rabbit's gallbladder resorbed half the volume of its content per hour(3).

Our data are in line with the above observations; namely, that certain constituents of the bile are retained in the gallbladder and reach concentrations considerably higher than that in bile from liver. Other investigators have noted a rapid loss of inorganic ions from the gallbladder(1). However, the high potassium concentrations in gallbladder bile have not previously been reported. Failure of the potassium to be resorbed and its accumulation in the gallbladder cannot be satisfactorily explained from the above determinations.

Summary. Measurements of sodium, potassium, pH, total solids and pigment in human gallbladder bile indicate wide variations. Potassium is present in higher concentrations than in extracellular fluids. The resorptive power as measured by pigment failed to correlate with the increase in potassium concentration and total solids.

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Placental and Mammary Transfer of Ingested Chlortetracycline in the Rat. (22874)

R. F. ELLIOTT AND A. R. WHITEHILL (Introduced by E. L. R. Stokstad)
Research Division of American Cyanamid Co., Pearl River, N. Y.

Results with animals indicate that the maximum growth response of antibiotics occurs when they are included in the diets from earliest possible age. The oral administration of antibiotics to young animals is difficult with certain species which do not consume food in the critical period following birth. An alternate means of administering antibiotics to such animals would be through either placental or mammary transfer or both. Placental transfer of penicillin was found to occur in human subjects by Charles(1) since significant amounts were found in the liquor amnii, cord blood and maternal blood following the injection of one million units of the antibiotic during labor. Chlortetracycline, chloramphenicol, oxytetracycline and streptomycin, when given in therapeutic amounts, were shown also to be transferred in therapeutic concentrations from the maternal to the fetal tissues. Carpenter and Larson(2) observed no transfer of chlortetracycline or penicillin across the placental tissues of the sow. Chlortetracycline, however, was detected in the milk of sows following oral administration of the antibiotic but the amounts were small and variable and did not influence the growth rate of suckling pigs. Placental transfer of either antibiotic presumably failed to occur since detectable amounts were not shown in the blood serum of the pigs at birth following antibiotic administration to the sows. An earlier publication by Carpenter (3) reported that the size or viability of litters of pigs was not affected by giving chlortetracycline to gestating sows nor was the growth of the pigs influenced during the suckling period which indicated that mammary transfer did not result from the amounts consumed by the sows. Maddock *et al.*(4) were unable to obtain appreciable concentrations of chlortetracycline in the blood serum or milk from sows 8 hours after feeding the antibiotic at levels up to 20 mg per pound of ration for a week. Blood levels of 0.74 γ per ml and whey levels as high as 2.21 γ per ml were obtained, however, 8 hours after administering chlortetracycline orally in single doses at levels from 2.0 to 4.0 g. Little correlation was found between the concentration of chlortetracycline in the serum and that in the whey and milk. Uram *et al.*(5) re-

ported that young suckling rats from dams receiving antibiotic-supplemented diets gained significantly faster than controls during the first 14-day period. This indicated that placental or mammary transfer might take place in this species. The following experiments were set up to explore this possibility and to determine if detectable amounts of chlortetracycline were present in the body tissues of newborn rats from dams that had consumed diets containing elevated levels of the antibiotic.

Methods. Thirty pregnant female rats of the Wistar strain were obtained from Carworth Farms, Kingston, N. Y., approximately 2 weeks before term and randomly distributed across the 3 treatments. A total of the 10 animals were assigned to each treatment, placed in individual cages and fed *ad libitum* a standard meal diet* containing either 0, 0.5, or 2.0 g of chlortetracycline per kilo. During the afternoon of the day prior to the end of gestation the females were sacrificed. The fetuses were removed by hysterectomy, sexed, weighed, placed in identified plastic bags, quick-frozen and held until they could be assayed for chlortetracycline by a modified pad plate method. The tissues were homogenized in acid acetone (5 ml/g of tissue). Pads of filter paper, 6.35 mm in diameter, were dipped into the homogenate and placed on agar plates that had been seeded with *Bacillus cereus* (ATCC 10702). The chlortetracycline content of the samples was determined by comparing the zone of inhibition with those plotted on a standard curve, obtained by serial dilutions of a standard antibiotic solution. In the second experiment, the young were born naturally, allowed to suckle for 24 hours, sacrificed and treated as outlined above. In the third experiment, the female rats were continued on their respective treatments, rebred, and allowed normal parturition. At the end of 24 hours, the young were sacrificed and then treated as in the second experiment. The females were sacrificed, the mammary glands excised, placed into plastic bags, quick-frozen and held until assayed for chlortetracycline.

* Ingredients: Beef Meal, Animal Liver Meal, Fish Meal, Brewers' Yeast, Condensed Fish Solubles, Soybean Oil Meal, Ground Yellow Corn, Wheat Bran, Dehydrated Alfalfa Meal, Linseed Oil Meal, Oatmeal, Wheat Germ Oil, Dried Whole Whey, Soybean Oil, Riboflavin, Niacin, Calcium Pantothenate, Choline Chloride, Vitamin A Feeding Oil, Irradiated Yeast, Iodized Salt, Calcium Carbonate, Copper Sulfate, Dicalcium Phosphate, Iron Sulfate, Cobalt Carbonate, Manganese Sulfate, and Vit. B_{12} Supplement.

TABLE I. Placental Transfer of Chlortetracycline to Rat Fetuses.

Level of chlortetracycline in dams diet.

	Control		0.5 g/kg		2.0 g/kg	
	γ/g					
Litter No.	♂	♀	♂	♀	♂	♀
1	0	0	0	0	.40	.41
2	0	0	0	0	.28	.40
3	0	0	0	0	.37	.34
4	0	0	0	0	.33	.35
5	0	0	0	0	.34	.28
6	0	0	0	0	.42	.40
7	0	0	0	0	.41	.48
8	0	0	0	0	.16	.30
9	0	0	0	0	.41	.38
10	0	0	0	0		
Avg					.35	.37

Results. Evidence of placental transfer of chlortetracycline. Placental transfer of the antibiotic was demonstrated by detecting significant amounts of chlortetracycline in the tissues of fetuses taken one day before term from female rats that had received a diet containing 2 g of the antibiotic per kilo of diet. As shown in Table I, the antibiotic concentration average (0.35 and 0.37 γ/g) of fetal tissue showed no significant difference between males or females. It is evident that substantial amounts of the antibiotic must be

TABLE II. Chlortetracycline in Tissues of Suckling Rats.

Level of chlortetracycline in dams diet.

	Control		0.5 g/kg		2.0 g/kg	
	γ/g					
Litter No.	♂	♀	♂	♀	♂	♀
1	0	0	0	0	.90	.55
2	0	0	0	0	.38	.46
3	0	0	.10	.30	.37	1.00
4	0	0	.09	.08	.75	.63
5	0	0	.07	.06	.21	.34
6	0	0	0	0	.19	.21
7	0	0	.42	.39		
8	0	0	.32	.35		
9	0	0	.20	.28		
10	0	0				
Avg			.13	.16	.47	.53

incorporated into the diet of pregnant female rats to accomplish *in utero* transfer since the antibiotic was not detectable in the fetuses from rats that received the diet containing 0.5 g per kilo.

Evidence of mammary transfer of chlortetracycline. In Table II, it is shown that the tissues of young rats that suckled during a 24-hour period, contained higher levels of antibiotic than those observed for fetuses (Table I). This additional amount of antibiotic in the tissues is assumed to have come from the ingestion of the milk which indicates mammary transfer. Further evidence of mammary transfer is signified since the tissues of the young contained detectable amounts of the antibiotic after suckling dams that had received a diet containing only 0.5 g per kilo of diet.

Maddock *et al.*(4) have shown in swine that at marginal levels of antibiotic feeding, chlortetracycline will appear in the milk of some swine and not in others. This accounts for the absence of antibiotic in the suckled young of some of the dams receiving the lower level of chlortetracycline even though their stomachs contained visible milk. To verify this, all dams were rebred and their excised mammary tissue was assayed for antibiotic at the same time as their young.

Significant amounts of chlortetracycline were found in the mammary tissues of rats consuming diets containing 2 g per kilo of diet. Again the tissues of the suckled young contained antibiotic from mothers at the same dietary level (0.5 g/kg) where placental transfer did not occur and the presence of chlortetracycline in the suckled young was associated with the presence of the antibiotic in the mammary tissue (Table III).

TABLE III. Chlortetracycline in Mammary Glands and Suckling Rats.
Level of chlortetracycline in dams diet.

	Control		0.5 g/kg		2.0 g/kg	
	γ/g					
Litter No.	Mammary	Suckling	Mammary	Suckling	Mammary	Suckling
1	0	0	.37	1.15	.40	.90
2	0	0	.20	0	.50	1.00
3	0	0	.18	.60	.47	.46
4	0	0	0	0	0	.21
5	.15*	0	0	0	.17	.85
6	.06*	0	0	0	.18	.68
7	0	0	0	0	.80	.90
8	0	0	0	0	.58	.21
9			.58	0	dest†	1.85
10					.09	.95
Avg	.02		.15	.19	.32	.80

* Probably due to natural mammary product because zone disappeared in 40 hr.
† des = destroyed.

TABLE IV. Effect of Chlortetracycline in Maternal Diet on Weight and Number of Fetuses Taken by Hysterectomy.
Level of chlortetracycline in dams diet.

	Control		0.5 g/kg		2.0 g/kg	
Litter No.	No./ litter	Wt/litter, g	No./ litter	Wt/litter, g	No./ litter	Wt/litter, g
1	10	39.0	2	10.0	11	42.0
2	10	37.0	11	39.5	10	37.5
3	10	39.0	9	32.5	11	41.0
4	4	15.5	10	39.0	11	41.0
5	5	16.0	10	38.5	12	42.5
6	2	7.5	9	35.5	9	33.0
7	9	32.5	7	23.5	12	45.0
8	10	38.5	11	41.0	8	29.5
9	10	35.0	7	38.0	9	37.0
10	10	37.0				
Avg	8	29.7	8.4	33.0	10.3	38.7

TABLE V. Effect of Chlortetracycline on Weight and Number of Young (Suckled for 24 Hr).
Level of chlortetracycline in dams diet.

	Control		0.5 g/kg		2.0 g/kg	
Litter No.	No./ litter	Wt/litter, g	No./ litter	Wt/litter, g	No./ litter	Wt/litter, g
1	9	53.0	9	49.0	8	51.0
2	8	42.0	9	51.0	11	64.0
3	9	54.0	8	43.0	8	50.0
4	6	35.0	12	63.0	11	64.0
5	8	52.0	10	54.0	10	58.0
6	9	55.0	8	46.0	4	28.0
7	8	46.0	8	46.0		
8	9	57.0	7	47.0		
9	4	26.0	8	50.0		
10	6	40.0				
Avg	7.6	46.0	8.8	49.9	8.6	52.5

Neither the numbers of fetuses nor their average weights were influenced by incorporating 0.5 or 2.0 g of chlortetracycline per kilo into the diets of pregnant rats for 2 weeks before term (Table IV). Although the average number of fetuses per dam was greater for those consuming the diet containing 2 g per kilo, the average differences were not significant. For all treatments the average fetal weights were approximately 2 g less

TABLE VI. Effect of Chlortetracycline on Weight and Number of Young (Second Litter of Dams in Table V).

Level of chlortetracycline in dams diet.

Litter No.	Control No./litter	Control Wt/litter, g	0.5 g/kg No./litter	0.5 g/kg Wt/litter, g	2.0 g/kg No./litter	2.0 g/kg Wt/litter, g
1	8	52.0	11	65.0	10	62.0
2	9	60.0	11	62.0	10	59.0
3	9	60.0	8	56.0	2	19.0
4	10	63.0	12	67.0	5	37.0
5	7	36.0	10	68.0	13	82.0
6	5	32.0	3	23.0	8	44.0
7	9	63.0	14	76.0	12	77.0
8	5	33.0	4	32.0	7	50.0
9			13	84.0	13	80.0
10					3	20.0
Avg	7.7	49.9	9.5	59.2	8.3	53.0

than for the young rats that were sacrificed at 24 hours after birth.

The high level of chlortetracycline supplement appeared to aid in conception on second breeding. In the second experiment (Table V), 4 of the 10 females received did not bear young. When these same females were continued on the same supplement while being rebred, all conceived within a 3-week period and went full term (Table VI). In the unsupplemented group conception was slower and only 8 of the 10 females conceived over a period of 3 months.

Summary. Pregnant female rats were fed diets containing different amounts of chlortetracycline. When the prenatal diet contained 2 g/kg the antibiotic was present in the fetal tissues. At levels of 0.5 and 2.0 g/kg of diet, detectable amounts of the antibiotic were present in the mammary glands of the females and in tissues of the suckled young. The higher level of antibiotic (2 g/kg) improved the conception rate but did not affect the size of the fetuses or the number of young per litter.

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Radioactive Tracer Studies on Uptake of Diamino-diphenyl-sulphone by Leprosy Patients. (22875)

K. R. CHATTERJEE AND R. K. PODDAR (Introduced by S. Banerjee)
School of Tropical Medicine and Institute of Nuclear Physics, Calcutta, India.

Diamino-diphenyl-sulphone and a number of its derivatives are now well-established in the treatment of leprosy. But there is much confusion as regards the mode of action of sulphone on leprous lesions(1-5). The present radioactive tracer study on uptake of sulphone by leprosy patients was undertaken with the hope that it might throw some new light and may be helpful in removing the confusion. The investigation has been concerned with (a) concentration of the drug in blood, bone-marrow and skin tissues, the rate of excretion through kidneys and (b) the localization, if any, of the drug in affected tissues and invading micro-organisms. This paper deals with the first part of the investigation.

Material and methods. We studied 22 cases of leprosy—8 lepromatous and 14 non-lepromatous. They were adult males, weighing 40-60 kg. Blood volume of each patient was determined by Evans blue and thiocyanate technics. *Diamino-diphenyl-sulphone (DDS)* tagged with S-35 was used. This was procured from Amersham, England and had an initial specific activity of 1 μc/mg. For *oral administration,* a single dose of 4 μc/mg was given as a powder. For parenteral use, 2-7 μc were injected subcutaneously as a 5% suspension in 0.1% agar solution. *Blood* was