A Method for Obtaining Rabbit Bone Marrow for Chromosome Studies.* (29027)

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Certain human mental disorders and congenital anomalies have been associated with chromosomal aberrations(1). Bone marrow has been used for chromosome studies because of its high mitotic index. In studies of the smaller laboratory animals the methods used in obtaining marrow have often involved sacrificing the animal or extensive surgery (2).

Many mutants in the Jackson Laboratory rabbit colony have pathologies analogous to human disorders. We have initiated studies to correlate these anomalies with possible chromosomal aberrations. The following technique has been developed for efficient chromosomal assay avoiding killing, doing permanent damage to the rabbit, or subjecting the animal to extreme trauma.

Materials and methods. All animals received intraperitoneally 1 ml/kg body weight of Colcemide (Ciba) solution (5 mg/cc distilled water). The relative nontoxicity of this Colchicum derivative and its rapid metabolism with subsequent elimination permit *in vivo* use(3). Thirty to forty-five minutes later a relaxing dose of nembutal sodium (60 mg/ml, approximately 0.35 cc/kg body weight) was given intravenously. The left leg in the area of the distal end of the femur was then washed with 70% alcohol. Novocain 2% Suprarenin 1:20,000 (Winthrop) was applied to anesthetize the surgical site. A sterile Univ. of Illinois biopsy needle (V. Mueller & Co.) was then introduced through the rabbit's skin. With the leg fully extended, the patella, patellar ligament, and the quadriceps femoris were displaced from the medial surface of the leg. The notch through which the ligament travels was next found and the point of the trephine directed to this site.‡ Pressure was applied to the trephine as it was rotated slowly in 180° arcs until bone penetration commenced. At this point, pressure was reduced slightly and drilling continued, using a full 360° rotation, until the point of the trephine was well into the marrow cavity. After removing the central core of the trephine a 5 cc syringe (this may or may not be heparinized) was attached to the trephine and approximately 1 cc of marrow was aspirated (Fig. 1). The marrow was immediately suspended in 5 ml 0.85% NaCl with 2 mg/liter of Colcemide added, the suspension diluted with 3 parts of 1% sodium citrate for each part of suspension, and allowed to remain for 20 minutes. The animal was given .1 cc Strep-Combiotic§/kg body weight and allowed to remain in a warm box for 18 hours before being returned to its cage.

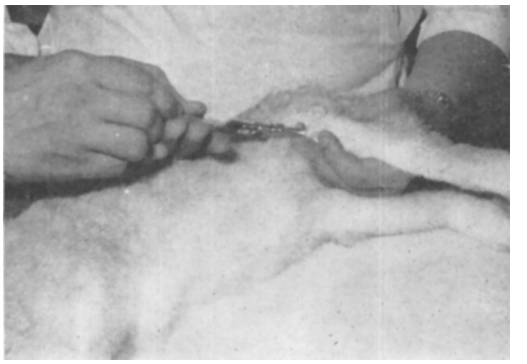

FIG. 1. Marrow being aspirated with a Univ. of Illinois biopsy needle inserted in distal end of left femur.

The material was then centrifuged at 2100 rpm for 5 minutes, the supernatant decanted

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‡ When the center of the femoral shaft was used as a biopsy site, the bone fractured and broke in 2 of the 3 animals tried.

§ Strep-Combiotic (Pfizer): 200,000 units Penicillin G procaine and 0.25 g Streptomycin as the sulfate in aqueous suspension.

and the pellet fixed in a 1:3 mixture of 50% glacial acetic acid and methyl alcohol, without being dislodged from the tube. The pellet was undisturbed for 20 minutes and was then suspended with a Pasteur pipette. After 3-4 minutes, the suspended material was centrifuged under the same conditions as above, then the pellet was resuspended in fresh fixative for 4 minutes and recentrifuged. The supernatant was discarded and the pellet dispersed in a very small quantity of fixative (the amount determined by cell density desired).

When the final suspension had been made, slides were prepared using the technique of Dr. Kenneth Yao (Inst. of Cellular Biology, Univ. of Nebraska) for air-dried preparations, dropping (from a height of about 3-5 cm) 1 to 2 drops of the suspension onto a clean slide previously dipped in distilled water. The slides were dried by a small fan blowing warm air, stained 15 minutes with aceto-orcein (2% orcein in 50% glacial acetic acid), and mounted in Diaphane after dehydration in an alcohol series (two 95% baths one minute each, followed by 15 minutes in 100%). Following drying the slides were cleaned and examined for mitotic figures.

Results and discussion. Of 55 animals aspirated none was lost due to bone damage. Three were lost to anesthesia when only nembutal was used. The combination of a relaxing dose of nembutal and a local anesthetic proved optimal. Post-mortem observation on killed animals indicates that bone regeneration commences 1 week after biopsy. Radiographic study of a few animals indicates that regeneration is almost complete at 5 weeks. No soreness, limping, or other signs of discomfort were noted. This method is much less traumatic than that reported by Steinberg and Martin(2).

It was also noted that there is an apparent relationship between time of day the marrow was taken and per cent of mitotic figures. Midday samples were much richer in figures than early morning samples. This is in accord with the leucocyte cycle as reported by Markowitz(4).

Summary. A technique for aspirating and preparing bone marrow for chromosome studies without harm or permanent injury to the rabbit is described. In consists of an intraperitoneal injection of Colcemide at midday, followed in 45 minutes by anesthesia (nembutal plus local novocain) and bone marrow aspiration by trephine. Aspiration is by an anterior medial puncture on the distal end of the left femur. Samples were obtained from 55 animals, of which none was lost due to the marrow recovery technique. Post-mortem observations indicated that bone regeneration commences within 1 week and is almost complete at 4 weeks. At 5 weeks the trephine hole is almost impossible to locate radiographically. This method may well be applied to other small mammals where current techniques require killing the animals.

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