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## Addendum

## DEMONSTRATION OF HERPES SIMPLEX VIRAL ANTIGEN BY IMMUNOFLOURESCENCE

As Sabin and Messori (1961) showed that indirect immunofluorescence will detect the antigen of herpes simplex virus in formalin-fixed tissue, this technique was applied to material from the cases described above, in the hope of getting

additional confirmation of the diagnosis. Since there is little information on stability of the antigen under prolonged storage, particularly in the tropics, the presence of herpes infection would not be excluded by a failure to show specific fluorescence in tissues which had been fixed for one to six years.

## MATERIALS AND METHOD

*Rabbit sera*

Rabbits were immunized by repeated injections of RK 13 cells infected with a type 1 herpes simplex virus. Normal rabbit serum was used for the control staining. As a check on specificity further sections were stained with antiherpes rabbit serum which had been absorbed with either normal BHK 21 cells or herpes-infected BHK 21 cells. This latter absorption abolished the specific staining. Sera diluted 1:20 were applied to sections for one hour.

*Anti-rabbit globulin*

This, conjugated with fluorescein (Nordic Diagnostics, Holland), diluted 1:80, was applied for 20 minutes.

*Microscopy*

The preparations were examined on a Vickers Patholette microscope, with iodine-quartz lamp, Barr & Stroud interference filter as primary and Schott OG.515 as secondary filter.

## RESULTS

Staining was considered specific if a cell, or group of cells, fluoresced with immune serum in the middle layer and did not fluoresce with normal serum, or antigen-adsorbed immune serum.

*Case 1*

No specific staining was seen in liver. In skin there were numerous vesicles, but no specific staining was observed.

*Case 2*

In liver scattered groups of cells showing specific staining, and in adrenal there were areas of diffuse specific staining in the cortex.

*Case 3*

No specific staining was seen in liver or adrenal.

*Case 4*

Many patches of liver cells showed specific staining, and in adrenal there were areas of necrosis with specifically stained cells at their edges in the cortex.

*Case 5*

In liver there were scattered groups of cells showing specific staining, mostly in portal tracts. In adrenal there were patches of clearly de-

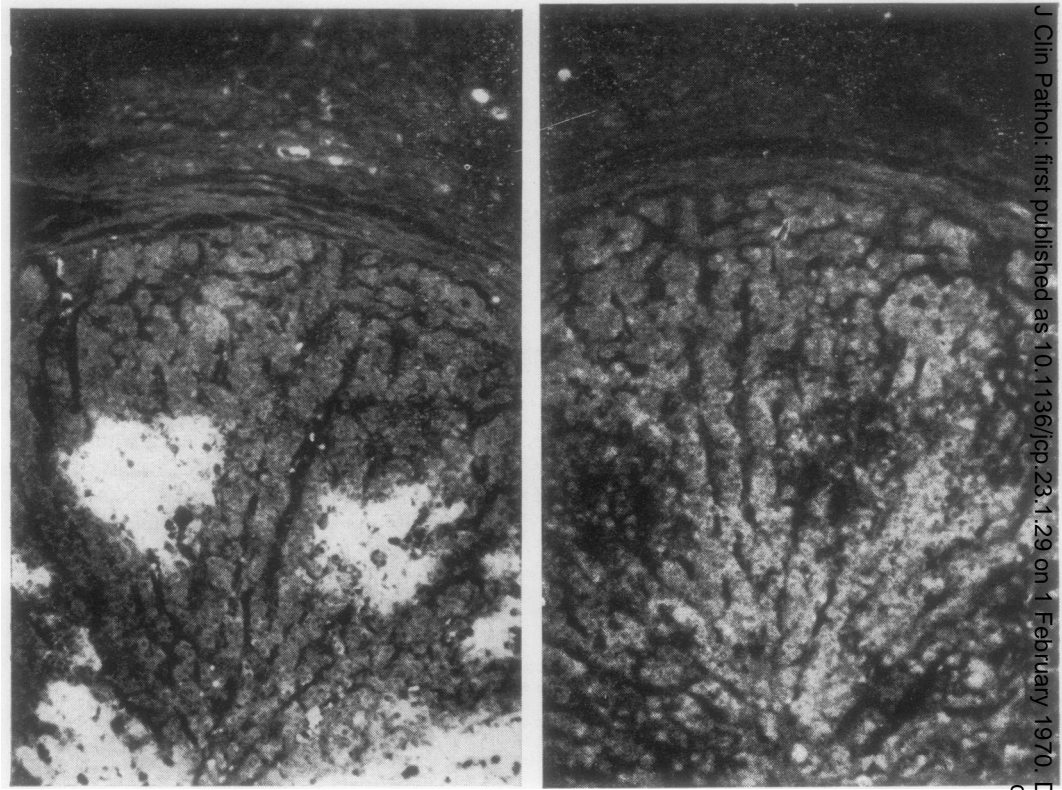


Fig. 4 Sections of adrenal of case 5: (left) treated with antiherpes serum and (right) control treated with normal rabbit serum.

marked, bright, specific staining (Fig. 4) in the cortex. No specific staining was seen in brain or kidney.

#### Case 6

Scattered patches of liver cells showed specific staining but no specific staining was seen in skin even in the base of vesicles.

#### CONCLUSION

The specific staining in tissue from cases 2, 4, 5, and 6 demonstrated the presence of herpes virus

antigen and provided further support for the diagnosis.

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