CHAPTER 13

Biopsy Darting

WILLIAM B. KARESH

REMOTE BIOPSY TECHNIQUES

The widespread use of biopsy punches adapted for remote sample collection began in the 1980s.[1,11] The technique allows for the collection of biologic materials without the need for, and the risks associated with, the capture and handling of animals. Before techniques for amplifying genetic material, such as polymerase chain reaction (PCR), were developed, biopsy samples were used in cell cultures to produce fibroblast cell lines and provide adequate amounts of deoxyribonucleic acid (DNA) for genetic analysis.

Subsequently, molecular genetic techniques advanced sufficiently to allow analysis without the need for cell culture. These same techniques have permitted genetic analyses to use other, noninvasively collected samples, such as feces, hair, or shed epithelial cells, and have led to some innovative approaches, such as netting sloughed skin from the surface of water around breaching whales or putting duct tape on the tip of a plastic syringe dart to pluck hair samples from primates.[5,13]

The ability to perform genetic analyses directly on biopsy material rather than requiring successful cell culture has also increased the utility of biopsy darting by eliminating the sample losses caused by bacterial and fungal contamination and making sample storage simpler. Concomitant with advances in genetic analytics, remote biopsy collection has become widely used when other options should not be employed. Increasingly, the technique is used to answer questions not related to genetics, such as those involving infectious and noninfectious diseases, toxicology, and biomarker assessment.[4,6,7,15]

Biopsy darts have been used in a wide range of vertebrate species, including more than 30 species of cetaceans, as well as many species of pinnipeds, carnivores, primates, ungulates, and birds[3,7-12] (Box 13-1). Darts have even carried by teams searching for "Sasquatch" in the United States. The most common delivery mechanism for the biopsy instrument uses a dart projector (e.g., pistol, rifle), a crossbow, or a mounting on the end of an extension pole (Figures 13-1 through 13-4).

Box 13-1

Partial Listing of Taxonomically Grouped Species Reported to Have Been Sampled Using Biopsy Darting Techniques

American alligator	Orangutan
Ostrich	Lowland gorilla
Bottlenose dolphin	Patas monkey
Common dolphin	Domestic horse
Striped dolphin	Zebra
Killer whale	Prezwalski horse
Humpback whale	African elephant
Fin whale	*Rhinoceros* spp.
Right whale	Gaur
Gray seal	American bison
Southern elephant seal	Giraffe
Southern fur seal	Okapi
South American sea lion	Greater kudu
Walrus	Impala
Lion	Waterbuck
Sun bear	Lechwe
Brown bear	Kob
African wild dog	Puku
Spotted hyena	Bighorn sheep

BIOPSY DARTING EQUIPMENT

Following the early development and production of biopsy darts by individuals or teams of researchers in the 1980s, biopsy darting equipment has become commercially available from a number of manufacturers (Box 13-2). Most of the biopsy tips produced commercially are adapted to drug injection darting equipment.

All systems use the concept similar to a standard biopsy punch: a metal cylinder with a sharpened edge that cuts through the skin and underlying tissues. The tissue is held within the biopsy punch tip by friction

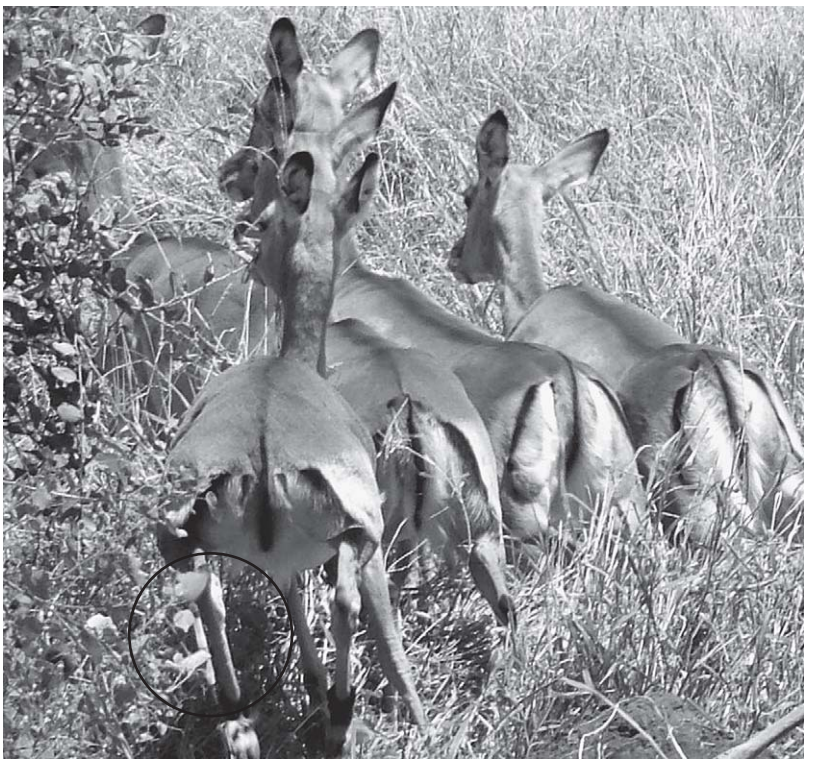


Fig 13-1 Darted impala. (See Color Plate 13-1.)

Fig 13-2 Darted zebra. (See Color Plate 13-2.)

or by barbs provided through the addition of either notches or protrusions from the inner wall of the punch cylinder, or by separate barbed hooks added inside the cylinder, such as the use of barbed broaches used in dentistry.

Figure 13-5 illustrates commercially available biopsy darts. The Telinject system (not illustrated) uses a cutting tip that fits one of the company's plastic syringe darts, combined with an insert piece that resembles three parallel-barbed dental broaches. The Palmer Cap-Chur biopsy tip screws on the company's metal dart barrels and uses standard coarse-grade barbed dental broaches. It may be mounted on a variety of projector devices, with fittings available from the company. This product is also available with a notched indent on the side of the biopsy punch that allows air to escape and provides additional tissue-holding strength. The Pneu-Dart biopsy tip attaches to the front of one of the company's projectile syringes, with the injection needle modified to serve as the barbed tissue holder. The Dan-Inject biopsy tip is longer and thinner than the other commercially available

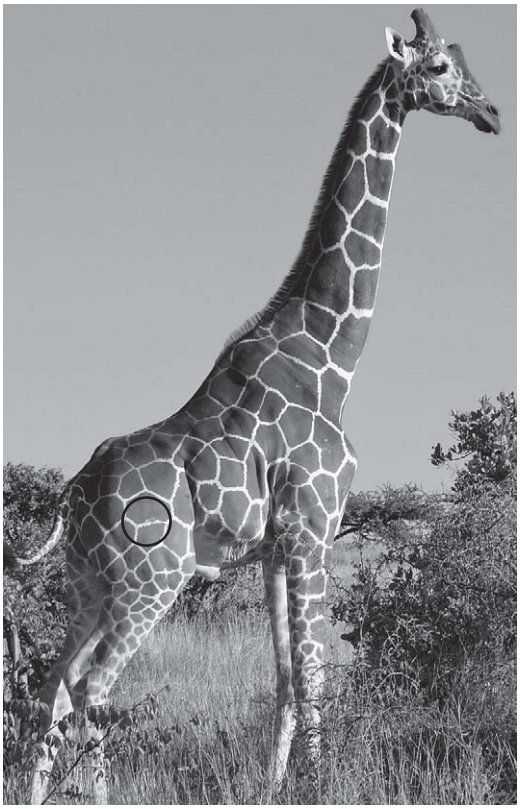


Fig 13-3 Giraffe darted in right hindquarter. (See Color Plate 13-3.)

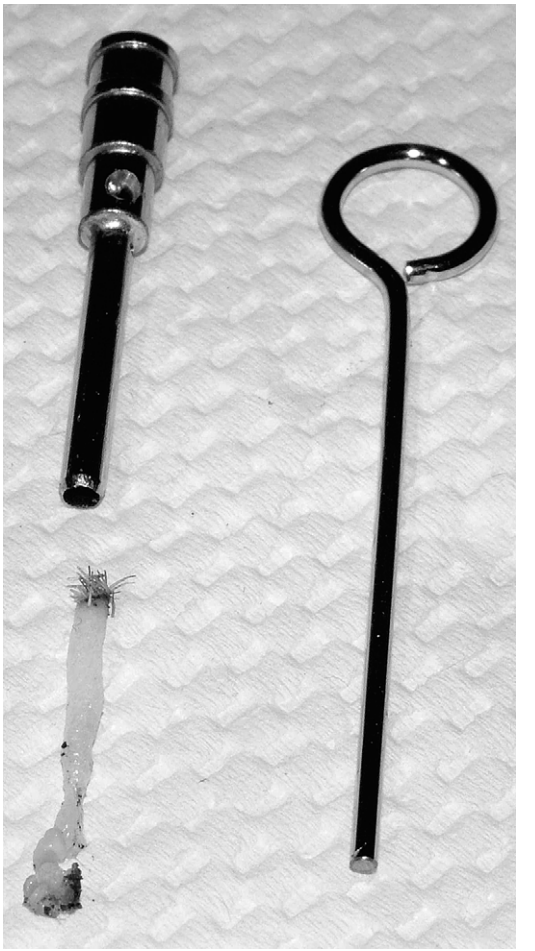


Fig 13-4 Biopsy sample of same giraffe in Figure 13-3. (See Color Plate 13-4.)

Box 13-2

Commercial Producers of Biopsy Darting Equipment and Supplies

Palmer Cap-Chur Equipment Co., Inc. (www.palmercap-chur.com): syringe dart, crossbow bolt, arrow or extension pole
Dan-Inject ApS (www.dan-inject.com): syringe dart
Pneu-Dart, Inc. (www.pneudart.com): syringe dart
Telinject GmbH (www.telinject.de): syringe dart

systems and uses friction to retain the biopsy sample within the tip. It has a side-port opening at its base to allow air pressure to escape behind the tissue sample, thus helping to prevent the sample from falling out of the tip.

For all these systems, the biopsy cutting tip works best when it strikes a relatively firm body surface lying in a plane perpendicular to the dart. Soft or pliable target areas, such as body areas with loose skin or abundant subcutaneous fat, may disperse impact energy and prevent the biopsy dart from making a complete skin incision. Most darting systems may effectively penetrate thick hair coats. For very-thin-skinned or more delicate animals, a rubber plunger from a 3-mL syringe may be slid down to the base of the biopsy tip to shorten the depth of penetration and serve as an impact absorber.

Unless the biopsy tip is attached to an extension pole or a retrieval cord, the projectile needs to recoil or bounce off the animal immediately, which is most common, or to fall out before losing sight of the darted individual. In thick-skinned animals such as elephants and rhinoceroses, the darts frequently stay in the skin until the animal's movement and gravity cause it to fall free. This could also be a result of the tendency to shoot long distances at large species; thus the dart hits with lower impact and less recoil energy to bounce out.

For aquatic use, the biopsy dart needs flotation material or a retrieval cord to prevent the loss of the darts and samples. Retrieval cords may also be useful in colony situations, where other group members would be disturbed if the researcher approached the animals to retrieve the dart from the ground.

Finding the biopsy dart once it has fallen off the animal may be quite challenging. In addition to

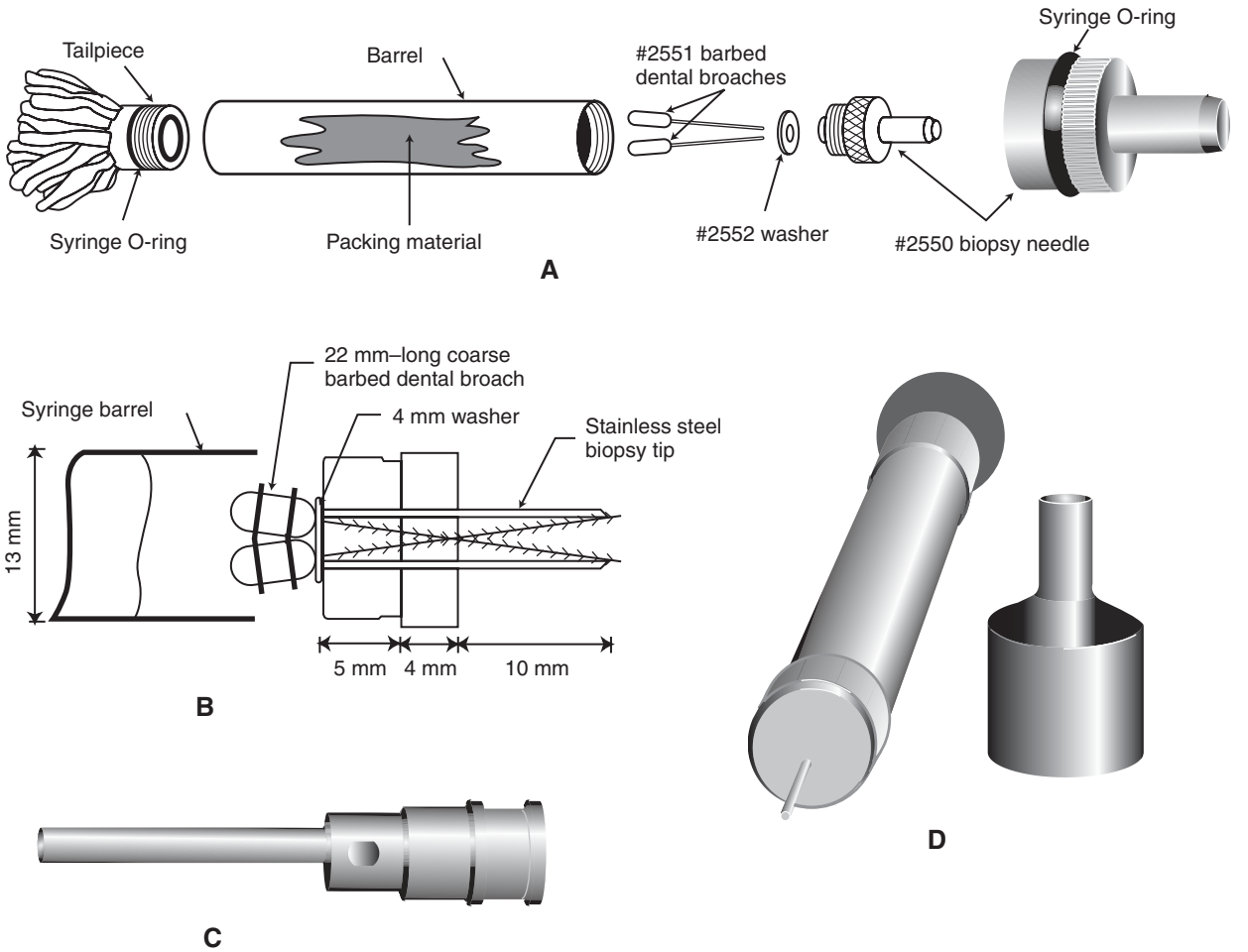


Fig 13-5 Commercially available biopsy darting systems. The Palmer Cap-Chur biopsy tip screws on the company's standard metal dart barrel **(A)** and uses standard coarse-grade barbed dental broaches **(B).** The Dan-Inject biopsy tip **(C)** fits on the company's projectile syringe and has an air exit hole at its base to prevent sample loss. The Pneu-Dart biopsy tip attaches to the front of one of the company's projectile syringes **(D),** with the injection needle modified to serve as the barbed tissue holder. (Images are not to scale.)

retrieval or tether cords, other methods include painting the dart a bright color, painting the dart with cold luminescent material and darting at dusk, using radio transmitter tailpieces for the darts, and using a metal detector. Dan-Inject also sells a dart syringe fitted with a passive diode for radio location by the Recco search system, providing a maximum search radius 15 to 20 m (50-66 ft).

DART SELECTION

Although reports of adverse reactions after biopsy darting are rare, researchers must always use the same level of caution required for any type of darting.[2,14] To avoid traumatic injuries to the animal, appropriate matching of the size, weight, and impact of the dart to the physical characteristics of the animal is essential. The fact that immobilization agents or other drugs are not being delivered in the darting system does not justify a lack of training and practice in darting procedures before working with live animals. Appropriate sterilization techniques are essential to prevent infectious agent transmission to or among animals being sampled. The discovery of prion-related diseases may pose additional considerations for sterilization techniques in the future.

Concern for behavioral disturbances must be applied, not only for the direct effect on the individual, but also for how the individual's reaction may affect others in the group, such as abandonment of young, displaced aggression, or stampeding. It must be recognized that dart tips, like injection needles, may break off in the animal, especially if they penetrate bone or other extremely hard tissues. As with any type of darting, the impact of overpowered darts may cause severe soft tissue damage, abdominal penetration, or skeletal fractures. Inaccurate darting or sudden movement of

animals may result in a dart hitting easily damaged body parts, such as eyes or genitalia.

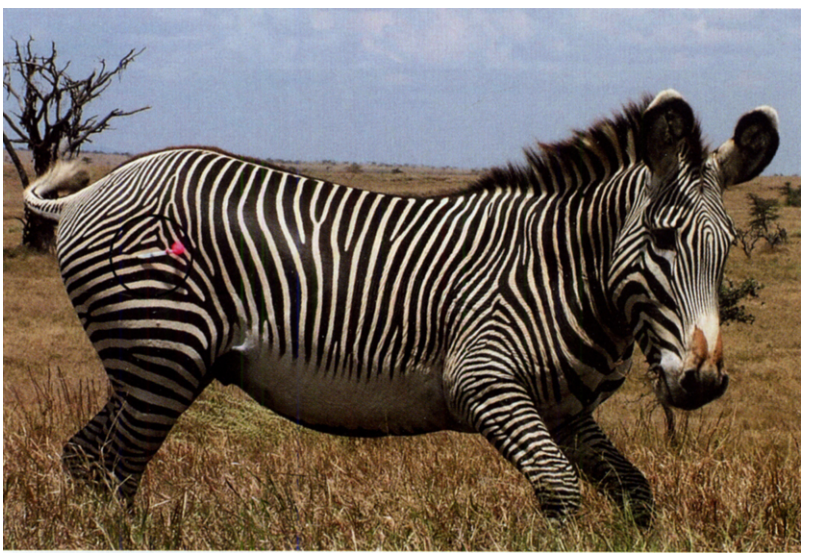
Personnel also need to be properly trained to prevent darting-related accidents in other staff and to handle samples properly to avoid zoonotic infections.

When used correctly, biopsy darting remains a valuable tool for obtaining samples for a variety of diagnostic and research purposes. It also provides an alternative to the capture and handling of wildlife and to lethal sample collection methods.

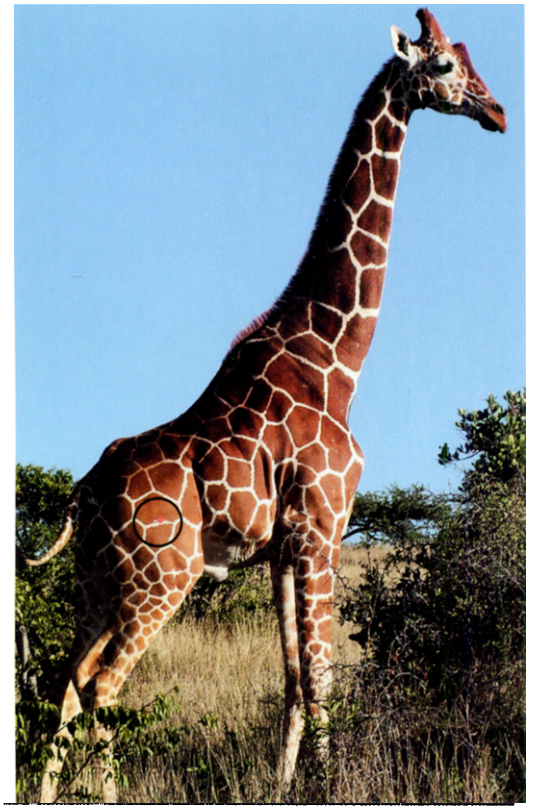
References

1. Aquilar A, Nadel J: Obtención de biopsias hipodérmicoas de cetáceos en libertad, *Invest Pesquera* 48(1): 23-29, 1984.
2. Bearzi G: First report of a common dolphin *(Delphinus delphis)* death following penetration of a biopsy dart, *J Cetacean Res Manage* 2(3):217-221, 2000.
3. Birungi J, Arctander P: Molecular systematics and phylogeny of the Reduncini (Artiodactyla: Bovidae) inferred from the analysis of mitochondrial cytochrome *b* gene sequences, *J Mamm Evol* 8(2):125-147, 2001.
4. Caraveo-PantioJ, Soto LA: Stable carbon isotope rations for the gray whale *(Eschrichtius robustus)* in the breeding grounds of Baja California Sur, Mexico, *Hydrobiologia* 539(1):99-104, 2005.
5. Dawn Harlan A, Würsig B, Baker CS: Skin swabbing for genetic analysis: application to dusky dolphin *(Lagenorhynchus obscurus), Mar Mamm Sci* 15(2): 409-425, 1999.
6. Fossi MC, Marsili L: Effects of endocrine disruptors in aquatic mammals, *Pure Appl Chem* 75(11-12):2235-2247, 2003.
7. Fossi MC, Marsili L, Neri G, et al: Skin biopsy of Mediterranean cetaceans for the investigation of interspecies susceptibility to xenobiotic contaminants, *Mar Environ Res* 50(2000):517-521, 2000.
8. Georgiadis N, Bischof L, Templeton A, et al: Structure and history of African elephant populations. 1. Eastern and southern Africa, *J Hered* 85(2):100-104, 1994.
9. Girman DJ, Mills MGL, Geffen E, Wayne R: A molecular genetic analysis of social structure, dispersal, and inter-pack relationships of the African wild dog *(Lycaon pictus), Behav Ecol Sociobiol* 40(3):187-198, 1997.
10. Hogg JT, Forbes SH: Mating in bighorn sheep: frequent male reproduction via a high-risk "unconventional" tactic, *Behav Ecol Sociobiol* 41(1):33-38, 1997.
11. Karesh WB, Smith F, Frazier-Taylor H: A remote method for obtaining skin biopsy samples, *Cons Biol* 1(3):261-262, 1987.
12. Kimwele CN, Graves JA, Burke T, Hanotte O: Development of microsatellite markers for parentage typing of chicks in the ostrich, *Struthio camelus, Mol Ecol* 7(2):249-251, 1998.
13. Valderrama X, Karesh WB, Wildman DE, Melnick DJ: Noninvasive methods for collecting fresh hair tissue, *Mol Ecol* 8:1749-1752, 1999.
14. Weller DW, Cockcroft VG, Würsig B, et al: Behavioral responses of bottlenose dolphins to remote biopsy sampling and observations of surgical biopsy wound healing, *Aquat Mamm* 23:49-58, 1997.
15. Wolfe ND, Escanlante AA, Karesh WB, et al: Wild primate populations in emerging infectious disease research: the missing link? *Emerg Infect Dis* 4(2): 149-158, 1998.

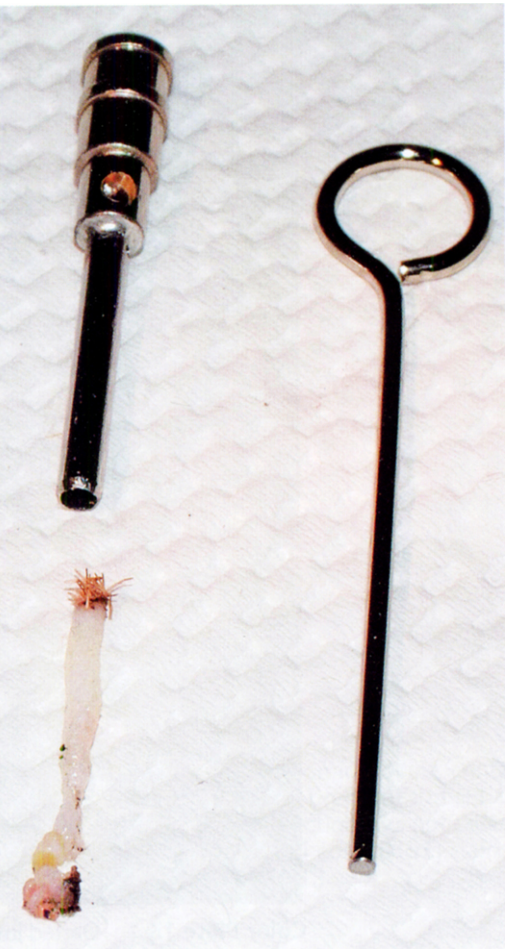
Color Plate 13-1 Darted impala. (For text mention, see Chapter 13, p. 106.)



Color Plate 13-2 Darted zebra. (For text mention, see Chapter 13, p. 106.)



Color Plate 13-3 Giraffe darted in right hindquarter. (For text mention, see Chapter 13, p. 107.)



Color Plate 13-4 Biopsy sample of same giraffe in Figure 13-3. (For text mention, see Chapter 13, p. 107.)