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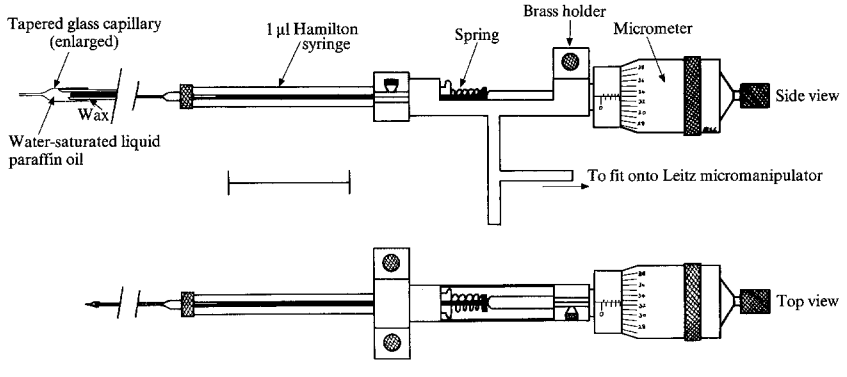
Summary. A piece of apparatus designed to measure nl-volumes of fluid has been constructed to fit onto a Leitz micromanipulator so that the small sample volumes can easily be transferred.

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.

then the sample (1 vol.) and finally 0.1 vol. of oil to enclose the sample.

We have successfully used this piece of apparatus for the transfer of small volumes of fluid for the estimation of inositol[3] and carnitine[4] in the fluid collected by micropuncture from the seminiferous tubules of the mammalian testis and epididymal duct.

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Ag-G staining, a rapid technique for producing combined silver staining and Giemsa banding in mammalian chromosomes

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Summary. A combination of the silver staining method with a Giemsa banding technique is described for mammalian chromosomes. This double staining simultaneously shows silver-stained NORs and a G-band pattern, and thus allows a rapid identification of the NOR-bearing chromosomes.

Ammoniacal silver (A-S) has been used to stain differentially chromosomes which are involved in the genetic activity of the NORs[1,2]. A number of techniques have been developed for the staining of NORs on human chromosomes[3-6]. These methods have the disadvantage that the chromosomes are only slightly, and uniformly, stained with the exception of the black-stained NORs. For identifying these NOR-bearing chromosomes additional techniques had to be applied. Lately combined methods have been described where silver staining was followed by Giemsa banding[7,8]. These combined procedures are very suitable for human chromosomes but less favourable for other mammalian chromosomes. In this paper we describe such a combined technique and its application to mammals of the family Bovidae.

Materials and methods. Lymphocyte cultures from peripheral blood from 3 individuals of cattle *(Bos taurus)* and goat *(Capra hircus)* were set up after routine methods. Chromosome preparations were stained with a modified silver method according to the Ag-staining of Mikelsaar et al.[9,10]. 4 drops of 50% aqueous $AgNO_3$ were put on air-dried chromosome preparations and covered with a cover-glass. The slide was placed for 10 min under a photoflood light (temperature optimum at 60 °C). After this procedure the slides were rinsed in several changes of distilled water and air dried.

For the next step, 2 drops of solution A (4 g $AgNO_3$ dissolved in 5 ml NH_4OH and 5 ml aqua dest.) and 2 drops of solution B (3,2 g Na-acetate in 97 ml aqua dest. and 3 ml formaldehyde) were mixed on a cover glass, and the slide

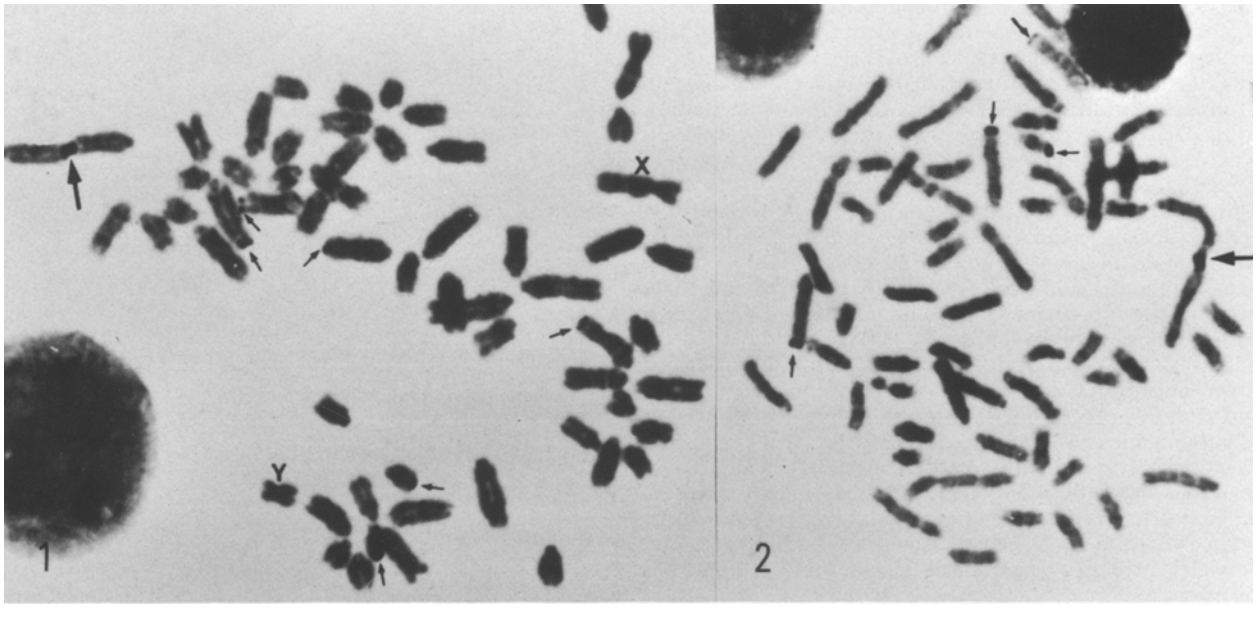

Fig. 1. Metaphase chromosomes of cattle (*Bos taurus*, 2n = 60) from Ag-G preparations. Small arrows indicate NOR sites on the telomeric regions of several chromosomes. Strong arrow indicates association between 2 NOR-bearing chromosomes. Fig. 2. Ag-G preparation from goat (*Capra hircus*, 2n = 60), showing chromosomes with Ag-NORs (small arrows). Association of 2 NOR chromosomes can be seen (strong arrow).