Placental Transfer of Radioactive Digitoxin in Pregnant Women and its Fetal Distribution

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With the technical assistance of John H. Curry, Jr. and Frederick D. Smith, Jr.

Placental transfer of radioactive C₁⁴-digitoxin was investigated in four pregnant women. The concentration of the labeled drug and its metabolic products in the various fetal organs was determined. Less than 1 per cent of the administered glycoside was detected in the fetus as unchanged digitoxin and less than 3.5 per cent as its metabolites. The fetal heart and kidney of 11 to 12 weeks gestation had the highest concentrations. However, at near term the fetal liver, gallbladder and intestine had somewhat similar concentrations. Metabolic conversion of the cardiac glycoside by fetal liver as well as biliary excretion is indicated from the tissue distribution data.

The placental transfer of digitalis in pregnant women has received little or no attention. Earlier studies conducted by our group on rats and guinea pigs indicated that C₁⁴-digitoxin crosses the placental barrier. In the guinea pig, approximately 22 per cent of the injected radioactivity was found in the fetuses one hour after intravenous administration of labeled digitoxin. In addition, on a tissue weight basis, the fetal heart of guinea pigs had six times the concentration of unchanged digitoxin as the maternal heart. One then wonders whether a similar phenomenon occurs in human subjects, and if so, whether an increased concentration of the glycoside in the fetal heart can have pathologic effects directly on the myocardium as demonstrated in experimental animals after chronic administration of digitalis.

The purpose of the present study was to investigate the passage of labeled digitoxin across the placental barrier of pregnant women and to determine the relative concentration of both the unchanged drug and its metabolic products in the various fetal organs.

Method

Four pregnant women hospitalized in the University of Chicago Lying-in Hospital were selected for this study. Three of the women required therapeutic abortion of the fetus for various clinical reasons. The fetus of the fourth woman was diagnosed as an anencephalic monster and carried to delivery. Uniformly labeled C₁⁴-digitoxin prepared by biosynthesis and having a specific activity of either 0.48 or 0.65 μC./mg. was administered intravenously to the four women. Doses of either 0.25 to 0.5 mg. were injected into the first three women 3 to 5 hours before removal of the fetus. Expulsion of the fetus was performed by hysterotomy during the eleventh and twelfth week of gestation. To the fourth woman, 0.5 mg. of the radioactive drug was administered approximately 2 to 3 hours before the expected time of delivery. However, the fetal heart beat stopped during delivery 2 hours after injection of the labeled drug. The stillbirth occurred during the thirty-fourth week of gestation.

During hysterotomy of the first 3 patients the amniotic sac was left intact, then rinsed with saline, and the amniotic fluid aspirated. Fetal cord blood and various fetal organs were obtained. Maternal blood samples, both immediately before administration of the radioactive drug and after removal of the fetus, as well as samples of maternal uterine muscle were obtained. From the fourth woman whose pregnancy was carried to near term, only maternal blood samples were collected. Fetal blood was obtained by cardiac puncture. In all cases, extreme caution was taken to prevent radioactive contamination of tissue samples by maternal blood as well as to reduce...
enzymatic degradation of the unchanged digitoxin molecule. Therefore, all tissue samples were removed as soon as possible after expulsion of the fetus, rinsed thoroughly with saline, immediately frozen with dry ice, and stored at −17°C. The extraction procedure that was employed for isolation of the unchanged drug from blood was similar to a method reported previously. The labeled drug in all solid tissues was extracted using a slightly modified procedure. Essentially, both methods involved the addition of nonlabeled carrier digitoxin to aid in the isolation of the micro amount of radioactive compound, followed by liquid-liquid solvent extraction and chromatographic separation. In the initial solvent extraction, hot methanol was used to liberate the drug bound to protein.

All radioactive fractions other than unchanged digitoxin were combined and considered to represent the radioactivity from metabolites of the parent compound. Determinations of radioactivity of the various extracted fractions, except the original tissue residue from the homogenate fraction, were made using an internal gas-flow Geiger counter. All counts were corrected for background, self absorption, and counter efficiency. The tissue residue was combusted to carbon dioxide in a vacuum combustion line and counted in an ionization chamber, using a vibrating reed electrometer according to the method of Brownell and Lockhart. All measurements of radioactivity were done by counting for periods long enough to give a standard error of less than ±5 per cent. Using a known amount of radioactive digitoxin as a control and subjecting it to the extraction procedure described, recoveries of 96 ± 5 per cent were obtained.

Results and Discussion

Placental transfer. Studies on the placental transfer of C14-digitoxin in four pregnant women indicate that the cardiac glycoside crosses the placental barrier. The amount of the radioactive glycoside and its metabolic products that can be detected in the fetus between 1.7 to 5 hours after intravenous administration of the labeled drug is shown in table 1. It can be seen from the data that during the eleventh and twelfth week of gestation less than 0.1 per cent of the injected dose can be found in the fetus and less than 0.33 per cent as its metabolic products. In the fetus that was carried to near term, 0.84 per cent was accounted for as the unchanged drug and 3.49 per cent as metabolites. The increase in the labeled compounds observed in the near term fetus can be attributed in all probability, to an increase in the size of the fetus since there is no increase in concentration on a tissue-weight basis as will be indicated later. Of the total radioactivity in the fetus, approximately 25 per cent was attributable to the unchanged parent compound.

Calculation of the total radioactivity in the fetal and maternal body on an equivalent body weight basis revealed that the near term fetus had approximately twice the concentration of the maternal body, whereas the 11 to 12 week fetuses had from three to six times that amount. However, as it was discussed above, there was less than 1 per cent of unchanged drug in the fetus and less than 3.5 per cent of its metabolic products.

One cannot make any definitive statement as to whether the concentration of the drug in the fetus is harmful from the present data. Since toxic symptoms have not been observed clinically in the newborn of cardiac mothers receiving digitalis, it is probable that the amount of the glycoside that enters the fetus may be nontoxic. However, the possibility cannot be dismissed that the fetal concentration of the drug may have subpathologic or biochemical effects that might become manifest at some later date.

Tissue Distribution. The distribution of labeled digitoxin and its metabolic products in the various organs is shown in figure 1. The results are expressed as μg. or μg. equivalent per 100 Gm. of tissue. The term “μg. equivalent” is used to express the amount of the original drug converted into metabolites, i.e., if 1 μg. of radioactive digitoxin has a certain number of radioactive disintegrations per minute and if a metabolic product of the drug contains the same number of disinte-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose (mg)</th>
<th>Approx. length of gestation (weeks)</th>
<th>Time after injection of drug (hrs)</th>
<th>Per cent of injected dose in fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>11</td>
<td>5</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.50</td>
<td>12</td>
<td>3.5</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>12</td>
<td>2.8</td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>34</td>
<td>1.7</td>
<td>0.84</td>
</tr>
</tbody>
</table>
PLACENTAL TRANSFER OF RADIOACTIVE DIGITOXIN

**Fig. 1.** Concentration of radioactive digitoxin and its metabolites in human fetal organs of 11 to 12 weeks gestation. One-half mg. of labeled digitoxin administered intravenously 2.5 to 5 hours before therapeutic abortion. Fetal tissues obtained from the patient receiving 0.25 mg. was corrected for 0.5 mg. dose. All figures represent the average value from tissues of three fetuses.

From the data in figure 1, it can be seen that the heart and kidney have relatively high concentration of the unchanged drug, 11.6 and 7.1 μg./100 Gm., respectively. Moderate amounts were detected in the liver and lung, with lower concentrations in the other organs. Selective removal of the parent compound from the blood by certain fetal organs is indicated by the higher concentration of the drug in these organs than in maternal or fetal blood. In all instances, a higher concentration of the metabolic products was noted in the tissues than the parent compound. The occurrence of digitoxin metabolites in fetal tissues suggests either a metabolic conversion of the cardiac glycoside by the fetal tissue or passage of metabolites across the placental barrier, or perhaps both. However, some of the metabolic products are believed to arise from detoxification of the drug by fetal liver. This is suggested by the high metabolite to digitoxin ratio found in this organ as compared with fetal placenta.

The passage of digitoxin across the blood-brain barrier is noteworthy and is demonstrated by its presence in the fetal brain. This correlates well with the known effects of digitalis glycosides on the central nervous system.

**Fig. 2.** Concentration of radioactive digitoxin and its metabolites in human fetal organs of 34 weeks gestation. One-half mg. of labeled C14-digitoxin administered intravenously 2 hours before death that occurred during delivery.

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TABLE 2.—Amount of C\textsuperscript{14}-Digitoxin and its Metabolites in Human Fetal and Adult Auricular Appendage

<table>
<thead>
<tr>
<th></th>
<th>Dose</th>
<th>Time after injection (hrs)</th>
<th>Digitoxin (μg per 100 Gm. tissue)</th>
<th>Metabolites (μg. eq. per 100 Gm. tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal heart (12 week gestation)</td>
<td>0.5</td>
<td>2.8-5</td>
<td>11.6</td>
<td>42.5</td>
</tr>
<tr>
<td>Fetal heart (34 week gestation)</td>
<td>0.5</td>
<td>1.7</td>
<td>2.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Adult auricular appendage</td>
<td>0.5</td>
<td>3</td>
<td>1.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

fetus as evidenced by the presence of these compounds in the liver, gallbladder, and intestine.

Since it was not possible to obtain samples of maternal myocardium, a direct comparison could not be made of the concentration of digitoxin in maternal and fetal heart. However, in a separate experiment a sample of an adult auricular appendage was obtained from a patient undergoing cardiac surgery.\(^*\) One-half mg. of C\textsuperscript{14}-digitoxin was administered intravenously three hours before removal of the myocardial tissue. A comparison of the concentration of labeled digitoxin and its metabolites in fetal and adult auricular appendage is shown in table 2. The heart of the near term fetus has approximately twice the concentration of unchanged drug as compared with the adult auricular appendage. However, the heart of 11 to 12 week fetuses has approximately a tenfold concentration.

As was discussed previously, placental transfer studies in experimental animals demonstrated that the fetal heart of guinea pigs had six times the amount of digitoxin on a tissue weight basis in comparison with the amount in the maternal heart.\(^1\) Wollenberger\(^1\) has suggested that this increased concentration may be only a reflection of differences in the size of the myocardial cell. If the mature and immature heart has approximately the same number of digitoxin molecules per heart cell, then the fetal heart would have a greater quantity of glycoside per unit mass due to its smaller size. Wollenberger thus believes that this may account in part for his findings that the heart of immature experimental animals tolerated a larger dose of ouabain than the heart of mature animals. If Wollenberger's findings can be extrapolated to human beings, it is possible that the higher concentration of the drug observed in fetal heart on a tissue weight basis may not necessarily entail the danger of over digitalization to the unborn child.

**Summary**

Studies on the placental transfer of biosynthetically-labeled C\textsuperscript{14}-digitoxin in four pregnant women indicate that less than 1 per cent of the administered drug was detected in the fetus as unchanged digitoxin and less than 3.5 per cent as its metabolic products.

On a tissue-weight basis, the fetal heart and kidney of the 11 to 12 week fetuses had relatively higher concentrations of digitoxin and metabolites than other organs. However, at near-term the fetal liver, gallbladder, and intestine had concentrations that approached those of the heart and kidney.

Metabolic conversion of digitoxin by the liver is suggested from the high metabolite to digitoxin ratio found in this organ.

Biliary excretion of both the unchanged drug and its metabolites were demonstrated in the fetus.

Tissues from 11 to 12 week fetuses had a higher concentration of the glycoside and its metabolites than corresponding tissues from near term pregnancy. However, part of this difference is reduced if the drug concentration is expressed in terms of the amount of drug per cell.

In all probability, the amount of digitoxin that crosses the human placenta may be considered to be nontoxic to the unborn child as evidenced by the low percentage of the administered drug found in the fetus. Although the fetal heart had a higher concentration of the glycoside than the adult auricular appendage on a tissue weight basis, the demonstration by Wollenberger that the immature heart...
myocardium is more resistant to the cardiac drug than that from the adult may in part reduce this difference.

**SUMMARIO IN INTERLINGUA**

Esseva studiate in quatro feminas pregnante le transferimento placental de digitoxina biosyntheticamente etiquettate con C14.

Minus que un pro cento del droga administrate esseva detegite in le feto como non-alterate digitoxina e minus que 3,5 pro cento como productos metabolic de illo.

Calculate pro equal pesos de histo, le corde e ren de fetos de 11 a 12 septimanas haveva relativemente plus alte concentrationes de digitoxina e metabolitos que altere organos. A periodos proxime a termino, hepate, vesica biliari, e intestino haveva concentrationes non multo inferior al concentrationes in corde e ren.

Conversion metabolic de digitoxina per le hepate pare conclucidile ab le alte proportion de metabolitos a digitoxina que es trovate in iste organo.

Excretion biliari de tanto le non-alterate droga como etiam su metabolitos esseva demonstrate in le feto.

Histos ab fetos de 11 a 12 septimanas haveva un plus alte concentration del glycosido e su metabolitos que correspondent histos ab fetos proxime a termino. Tamen, un parte de iste differentia se reduce si le concentration del droga es exprimite como quantitate del droga per cellula.

Il es molto probabile que le quantitate de digitoxina que transversa le placenta human pote esser considerate como nontoxico al feto. Iste conclusion es supportate per le basse percentage del droga administrate que es retrovate in le feto. Ben que le corde del feto haveva un plus alte concentration del glycosido que equal pesos de histo del adulte appendage auricular, le demonstration per Wollenberger que le myocardio immatur es plus resistente al droga cardiac que le myocardio de adultos reduce in parte le importantia de iste distinction.

**REFERENCES**


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