et al. 1969). These observations suggest, that in comparison to lynestrenol, larger amounts of the administered mestranol may disappear from the body with faecal excretions. Unfortunately an accurate estimation of the radioactivity excreted with the faeces has not yet been achieved.

In comparing the relative resorption and excretion of radioactivity of lynestrenol and of mestranol, it is important to realise, that the amount of lyne-
strenol (5000 µg) in the administered Lyndiol®-tablets was 33 times higher than the amount of mestranol (150 µg). Even if the resorption of radioactivity from these two steroids may be slightly different, the resorption of the mass amounts of mestranol and lynestrenol is at a completely different level.

**Plasma**

The curve of the radioactivity in the plasma after administration of ¹⁴C-mestranol (Fig. 1) indicates a half-life for the disappearance of »mestranol and metabolites< from the plasma in the order of 40–60 h, for the second part of the disappearance curves, 24–48 h after the administration (Table 2). These half-lives are in the order of those found for »lynestrenol and metabolites< of about 40 h, and fall in the range of the half-lives, that have been estimated for the physiologically occurring steroid conjugates, but are in contrast to those in the order of 1 h, for the physiologically occurring free steroids (Van der Molen et al. 1969). Although these half-lives for »mestranol and metabolites< in the peripheral plasma appear to be comparable to those of »lynestrenol and metabolites<, the disappearance curves in Fig. 1 differ from those found for ¹⁴C-lynestrenol. After the initial increase in plasma radioactivity following ¹⁴C-mestranol administration, the disappearance curve of radioactivity in Fig. 1, is composed of two straight parts. A first rapid decline in plasma concentrations of radioactivity occurs until 24–48 h after administration (with a half-life of 24–36 h) followed by a second slower disappearance (with half-life of 40–60 h; Table 3). After lynestrenol administration the plasma radioactivity shows an almost linear disappearance curve. This may indicate a difference in distribution or metabolism for mestranol and lynestrenol.

In order to allow comparison with the lynestrenol data (Van der Molen et al. 1969), the metabolic clearance rates for the disappearance of radioactive »mestranol and metabolites< have been calculated (using single pool kinetics) for the final part of the disappearance curve: 

\[
\text{MCR} = \frac{\log_{10} 2}{\text{half-life}} \times \frac{1}{D'} \times 24 \text{ l/day}
\]

\(D'\) = fraction of administered radioactivity per litre of plasma, that was obtained by extrapolation of the final part of the disappearance curve to time zero).

The data of Table 3 demonstrate that the metabolic clearance rates after ¹⁴C-mestranol administration show large variations (26.1–75.6 l plasma/day) between the individual subjects. The smaller fraction of the radioactivity of mestranol that appears in the plasma, may explain the larger metabolic clearance rates for »mestranol and metabolites< (Table 3) as compared to the metabolic clearance rates for »lynestrenol and metabolites<, even when half-lives for the final disappearance from the plasma appear to be of the same magnitude (Van der Molen et al. 1969).
Milk

The amount of milk that could be collected in these non-nursing women was extremely low (Table 4). As the milk was collected with a breast-pump, it is possible that the absence of suckling could explain this small production of milk. However, during our studies in which the excretion of $^{14}$C-lynestrenol was investigated, normal milk values (63-405 ml per day) could be collected. No explanation can be offered for this discrepancy, the experimental conditions being exactly the same: in both series of experiments Lyndiol® administration was started on the day of delivery or on the first day after delivery, and the pretreatment period with Lyndiol®-tablets was always 4 days. However, because of these small milk volumes the absolute amounts of radioactivity that were excreted in the total milk fractions were low, 0.0002-0.013 % of the administered dose, whereas the lynestrenol experiment yielded total excretion values of 0.022-0.088 %.

In order to allow calculation of the amount of »steroid + metabolites« that may be transferred with the milk from mother to child, we have previously discussed the following assumptions (Van der Molen et al. 1969), that we have also applied to our studies with mestranol:

1. The metabolism of mestranol does not vary in a quantitative sense during the period of milk collection (»steady state«);
2. The concentration of mestranol and metabolites in the milk is independent of the volume of milk that can be collected at a certain time.

Considering the concentration of radioactivity in the milk (in pc/ml milk), the cumulative amount of »$^{14}$C-mestranol and metabolites« that may be excreted per ml milk during daily administration of 150 µg $^{14}$C-mestranol (in a Lyndiol®-tablet) may be calculated from Fig. 2. The data suggest that the concentration of radioactivity in milk could reach a value of 10-20 pc/ml if 5 µc of $^{14}$C-mestranol was administered daily. From the specific activity of the administered $^{14}$C-mestranol (= 5 µc/150 µg) it can be calculated that the amount of »mestranol and metabolites« that will be excreted per 100 ml of milk is equivalent to 1000-2000 pc, or 0.03-0.06 µg (= 0.02-0.04 % of the daily administered dose). During our previous experiments with $^{14}$C-lynestrenol it was calculated in a similar way, that 0.9-1.3 µg of »lynestrenol and metabolites« would be transferred per 100 ml milk. Although these values are much higher than for mestranol, one should realize that the dose administered per tablet differs greatly. Since the Lyndiol®-tablets contained 5000 µg of lynestrenol, the amounts transferred per 100 ml milk are of the same order on a percentage weight basis: i. e. 0.02-0.03 % for lynestrenol.

If it is assumed that a normal infant during the first 3 months of life will consume about 600 ml of mother milk per day, then on the basis of the above calculation, 0.18-0.36 µg of »mestranol and its metabolites« might be transferred from the mother to the infant daily. As to the effects of the daily in-

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Cumulative amount of radioactivity in milk (\( \text{E} \text{ pCi/ml} \))

**Fig. 2.**
Cumulative amount of \(^{14}\text{C}\)-radioactivity per ml milk if subjects received one tablet with 5 \( \mu \text{c} \) of \(^{14}\text{C}\)-mestranol daily.

Suggestion of this amount of »mestranol and its metabolites«, little can be said as long as the exact nature and pharmacological activities of the substances in the milk are unknown. Moreover, little is known about the excretion of endogenously produced ovarian oestrogens into the milk of nursing mothers, whose ovaries resume their normal activity after the post-partum amenorrhoea.

As mentioned in the previous publication the presence of oestrogenic activity in the milk of lactating women who were using oral contraceptives could not be demonstrated by some investigators (Pincus et al. 1966; Duncan et al. 1967). In other investigations, however, the presence of oestrogens in the milk of such mothers was inferred from one observation (out of 58 cases) on breast development (Curtis 1964) and from vaginal changes (Lauritzen 1967) in children during the period of breast feeding.

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