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PRO EXPERIMENTIS

Trichromatic fluorescent vital labeling of bone in the fetal macaque[1]

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Summary. Mineralizing tissue was labeled in the macaque fetus by administering sequential vital bone labels to the pregnant female. The 3 labels used (DCAF, xylenol orange and minocycline) fluoresce different colors, thereby facilitating identification of discrete lines in the rapidly growing bone and a quantitative analysis of bone deposition in utero.

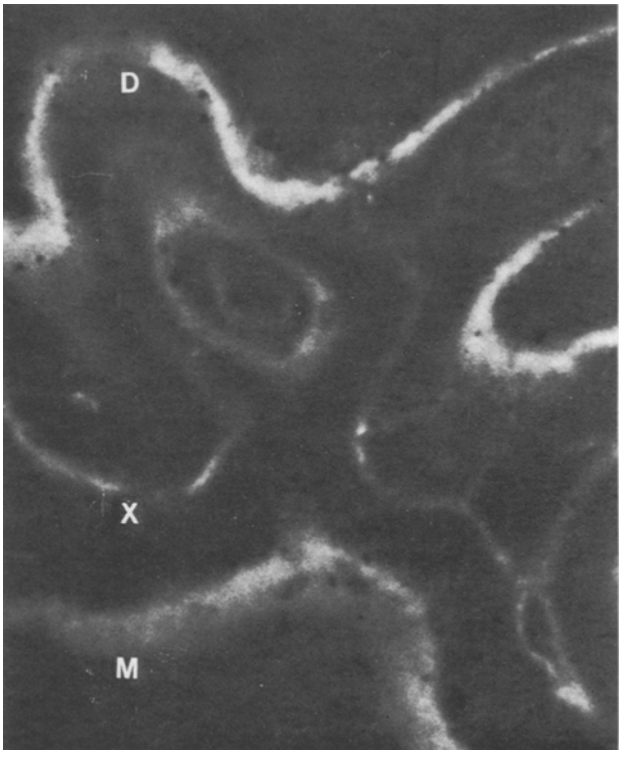
Various fluorescent compounds, particularly antibiotics of the tetracycline group, have been used to label mineralizing bone and dentin in diverse species. Although transplacental transmission of various tetracyclines has been demonstrated in both the rat and human fetus[2,3], there has been no systematic attempt to label mineralizing fetal tissues for the purpose of analysis. We report here a method for labeling in utero bone in the fetal macaque *(Macaca nemestrina)* using 3 fluorescent substances administered sequentially. The substances were DCAF (2,4Bis) N,N'Di (carboxymethyl) aminomethyl fluorescein[4] obtained from ICN Pharmaceuticals, Inc., xylenol orange[5] from Mallenckrodt, Inc., and minocycline hydrochloride from Lederle Laboratories Division, chosen on the basis of reported minimal interference with bone growth compared with that of other vital labels[6,7].

Four pregnant macaques *(Macaca nemestrina),* 1 of known conception date and 3 of estimated conception dates, were started on the marking schedule when the fetus was estimated to be about 145 gestational days. The female was anesthetized and 100 cm^3 of saline solution containing the label compound was administered i.v. by slow drip; fetal heart beat was monitored throughout. After birth the left humerus was fixed, dehydrated in alcohol and dioxane, and embedded in Bioplastic® from Wards Biological Supply. Blocks 250-µm-thick were cut at midshaft with a diamond saw and ground to 40 µm. The sections were mounted and viewed with a bright field condenser microscope with a high pressure mercury lamp as the UV light source. A

In utero trichromatic vital bone labeling of the macaque fetus

Label	Dosage (mg/kg)	Birth	Interval	Age
D, X, M	50, 50, 35	dead	3	170
D, X, M	50, 50, 35	viable	9	170 E
D, X, M	35, 35, 35	dead	1	145 E
D, X, M	20, 20, 20	viable	21	160 E

Labels are given in order of administration at 9-day intervals; D = DCAF, X = xylenol orange, M = minocycline. Interval refers to the number of days between the last prenatal label and parturition. Age given is gestational days at parturition; E = estimated gestational age as obtained by age-predictive regression equations[9].



Contrasting trichromatic fluorescent labels in the humerus from the fetus of estimated 160 gestational days at birth (D, DCAF; X, xylenol orange; M, minocycline). Midshaft transverse section, original magnification: × 52.

BG12 exciter filter (maximum transmission approximately 400 nm) and a 530 barrier filter permitted simultaneous observation of the 3 labels. DCAF fluoresces blue-green; xylenol orange, orange; and minocycline, yellow.

The first 2 fetuses were labeled at 9-day intervals with 35–50 mg solution per kg pregnant female weight; 1 was a viable fullterm birth, 1 a stillbirth. The dosage was then reduced to 35 mg/kg and a premature, stillborn fetus was delivered at about 145 days. Since the conception date had been underestimated, death of the fetus may have been related to its immaturity throughout the labeling period. The 4th animal was labeled at 20 mg/kg and was a viable birth at about 160 gestational days. Each dosage level yielded marks that fluoresced satisfactorily.

We conclude that in utero vital labeling of bone is feasible in the macaque fetus during the last quarter of the gestational period at dosage levels of 35 or 20 mg/kg pregnant female weight. The trichromatic system presents distinct advantages over sequential monochromatic labeling when bone is mineralizing rapidly and the interval between doses is short. Unique longitudinal information may be obtained on rates and sites of absorption and deposition in the fetus, as has been demonstrated in a study of the growth of the cranial base[8].

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A simple device for measuring nanolitre volumes of fluid[1]

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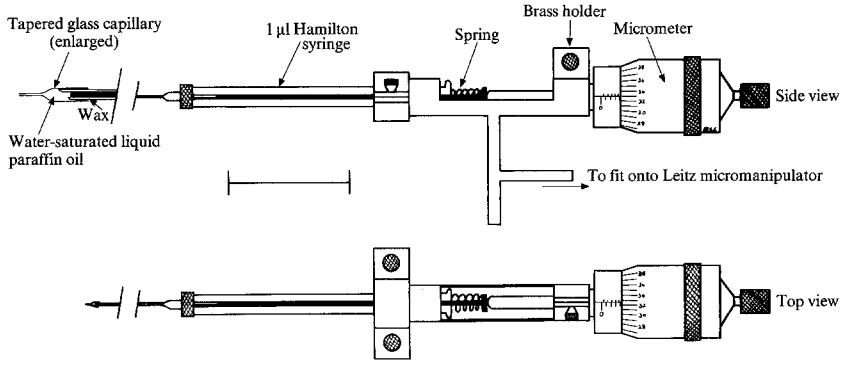
Summary. A piece of apparatus designed to measure nl-volumes of fluid has been constructed to fit onto a Leitz micromanipulator so that the small sample volumes can easily be transferred.

For measuring sample volumes of up to 100 nl, a piece of apparatus has been constructed based on an idea from Little[2]. A 1-µl Hamilton syringe was attached to a micrometer (Moore & Wright, Sheffield, U.K.) and mounted onto a brass holder designed to fit onto a Leitz micromanipulator (Leitz, Luton, U.K.) (figure). A spring was placed around the plunger of the syringe and a piece of tapered glass capillary (S.G.A. Scientific Inc., New Jersey, USA; 1.0 mm outer diameter, 0.7 mm inner diameter, tapered to about 30–50 µm tip diameter, length of taper from shoulder to tip about 4–5 mm) was fitted to the tip of the needle by melting dental wax around the join between the capillary and the needle (figure). The glass capillary had been siliconized using 1% aqueous solution of Siliclad (Clay Adams, New Jersey, USA). The syringe and glass capillary were filled with water-saturated liquid paraffin oil (0.87–0.89 g/ml; Hopkin & Williams, Essex, U.K.) taking care that no air bubbles were introduced into the system.

The tip of the glass capillary was inserted into the sample positioned on the bottom of a clean siliconized glass dish under water-saturated liquid paraffin oil, and by revolving the micrometer the sample could be drawn up into the lumen of the glass capillary. One complete revolution of the micrometer head corresponded to 10 nl. A check on the accuracy was made using tritiated water (1 mCi/ml; Amersham, Bucks, U.K.). When 10 nl of tritiated water was measured 10 times, the final calculated volume was 10.3 ± 0.05 nl (mean ± SEM) and the coefficient of variation was $< 1.5\%$. Similar results have been obtained when volumes of 5–100 nl of fluid have been measured.

If the measured sample is to be deposited into larger volume of buffer or other diluent, it is best to wash out the sample from the glass capillary with that diluent. To achieve this, approximately 2 vol. of diluent were first drawn up into the glass capillary, followed by 0.1 vol. of oil,



Schematic diagram of measuring apparatus. The scale bar represents 5 cm.