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Placental Transfer of Radioactive Digitoxin in Rats and Guinea Pigs.* (19682)

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The placental transfer of digitalis has received little or no attention in the past. Since the availability of radioactive digitoxin, studies on the penetration of digitoxin across the placental membrane in laboratory animals have become possible.

Method. Rats and guinea pigs were selected as test animals since their placenta is in some respects morphologically similar to that of man(1). Uniformly labeled C^{14}-digitoxin prepared by biosynthesis(2) and having a specific activity of 620,000 counts per minute per milligram (0.43 μc per mg) was administered intravenously to each animal. The dose used for each Sprague-Dawley rat was 0.175 mg (approximately 0.5 μg/g) and for each guinea pig 0.25 mg (approximately 0.25 μg/g).

The animals were sacrificed one hour after intravenous administration of the drug and the fetuses and maternal hearts were removed. In addition the guinea pig fetal hearts were isolated. The tissues were thoroughly washed in isotonic saline to remove blood, then dried between filter paper, weighed, and homogenized in Potter and Elvehjem homogenizers with 50% ethanol. Guinea pig fetuses (minus the hearts) were prehomogenized in a Waring blender and an aliquot transferred to Potter and Elvehjem homogenizers. The fetal hearts of the rats were not isolated because of their small size.

The extraction procedure employed for the isolation of unchanged digitoxin and fractionation of its metabolic products consisted of diluting the homogenates with 50% ethanol, adding a few milligrams of non-radioactive digitoxin as carrier, and making a liquid-liquid extraction with chloroform(2). The chloroform fraction was evaporated to dryness, taken up in 50% methanol, extracted with carbon tetrachloride to remove fats and lipids, and re-extracted with chloroform. The latter chloroform fraction was evaporated to dryness, again taken up in chloroform (C.P.), and then allowed to pass through an alumina column. Successive elutions were made with the following solvents: 1% ethanol in chloroform, 10% ethanol in chloroform, and 40% aqueous ethanol. Unchanged digitoxin was found to occur in the 10% ethanol in chloroform eluate. Identification of the drug was accomplished using paper partition chromatography(2), polarographic analysis(3), and color reaction tests(2). All radioactive samples were analyzed using an internal gas-flow Geiger counter(4). The maximum counting error was 10% with the error for most counting samples being less than 5%. The various samples showing radioactivity other than unchanged digitoxin were considered to contain metabolic products of the parent drug. The

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TABLE I. Placental Transfer of C^{14} Digitoxin in Pregnant Guinea Pigs.

Pregnant guinea pigs*	Approximate period of pregnancy (term = 1)	Fetus: Unchanged digitoxin: % original dose injected	Fetus: Unchanged digitoxin: % of total C^{14} in fetus	Fetus: Metabolites: % original dose injected	Fetus: Metabolites: % of total C^{14} in fetus	Total C^{14} in fetus: % original dose injected
G.P. 1	.40	.28	1.6	17.3	98.4	17.58
2	.70	.55	2.1	26.4	97.9	26.95
3	.90	.35	1.7	20.05	98.3	20.40
4	.95	.73	3.0	23.70	97.0	24.43
Avg	.74	.48	2.1	21.9	97.9	22.34
Stand. dev.		±.10	±.32	±1.9	±.32	±2.1

* Animals sacrificed one hr after administration of drugs.
Amt. C^{14} digitoxin/G.P. = 0.25 mg; Sp. Activity = 620 cpm/μg; route administration = I.V.

TABLE II. Placental Transfer of C^{14} Digitoxin in Pregnant Rats.

Pregnant rats*	Approximate period of pregnancy (term = 1)	Fetus: Unchanged digitoxin: % original dose injected	Fetus: Unchanged digitoxin: % of total C^{14} in fetus	Fetus: Metabolites: % original dose injected	Fetus: Metabolites: % of total C^{14} in fetus	Total C^{14} in fetus: % original dose injected
1	.90	.05	5.	1.03	95.0	1.08
2	.80	.03	8.2	.39	91.8	.43
3	.50	.02	6.2	.35	93.8	.37
4	.85	.04	6.1	.69	93.9	.73
Avg	.76	.04	6.4	.61	93.6	.65
Stand. dev.		±.007	±.66	±.16	±.76	±.03

* Animals sacrificed one hr after administration of drugs.
Amt. C^{14} digitoxin/rat = 0.175 μg; Sp. activity = 620 cpm/μg; route administration = I.V.

nature of these metabolic products is not known at the present time.

Results and discussion. The results listed in Tables I and II indicate that in all instances digitoxin crosses the placenta of guinea pigs and rats. Although guinea pigs and rats have morphologically similar fetal membranes, the data indicate that in the guinea pig digitoxin and possibly its metabolic products are able to cross the placenta much more readily than in rats. One hour after administration of digitoxin the average placental transfer for guinea pigs was 22.3 ± 2.1% of the original dose as compared with 0.65 ± .03% for rats.

Less than 9% of the carbon 14 found in the fetuses of both species represented unchanged digitoxin, while the rest was in the form of its metabolites. The metabolite-digitoxin ratio in the fetal guinea pig is approximately 47-fold, while in the fetal rat it is approximately 15-fold. Fischer and coworkers (5) have shown that one hour after administration of radio-digitoxin to normal rats, the major portions of the radioactivity in the organism was in the form of unchanged digitoxin. Therefore, the extremely high metabolite-digitoxin ratio in the fetus may be due either to a high conversion rate of the drug by the embryonic tissue or to a selective penetration of the metabolites across the placental barrier, or both.

Although the present study was not designed to determine at which period of gestation maximum rate of placental transfer was effected, our data suggest that as the gestation period increases there is a corresponding increase in the rate of transfer of digitoxin across the placenta.

To determine whether there is an uptake of digitoxin and its metabolites in the embryonic heart, fetal hearts of guinea pigs were assayed and compared with the maternal heart. The data shown in Table III indicate that embryonic hearts from 3 of the guinea pigs contained unchanged digitoxin, while embryonic hearts from all of the guinea pigs contained its metabolic products. Although on an organ basis the embryonic hearts accumulated less of the drug than the maternal heart, on a weight basis it is seen that the fetal heart has a higher concentration. The latter fact is interesting for it suggests the possibility of digi-

TABLE III. Comparison Between Fetal and Maternal Heart on Uptake of C^{14} Digitoxin and its Metabolites in Guinea Pigs.

	Unchanged digitoxin				Metabolites			
	Fetal hearts		Maternal heart		Fetal hearts		Maternal heart	
Animal*	μg/heart	μg/g	μg/heart	μ/g	μg equiv†/ heart	μg equiv/ g	μg equiv/ heart	μg equiv/ g
G.P. 1	N.S.C.‡	—	.03	.01	.06	.46	.23	.09
2	.05	.09	.03	.01	.07	.12	.45	.16
3	.03	.07	.02	.01	.09	.21	.28	.18
4	.02	.03	.02	.01	.05	.06	.19	.10
Avg	.03	.06	.03	.01	.07	.21	.29	.13
Stand. dev.	±.007	±.013	±.004	±.0	±.006	±.09	±.003	±.08

* Animals sacrificed one hr after administration of drug.
† Amount of original drug converted into metabolic products.
‡ No significant counts.

talizing an embryonic heart beyond the therapeutic level.

Summary. 1. Digitoxin, and possibly its metabolic products, crosses the placenta of rats and guinea pigs. 2. Larger amounts of both unchanged digitoxin and its metabolites were found in the fetuses of guinea pigs than in the fetuses of rats. 3. Embryonic tissue may have a marked ability to catabolize the drug, or there may be a selective penetration of the metabolites across the placenta, as noted by its high metabolite-digitoxin ratio. 4. On a tissue weight basis, digitoxin and its metabolites were found in higher concentrations in the embryonic heart than in the maternal heart.

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Factors Influencing Development of Hypophyseal Tumors in Mice After Treatment with Radioactive Iodine.* (19683)

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It has been shown(1) that quantities of radioiodine of approximately 200 μc given to mice destroy, or almost destroy, the thyroid gland. This treatment is followed after an interval of about a year by development of fatal tumors of the pituitary gland. Gonadectomy neither favors nor inhibits the growth of the pituitary tumors(2).

In further exploration of the reasons for the tumorous growth, the following 2 series of experiments were initiated.

When C_{57} mice are fed the Remington "low-iodine" diet for one or 2 weeks before they are given I^{131}, 60% of the radioiodine accumulates in the thyroid gland. With such use of the Remington diet it is possible to produce the same thyroidal destruction with 30 μc of I^{131} as is produced in mice fed normal (Purina chow) diets by 200 μc(2) of I^{131}. The systemic, or "whole-body" radiation in the 2 cases is, however, very different,

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