Immunohistochemical Demonstration of Avian Leukosis Virus Antigens in Paraffin Embedded Tissue[1] (37187)

HENRY S. DI STEFANO, AMERICO A. MARUCCI, AND ROBERT M. DOUGHERTY

Departments of Anatomy and Microbiology, State University of New York, Upstate Medical Center, Syracuse, New York 13210

An immunohistochemical procedure was described for demonstration of avian leukosis virus (ALV) antigens in cell culture (1), utilizing the "unlabeled antibody enzyme method" developed by Sternberger *et al.* (2). We now have applied this procedure to detect ALV antigen in paraffin-embedded tissues of the intact host.

Antiviral antisera were obtained from chickens infected either with RAV-1, which belongs to ALV serotype A, or with RAV-6, which is serotype B. In neutralization tests the antisera reacted only with viruses of the homologous serotype. Pancreas, which is a particularly rich source of ALV in infected birds, was obtained from chickens infected with the F-42 strain of ALV, which belongs to serotype A. Pancreases of uninfected birds from a flock known to be free of ALV infection were used as controls. Tissue samples, fixed overnight with 4% formaldehyde in 0.1 *M* phosphate buffer (pH 7.2), were washed in the buffer, dehydrated by passage through a graded ethanol series, cleared with cedarwood oil, and embedded in paraffin using routine histologic procedures. Xylene or similar solvents are most commonly used as clearing agents for paraffin embedment, however, the prolonged treatment necessary for clearing the tissue samples apparently modified the viral antigen and greatly reduced the intensity of specific staining. This was avoided by the use of cedarwood oil as a clearing agent. Sections 5 μm thick were mounted on coverglasses and the paraffin was removed with xylene. This brief treatment with xylene did not detectably affect the intensity of staining as compared with sections in which ether was used to remove paraffin. The sections were rehydrated through graded ethanol, and the immunohistochemical pro-

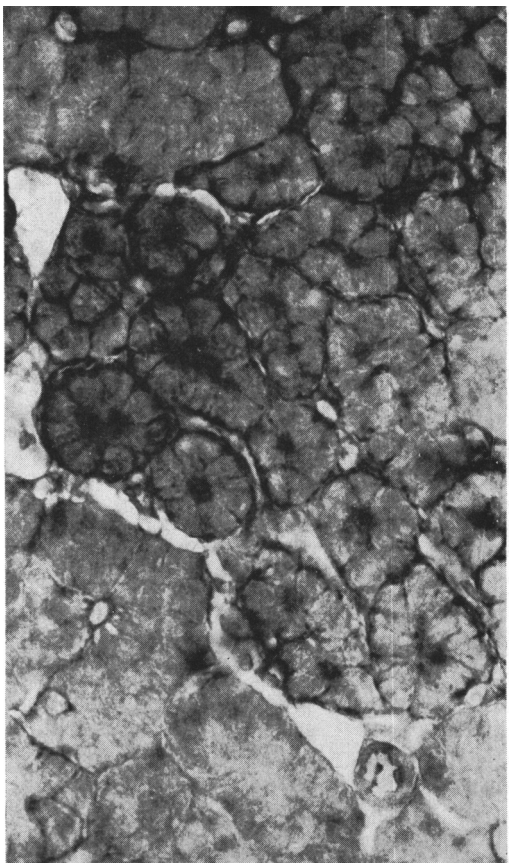

FIG. 1. Localization of type-specific viral antigen in pancreas from a chicken infected with ALV belonging to serotype A and reacted with a homologous chicken serum against ALV of serotype A. The antigen appears in acinar lumena and surrounds many of the epithelial cells (× 550).

[1] Supported by U.S. Public Health Service Grant CA 10148, and National Science Foundation Grant GB 14061.

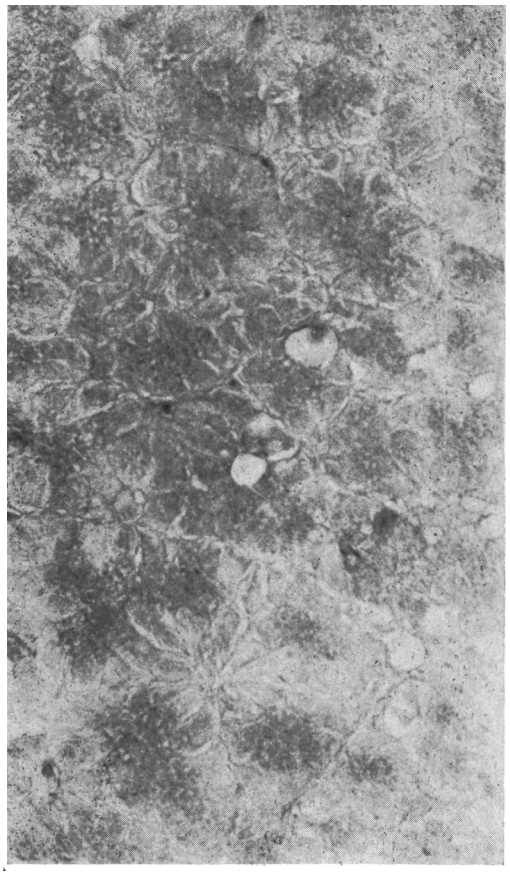

FIG. 2. Section of pancreas from the same bird used in Fig. 1, but reacted with a heterologous chicken serum directed against ALV of serotype B. No reaction product (× 550).

cedure carried out as described in detail previously (1). Briefly, the tissues were first allowed to react with antiviral antibodies prepared in chickens. After washing, the reacted tissues were treated with rabbit antichicken globulin applied in antibody excess. Under these conditions only one of the rabbit antiglobulin binding sites reacted with the bound antiviral chicken globulin leaving one unreacted antiglobulin valence. Finally, a soluble immune complex of peroxidase and chicken antiperoxidase (PAP) was allowed to react with the free rabbit antiglobulin binding sites which resulted in localization of peroxidase at the site of viral antigens. The bound peroxidase was then made visible according to the procedure of Graham and Karnovsky (3), using 3,3′-diaminobenzidine tetrahydrochloride to develop the color. No endogenous peroxidase activity was detected in blank sections, not exposed to antibody and PAP, however, erythrocytes, which do not contain peroxidase, gave a strong color reaction. This was abolished by exposure of the sections either to absolute methanol before rehydration, or to 0.5% H_2O_2 after rehydration, but before the immunological procedure. Either treatment also abolished the specific peroxidase reaction if performed immediately before the Graham–Karnovsky procedure.

The localization of ALV type-specific antigen in the pancreas of a chicken infected with the F-42 strain of ALV and reacted with homologous type A antiserum is shown in Fig. 1. Extracellular viral antigen was concentrated in the lumena of pancreatic acini, and along the surface of the acinar

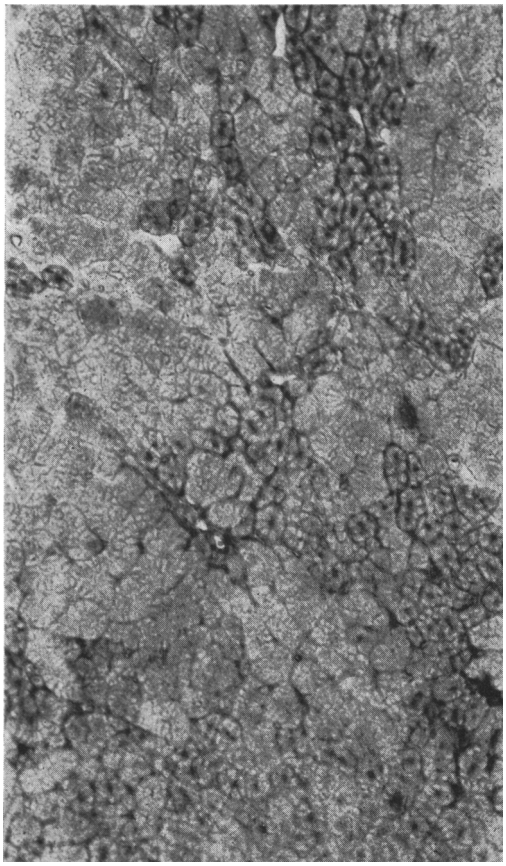

FIG. 3. Low power field from a section of the same pancreas used in Fig. 1 showing the focal nature of ALV infection (× 140).

cells, especially the basal surface, outlining the acini. This corresponded to the distribution of virions revealed by electron microscopic examination of samples from the same pancreas (4). No intracellular viral antigen was detected, suggesting that little virus precursor protein accumulates in pancreatic cells prior to virus maturation. This contrasts with findings in cell cultures infected with ALV, where intracytoplasmic, type-specific proteins are readily demonstrable by the PAP technique (1) or with fluorescent antibodies (5), and underlines the difficulty of predicting what may occur *in vivo* on the basis of *in vitro* observations. The intensity of the reaction was unaltered when mounted sections or uncut blocks were stored at room temperature for 6 months prior to PAP staining. Thus the antigenic specificity of viral proteins remained stable in the embedded tissue.

The specificity of the reaction was confirmed by exposing sections of pancreas infected with type A virus to chicken antiserum directed against the type B ALV antigen. Under these conditions no specific staining developed when the histochemical reaction was carried out (Fig. 2). Sections of pancreas from uninfected control birds were also negative with either type A or type B antiviral serum.

One feature of ALV infection *in vivo*, not evident by electron microscopic examination, but readily seen with low-power examination of PAP stained sections, is the focal nature of ALV infection (Fig. 3). Large areas of apparently uninfected tissue remained, despite the fact that this congenitally infected chicken had been persistently viremic and shedding virus throughout its 2-year life span.

The authors acknowledge the technical assistance of Mrs. Ursula Feller.

1. Dougherty, R. M., Marucci, A. A., and Di Stefano, H. S., J. Gen. Virol. **15**, 149 (1972).

2. Sternberger, L., Hardy, P. H., Cuculis, J. J., and Meyer, H. G., J. Histochem. Cytochem. **18**, 315 (1970).

3. Graham, R. C., Jr., and Karnovsky, M. J., J. Histochem. Cytochem. **14**, 291 (1966).

4. Di Stefano, H. S. (unpublished observation).

5. Rubin, H., and Vogt, P. K., Virology **17**, 184 (1962).

Received Nov. 30, 1972. P.S.E.B.M., 1973, Vol. 142.