Fetal Marking *in Utero*[1] (37148)

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In utero marking of a fetus to detect it among litter mates after birth became a necessity in our laboratory. The technique needed to be one which had no adverse effect on the fetus marked. Vital staining of fetuses has been attempted with varying results (1, 2). Most mammalian fetuses that have been checked were found to drink amniotic fluid while *in utero*. The technique is dependent upon fetal drinking *in utero* (1, 3–5) and absorption of the dye into the blood stream. Most "success" has been obtained with Trypan Blue. However, mammalian fetuses have a low tolerance limit for the dye.

Materials and Methods. A saturated solution of Blue Dextran 2000 (2.506 g dissolved in 63–65 cc of H_2O) (furnished by Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and Cibacron Blue F 3G-A dye (18 mg/cc of H_2O) (furnished by CIBA, Basal, Switzerland) were prepared. As far as we could determine the latter dye is the one used to make the Blue Dextran 2000.

Coeliotomies were performed on pregnant females, exposing the uterine horns with their fetuses; 0.1 cc of the dye was injected through the uterine wall into the amniotic fluid of selected fetuses with a #27 hypodermic needle.

Results. We surveyed many "vital" stains for marking fetal cotton rats (*Sigmodon hispidus*). All ended with failure except the saturated solution of Blue Dextran 2000 and the Cibacron Blue F 3G-A (6). Each dye marked a fetus equally well. The fetuses were not vitally stained, but the dye was concentrated in the lower intestine. The dye could be seen with ease through the abdominal walls in the inguinal area. Two females were killed before parturition to ascertain that the marked fetus was indeed the one whose amniotic fluid received the injection. We tried this marking technique in standard laboratory animals with the same results. The dye could be seen in young cotton rats for 29 hr postpartum, while young white rats (*Rattus norvegicus*) were still visibly marked 4 days after birth.

No harmful metabolic effects of either dye were detected in the marked cotton rat or white rat fetuses. Table I summarizes the marking and growth of some fetuses with both dyes. The relatively poor success with rabbit (*Oryctolagus cuniculus*) and hamster (*Cricetus auratus*) was probably due to poor operating technique.

Discussion. Dissection of marked and unmarked white rat and cotton rat young showed that only the marked animals contained the dye and it was indeed concentrated in the lower intestine. We think the lower intestine is vitally stained because the dye does not pass out with the feces.

Parkinson (7) found dextranase activity in the proximal two-thirds of the white rat intestine. Fischer and Stein (8) say mammalian dextranases degrade dextran by endwise splitting of glucose residues back to branching points, and the extent of splitting ranges 25–30%. Pharmacia claims their Blue Dextran 2000 has a molecular weight of about 2 million. Considering the above, the molecular weight of the Blue Dextran dye remaining in the intestine is still large, and

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TABLE I. Fetal Marking Experiments.

Species (dye)	An. No.	Day of operation (p.c.)	No. fetuses ( )=No. marked L. Horn	R. Horn	No. born alive ( )=No. marked	Av. 20-day wt. of young (g) Unmarked	Marked
Cotton rat							
(Dextran Blue)	1	17	6 (1)	1 (1)	7 (2)	34	30
	2	18	1 (1)	7 (1)	6 (2)	41	40
	3	19	8 (2)	0	—[a]		
	4	20	4 (1)	5 (3)	8 (4)	26.5	29
	5	20	4 (1)	3 (1)	6 (1)	27.6	28
	6	22	5 (1)	3 (1)	7 (2)	35	31
	7	24	5 (1)	2 (1)	7 (2)	21.3	26
	8	24	4 (1)	3 (1)	5 (1)	32.8	36
Cotton rat							
(Cibacron Blue)	1	20	2 (1)	4 (1)	—[a]		
	2	20	2 (1)	4 (1)	3 (1)	53	47
	3	22	3 (1)	5 (2)	5 (2)	35.3	35.5
White rat							
(Dextran Blue)	1	17	9 (3)	1 (0)	7 (1)		
	2	19.5	5 (2)	3 (2)	6 (2)		
	3	19.5	3 (2)	5 (2)	6 (3)		
	4	20.6	2 (2)	6 (2)	7 (4)		
Hamster							
(Dextran Blue)	1	13	3 (1)	5 (1)	8 (2)		
Rabbit							
(Dextran Blue)	1	23	3 (1)[b]	4 (1)	7 (1)		

[a] Female sacrificed prior to parturition to check position of fetuses.

[b] 0.5 cc of dye injected into each amnion.

should not be absorbed from the intestine. Although the Cibacron Blue F 3G-A has a molecular weight of only 839 g, there is no indication that it is absorbed in the intestine.

Dextranase may not exist in fetuses and young rats. If it is present, then much of the dye could be set free from the dextran. This "free" dye could then stain as the Cibacron Blue F 3G-A does.

We conclude that this marking technique, involving fetuses of polytoccus species, is successful and useful when controls are needed for fetuses treated *in utero*. We have seen no indication that the technique has an adverse influence on a fetus.

1. Wislocki, G. B., Contr. Embryol. Carnegie Inst. Wash. **11**, 45 (1920).

2. Wislocki, G. B., Contr. Embryol. Carnegie Inst. Wash. **13**, 89 (1920).

3. Becker, R. F., Windle, W. F., Barth, E. E., and Shulz, M. D., Surg. Gynec. Obstet. **70**, 603 (1940).

4. Huggett, A. St. G., Cold Spring Harbor Symposia on Quant. Biol. **19**, 81 (1954).

5. Reynolds, S. R. M., "Physiology of the Uterus," p. 387. Paul B. Hoeber, New York (1949).

6. Turnipseed, Marvin R., "Fetal and Ovarian Effects of Gamma Irradiation in Female Cotton Rats (*Sigmodon hispidus*)," p. 11. Ph. D. Thesis, University of Georgia, Athens (1969).

7. Parkinson, T. M., Nature (London) **215**, 415 (1967).

8. Fischer, E. H., and Stein, E. A., *in* "The Enzymes" (P. D. Boyer, H. Lardy, and K. Myrback, eds.), 2nd ed., Vol. 4. Academic Press, New York (1960).

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