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# USING SCRAPINGS FROM FORMALIN-FIXED TISSUES TO DIAGNOSE LEPTOSPIROSIS BY FLUORESCENT-ANTIBODY TECHNIQUES[1]

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ABSTRACT. **Small specimens of formalin-fixed tissues approximatey 1 × 1 × 0.2 cm were cut from the suspect specimen. Several clean microscope slides were dipped in 1% aqueous gelatin and air-dried or dried on a slide warmer. Each tissue specimen was washed in running tap water for 2–3 min and then lightly scraped with a straight knife blade, cutting edge perpendicular to the surface of the specimen. The scrapings were allowed to build up and cling to the knife blade, which was then turned so that the broad surface contacted the slide; thus, the scrapings could be smeared onto the slide in a single motion. Sufficient pressure was applied to embed the tissue fragments in the gelatin coating. Smears, dried in air or on a slide warmer, were stained immediately by a standard direct or indirect technique to detect fluorescein-labeled antigens. This scraping method, adapted to the study of leptospirosis by fluorescent-antibody technique, could reduce the need for cryostat-cut tissues and facilitate the observation of individual leptospires.**

A major consideration in preparing specimens for fluorescent-antibody techniques (FAT) is not only to localize but also to retain antigens with minimal morphologic and biologic alterations and to permit reaction with specific antibody. Ideally, tissues are collected and quickly frozen in a slurry of liquid nitrogen and isopentane (Sainte-Marie 1962), although a dry-ice ethanol mixture (Coffin and Maestrone 1962; and Coons and Kaplan 1950) is also suitable. Most investigators reporting during the last 15 years have preferred tissues cut in a cryostat. Several, however, have successfully used chemical fixatives and paraffin embedding of tissues (Goldman 1968; Nairn 1969; and Sainte-Marie 1962).

Viable leptospires present in sufficient numbers in animal tissues or body fluids may be detected by dark-field microscopy. Freshly collected, uncontaminated tissues can be placed on culture media or inoculated in susceptible laboratory animals. Silver-stained preparations of paraffin-embedded tissues may also be of value.

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Unfortunately, in routine necropsies adequate tissue specimens are sometimes not taken, and quick-freezing equipment and refrigeration facilities are not always available. Often 10% formalin (4% HCHO) is the only available tissue fixative. Formalin kills leptospires quickly and reduces the risk to technicians working with infected specimens. In view of the hazards of techniques requiring fresh specimens, formalin is especially attractive as a fixative for leptospire-infected tissues.

If a laboratory's concern is primarily to detect an antigen and not immediately to determine its localization, the technique described here can be helpful.

## Materials and Methods

Cultures of 1 serotype of *Leptospira biflexa* (Patoc 1) and of 9 serotypes of *L. interrogans:* australis (1556), autumnalis (13 KKB), bataviae (1415), canicola (Moulton, M-clone), canicola (108), grippotyphosa (1540), hebdomidus (198), icterohaemorrhagiae (1498), and pomona (pomona, HO 1) were provided by Dr. A. D. Alexander of the Walter Reed Army Institute of Research. In addition, *L. biflexa* (Sao Paulo) antiserum was used.

Sixty-six hamsters[a], 21–23 days old, were inoculated intraperitoneally with fresh whole blood from hamsters in the septicemic stage of leptospirosis. Seventy-two hours following inoculation we collected tissue blocks fixed in phosphate-buffered 10% formalin. Thirteen control animals were kept under similar conditions and killed along with the inoculated groups. Tissues collected for this study included the livers and kidneys. In accumulating data for this report, more than 1000 individual tissue scrapings were made. Small tissue blocks measuring approximately 1 × 1 × 0.2 cm were prepared from the formalin-fixed tissues. Several clean microscope slides were dipped in a 1% aqueous gelatin solution and then air-dried or dried on a slide warmer. The specimens were washed for up to 5 min in tap water. The washed specimens were lightly scraped with a straight-bladed knife, its cutting edge perpendicular to the surface of the specimen. Scrapings were allowed to build up and cling to the knife blade. The knife was turned on its side and the tissue scrapings smeared on the slide in a single motion, with sufficient pressure to embed tissue fragments in the gelatin coating. Smears were dried in air or on a slide warmer and immediately stained by a standard direct or indirect technique to detect fluorescein-labeled antibody (Goldman 1968; Nairn 1969).

## Results

Little tissue was lost from the slides during staining procedures. Specific staining clearly showed the leptospires as individual organisms free from debris (figure 1). Therefore, nonspecific staining mattered little here.

Tissue smears provided an opportunity to study individual leptospires but not lesions, although relationships to individual cells in tissues could be ob

[a] In conducting the research described in this report, the investigators adhered to the *Guide for Laboratory Animal Facilities and Care*, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences National Research Council.

served readily (figure 2). In the hamster liver (during the septicemic stage), leptospires intimately associated with hepatocytes were best observed around the edges of the cell. Fewer were present in blood vessels and liver-tissue stroma. In the kidney, leptospires were observed at all levels of the nephron but



FIG. 1 (*top*). Individual leptospires (arrows) stained by fluorescent-antibody technique to demonstrate characteristic conformation. Serotype canicola; × 2000. AFIP Neg. 70-7651-1.

FIG. 2 (*bottom*) Formalin-fixed tissue scraping stained by the Warthin-Starry method to show relationships of individual leptospires (arrows) to hepatocytes Serotype bataviae; × 1000. AFIP Neg 70-7651-2.

were found more often concentrated near basement membranes on and . the cells of the convoluted tubules.

## Discussion

Recently we reported on the effects of formalin on genus specificity of leptospiral antigens in tissues and on the use of the FAT to detect leptospires subsequently (Cook *et al.* 1971). In that study we relied almost entirely on cryostat-prepared tissue specimens. The sections, cut at 2–5 μ, were placed on microscope slides previously dipped in a 1% gelatin solution. Cryostat-cut sections were valuable, but their use presented certain disadvantages:

1. The antigen could be detected readily in formalin-fixed tissues, but it was difficult to recognize individual leptospires.
2. Both cryostat and a skilled technician must be available.
3. Many cryostat-sectioned tissues can become separated from the microscope slides during the necessarily prolonged washing and staining required to detect leptospires in formalin-fixed tissues.

It seemed evident that it would be highly desirable to find a technique that would not require use of the cryostat and that would allow recognition of individual leptospires rather than antigen alone.

Tissue scrapings have long been advocated and successfully used for other purposes; however, formalin-fixed leptospirosis-infected tissue scrapings have not been reported previously for use in the FAT.

In this study we found this scraping technique to be simple and rapid, requiring little equipment. In diagnostic situations and in those in which tissue changes can be evaluated in paraffin-processed tissues, the technique precludes using a cryostat. Also the technique greatly reduces the problem of specimens separating from the microscope slides while undergoing the prolonged staining and washing phases. It reduces nonspecific background staining (we observed leptospires with specific structure and staining specificity rather than amorphous antigen). It allows titration of end points for both antisera and conjugate, based entirely on staining of individual leptospires without the antigen's being masked by cells and debris. The technique is especially adaptable to photography.

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