Electrophoretically Pure Mouse Interferon Inhibits Growth, Induces Liver and Kidney Lesions, and Kills Suckling Mice

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Suckling Swiss mice were injected daily for 8 days with either electrophoretically pure (EP) mouse interferon (s.a. 4.7 × 10^8 units/mg protein), the major impurities obtained in the course of purification, or partially purified mouse interferon (s.a. 1.3 × 10^7 units/mg protein). Only EP or partially purified interferon inhibited growth, induced liver and kidney lesions, and killed mice. The authors conclude that interferon itself is responsible for these effects. (Am J Pathol 1981, 102:396–402)

DAILY ADMINISTRATION of potent mouse interferon preparations to suckling mice resulted in inhibition of growth, extensive liver cell necrosis, and death.[1] When interferon treatment was discontinued, surviving mice developed a severe glomerulonephritis.[2] Although the experimental evidence strongly suggested that interferon was the responsible factor, these experiments were undertaken with partially purified mouse interferon in which interferon protein constituted approximately only 1% of the total protein content of the preparation. It might be argued therefore that the very marked pathologic changes in liver and kidney were due to substances other than interferon present in these preparations. The availability of relatively large amounts of electrophoretically pure (EP) mouse interferon[3] has permitted us to show that interferon itself was responsible for a number of biologic effects previously observed with partially purified interferon.[4] We show herein that the daily inoculation of suckling mice with approximately 1 μg of EP mouse interferon protein per day for 8 days results in a marked inhibition of growth, diffuse liver cell necrosis, and death. When this treatment was stopped on Day 8, all of the few surviving mice developed glomerulonephritis and died. The identical syndrome was observed in mice inoculated with the partially purified interferon preparation, whereas inoculation of the impurities obtained in the course of purification of this partially purified interferon were without effect.

Materials and Methods

Mice

Swiss mice were obtained from a pathogen free colony at the Institut de Recherches Scientifiques sur le Cancer.

Preparation of Mouse Interferon

Partially purified interferon

A mouse interferon preparation from suspension cultures of C243 cells induced with Newcastle Disease Virus (NDV) was concentrated and semipurified as previously described.[5] The titer of this preparation was 1.28 × 10^{-7} (expressed in terms of mouse reference units), and the protein concentration was 930 μg/ml. The specific activity was 1.37 × 10^7 units/mg protein.

Interferon preparations were assayed on mouse L cells using vesicular stomatitis virus as previously de-

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scribed.[6] Protein concentrations were determined according to the method of Lowry.

Electrophoretically pure (EP) Interferon

This partially purified interferon preparation was then passed on an agarose-sheep-antimouse-interferon antibody column (Affigel 10, Biorad).[3] The unabsorbed material constituted the preparation labeled "impurities." The interferon titer was 6.4×10^{-2}, and the protein concentration was 600 μg/ml. The specific activity was 1.06×10^{3} units/mg protein.

Interferon was eluted from the antibody column by 0.1 M citrate–1.0 M NaCl buffer, pH 2.0, dialyzed and passed on a CM-Sepharose CL-6B column (Pharmacia). The eluted material had a titer of 1.04×10^{-8} and a protein concentration of 220 μg/ml. (The specific activity was 4.7×10^{8} units/mg protein).

SDS polyacrylamide gel electrophoresis (SDS PAGE) was performed according to the method of Laemmli.[7]

Light and Fluorescent Microscopy

All tissues (except the kidney) were fixed in Bouin's fixative and embedded in paraffin; the sections were stained with hematoxylin and eosin (H&E). Renal tissue was fixed in Dubosq Brazil fixative (alcoholic Bouin) for 2 days and then in 4% formalin in saline and was cut at 3 μ. Immunofluorescence was done on snap-frozen specimens cut in a cryostat with the use of reagents as previously described.[2]

Electron Microscopy

Small blocks of kidneys and livers of control and interferon-treated mice were fixed and embedded as previously described.[8]

Ultracryotomy

Kidneys of control and interferon-treated mice were cut in small blocks fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2–7.4, for 60 minutes, then washed in this buffer and encapsulated in 20% gelatin.[9] Ultrathin frozen sections were stained with 1% phosphotungstic acid (PTA-HCl solution) and rinsed in N HCl.[10]

Results

Electrophoretic Profile of Partially Purified and EP Interferon

The electrophoretic patterns of the partially purified interferon (A), the impurities (B) and the EP interferon (C) are shown in Figure 1. Whereas no gross difference can be seen between A and B, it is evident that a marked purification was achieved in preparation C.[11] Three bands were observed by staining with Coomassie blue (corresponding to apparent molecular weights of 35,000, 28,000, and 22,000[11]). Upon elution from 2-mm gel slices antiviral activity was associated only with these three bands. We conclude therefore that each of these three bands corresponds to a different molecular form of interferon.

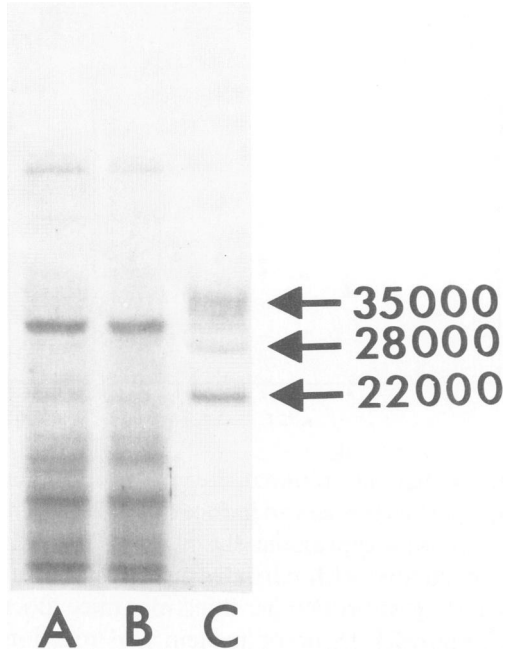

Figure 1—Electrophoretic profile of partially purified mouse interferon (**A**), impurities (**B**), and EP interferon (**C**) in SDS PAGE. Arrows indicate molecular weights. Homogenous slab gels of 12.5% acrylamide 0.8 mm thick, 90 mm long, and 130 mm wide were overlaid with a 10-mm-long 3% stacking gel and run with a constant current of 10 mÅ in the presence of 0.1% SDS (BDH). The samples were dialyzed against 0.125 M Tris/HCl (pH 6.8) containing 1% SDS and heated for 2 minutes in a boiling water bath after addition of 1% 2-mercaptoethanol (Eastman). The sample size was 25 μl.

Effect of Daily Administration of EP Interferon on Suckling Mice

There were 5 groups of newborn mice of 2 litters (15 mice) each: uninoculated mice; mice inoculated with phosphate-buffered saline (PBS); mice inoculated with partially purified interferon (Figure 1A); mice inoculated with impurities present in this preparation (Figure 1B); and mice inoculated with EP interferon (Figure 1C). Mice were given daily subcutaneous injections (interscapular region) of 25 μl.

The interferon titer of the 2 interferon preparations as used in the experiment was 2.56×10^{-7} mouse reference units, and the interferon titer of the preparation of impurities was 6.4×10^{-2}. (We have previously em-

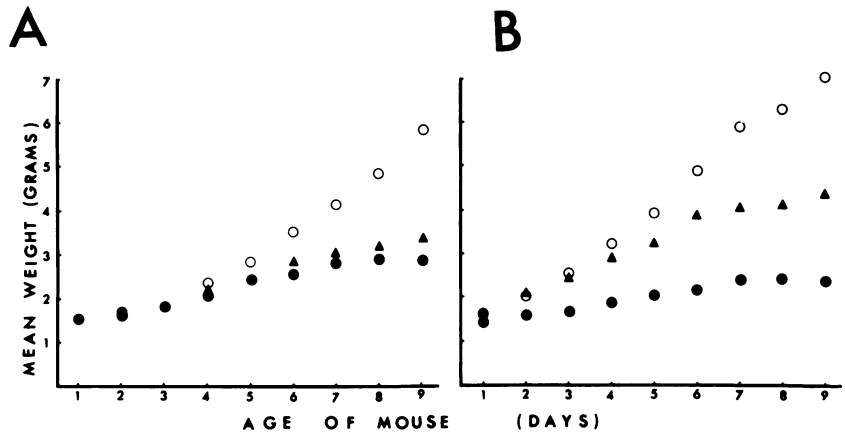

Figure 2—Growth of suckling Swiss mice inoculated once daily with partially purified interferon ●——●; impurities O——O; or EP interferon ▲——▲. In **A** there were 8 mice per litter and in **B** 7 mice per litter.

phasized that the minimal titer of mouse interferon preparations necessary to induce overt disease in suckling mice was approximately 4×10^{-5})[1,2]. Suckling mice inoculated with partially purified interferon received 23 μg of protein per injection; mice inoculated with impurities 15 μg of protein per injection; and mice injected with EP interferon 1.4 μg of protein per injection.

Treatment was continued for 8 days.

Effect on Growth

A clear-cut inhibition of growth was seen in mice inoculated with either the partially purified or EP interferon preparations (Figure 2), whereas mice inoculated with impurities grew normally (compared with PBS-inoculated mice or mice not inoculated, data not shown).

Mortality

As can be seen in Table 1, only interferon-treated mice died. Most deaths occurred between the seventh and seventeenth days as previously reported.[1] Although interferon treatment was discontinued on the eighth day, surviving mice gained weight slowly, appeared ill, and died between the seventeenth and seventieth days.

Microscopic Examination of Different Organs

At 9 days: Three mice in each group were sacrificed on Day 9. On macroscopic examination the livers of interferon-treated mice were pale and fatty. The brain, heart, lungs, thymus, kidney, spleen, liver, pancreas, and striated muscle were fixed and examined. On light-microscopic examination the only lesions were found in the livers of mice treated with partially purified or EP interferon preparations (Table 1). Histologic sections showed almost total disruption of the liver architecture with diffuse and extensive liver cell necrosis (Figure 3B). No lesions were seen in the livers of mice inoculated with impurities (Figure 3A). On electron-microscopic examination the cytoplasm of liver cells from interferon-treated mice was filled with fat vesicles (Figure 4B). Furthermore, glycogen was absent in the liver cells from interferon-treated mice, and the lumens of the endoplasmic reticulum were dilated.

By light microscopy, the kidneys of interferon-treated mice appeared normal, although many subcortical glomeruli were immature, compared with the

Table 1—Effects of Partially Purified and EP Interferon on Suckling Swiss Mice

Group	Mortality*	Lesions in mice sacrificed at†		
		9 days (liver)	37 days (kidney)	118 days (kidney)
Uninoculated	0/9	NT	0/3	0/9
Inoculated with				
PBS	0/9	0/3	0/3	0/9
Impurities	0/9	0/3	0/3	0/9
Partially pure interferon	9/9	3/3	3/3	No survivors
EP interferon	9/9	3/3	3/3	No survivors

* There were 15 mice in each group. Three mice in each group were sacrificed at Days 9 and 37, and these 6 mice were therefore excluded from those in the column labeled "mortality." Interferon treatment was discontinued on the eighth day. Mice inoculated with partially purified interferon died on Days 7, 8, 9, 10, 16, 30, 65, 65, and 70. Mice inoculated with EP interferon died on Days 9 (7 mice) 17, and 37.

† Number of mice with lesions per number of mice sacrificed.

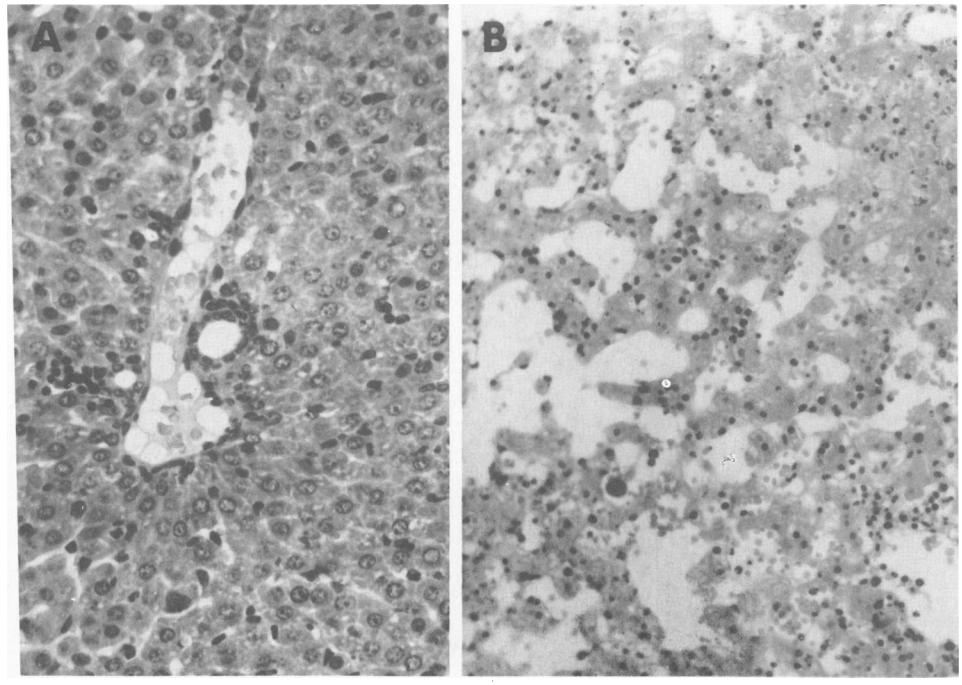

Figure 3—Sections of liver of 9-day-old Swiss mice treated with impurities (**A**) or EP interferon (**B**). (**A**, H&E, ×250; **B**, ×63) Note the normal liver in **A** and extensive disruption of liver architecture and cell necrosis in **B**.

glomeruli of control mice. By electron microscopy, the glomeruli from mice treated with partially purified or EP interferon showed a marked thickening of the lamina rara interna of the basement membrane (GBM) (Figure 5B) containing fibrils and electron-dense areas previously described for partially purified interferon.[8] Use of PTA-HCl-staining indicated that this marked thickening of the GBM in interferon-treated mice contained glycoproteins (Figure 5D).

At 37 Days: By light microscopy, the kidneys of mice given injections of impurities or PBS or left untreated were normal, whereas the kidneys of mice in-

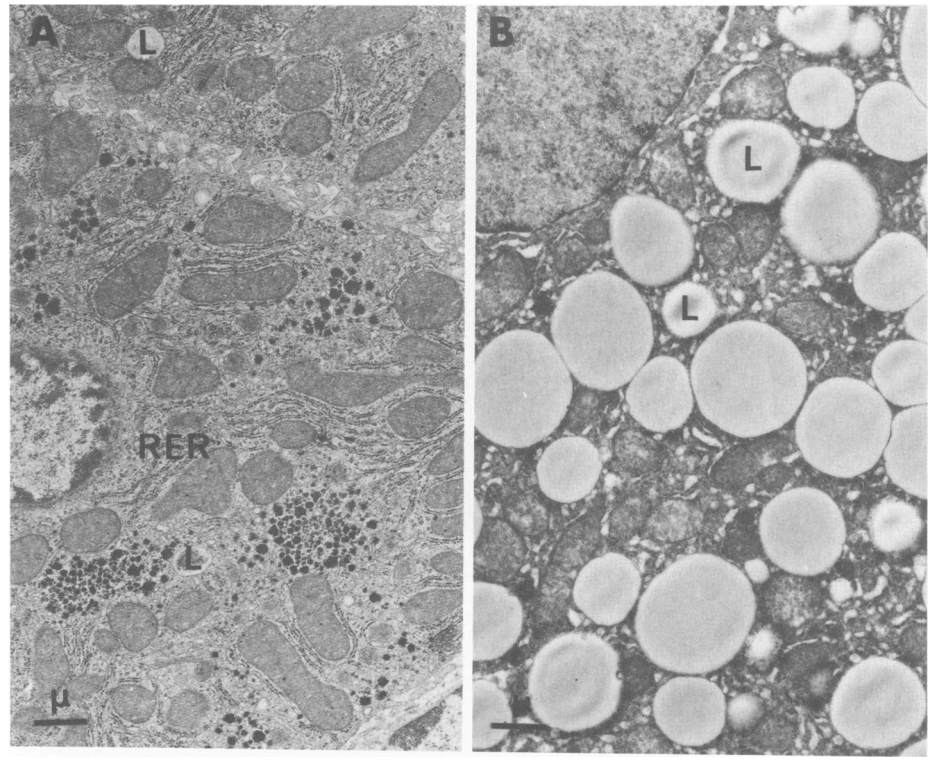

Figure 4—Livers of 9-day-old mice. (Uranyl and lead staining of thin sections of tissue embedded in Epon, ×7200) **A**—Mouse injected with impurities. Low-power photomicrograph showing parts of two hepatocytes. In the cytoplasm, the rough endoplasmic reticulum (RER) forms stacks of flattened cisternae. Rosette-like aggregates of glycogen and a few fat droplets (*L*) are present between mitochondria. **B**—Mouse inoculated with EP interferon. Most of the field is occupied by fat droplets (*L*). Glycogen is absent, and the RER has a finely vesicular appearance.

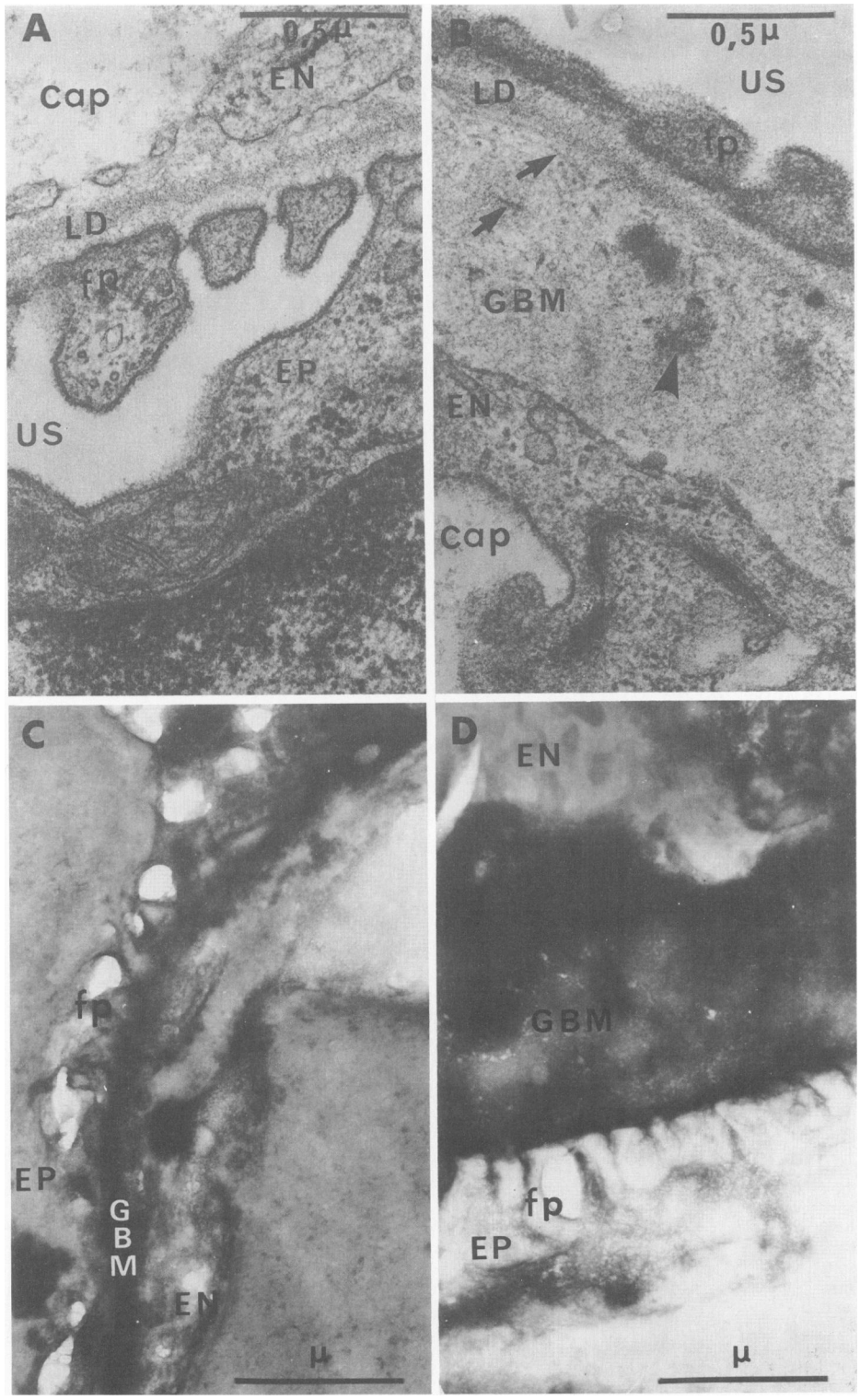

Figure 5—**A** and **B** are glomeruli from 9-day-old mice. Conventional electron microscopic techniques were used. *EP* = epithelium; *fp* = foot processes; *LD* = lamina densa; *EN* = endothelium; *Cap* = capillary lumen; *US* = urinary space; *GBM* = glomerular basement membrane. (Uranyl and lead, ×48,000) **A**—Mouse inoculated with impurities. The capillary loop basement membrane appears within normal limits. **B**—Mouse inoculated with EP interferon. Note marked thickening of the lamina rara interna of the capillary loop basement membrane, which contains electron-dense deposits (*arrowhead*) and fibrils (*arrows*). **C** and **D** are ultrathin frozen sections of kidneys stained with PTA-HCl. (×24,000) **C**—Mouse inoculated with impurities. The unthickened GBM is stained homogeneously. **D**—Mouse inoculated with EP interferon. Note irregularly thickened GBM, which is intensely stained.

oculated with partially purfied or EP interferon showed advanced disease (Table 1). Virtually all glomeruli were sclerotic, and the tubules showed diffuse atrophy of epithelium and dilated lumens filled with proteinaceous casts (Figure 6). By immunofluorescence there were granular coarse deposits of IgG, IgM, and C3 along the GBM of kidneys from interferon-treated mice as previously described.[2]

At 118 Days: The remaining 9 mice in the groups of mice left untreated or inoculated with PBS or impurities were sacrificed. No gross or microscopic lesions were present in either the liver or kidney of these mice (Table 1).

Discussion

The experiments reported herein show that EP interferon is responsible for inhibition of growth, liver and renal lesions, and death in suckling mice. The impurities containing at least 10 times more protein and displaying an electrophoretic profile virtually identical to the partially purified interferon preparation (Figure 1) were without discernible effect.

We do not know at present how interferon effects such dramatic changes in the suckling mouse; why the liver is the sole site of such extensive parenchymal damage; whether the marked thickening of the glomerular basement membrane, which can be seen as early as 4 days (Woodrow et al, unpublished data), is secondary to liver cell necrosis or not; and why all other organs appear to be spared. Our previous work showed that comparable amounts of interferon did not induce liver or renal lesions in mice older than 1 week,[1,2] so that the immaturity of the mouse in the neonatal period is clearly an essential component.

Two points appear worth stressing. The first pertains to the use of interferon in newborn infants. These very marked pathologic effects observed in suckling mice were obtained by the daily injection of as little as ≤ 1.4 μg of interferon protein per day for 8 days. Our previous work suggested that 1 LD_{50} of interferon (administered as 1 injection/mouse/day for 7 days) was about $1/16$ of the amount of interferon injected in this experiment[1] (ie, the LD_{50} would be approximately ≤ 88 ng/mouse/day). If one can extrapolate these results directly to the human newborn, it would seem that the daily injection of potent human interferon preparations might be associated with untoward effects. The second point concerns the relevance of our observations to human pathology. We have shown that the disease induced in suckling mice by neonatal infection with lymphocytic choriomeningitis (LCM) virus is in large part due to the endogenous interferon induced by the virus.[12,13] Mice treated for the first week of life with interferon[2] or injected as neonates with LCM virus[13] develop glomerulonephritis later in life, long after interferon has been eliminated. Thus the early lesions induced by interferon in the GBM of the developing glomerulus (Figure 5) lead to a progressive glomerulonephritis.[2]

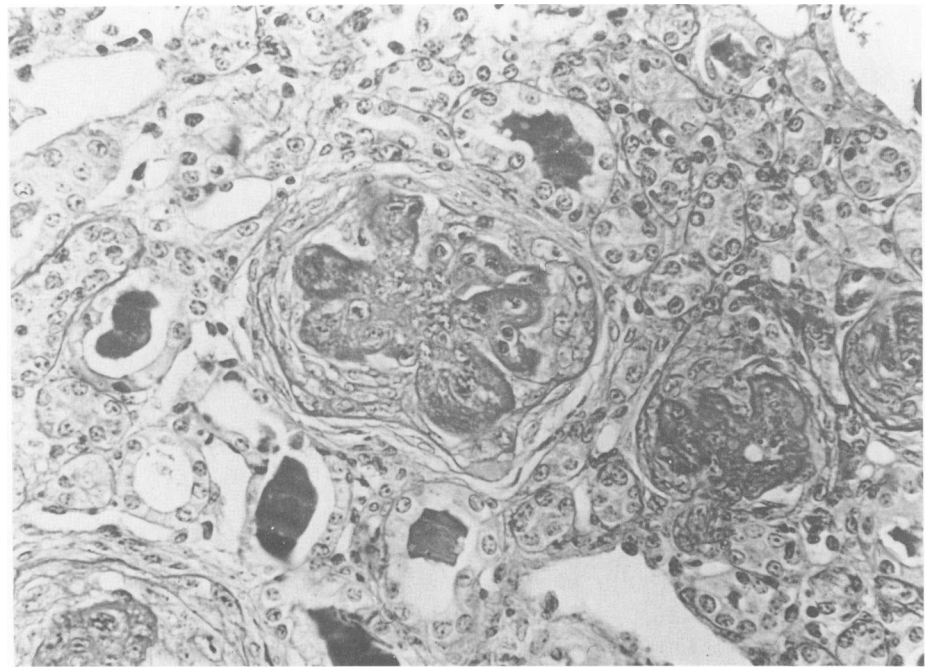

Figure 6—Kidney of Swiss mouse sacrificed at 37 days. Inoculated as a suckling with EP interferon. (PAS, ×250)

Likewise, it seems possible that in some virus infections of man, lesions may be due to virus-induced cell products, such as interferon, rather than to direct virus cytopathic effects *per se*, and that in some instances, the manifestations of disease only become apparent much later in life.

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