Strain Differences in Shope Fibroma Virus

An Immunopathologic Study

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The pathogenic effects of plaque-purified Boerlage and Patuxent strains of Shope fibroma virus (SFV) in neonatal rabbits are compared with results of previous reports which used nonpurified SFV. Clinically, the Boerlage strain produced large tumors; whereas the same dose of Patuxent strain SFV induced much smaller tumors locally. Neither virus caused metastatic or extensively invasive local spread in our study. Some Patuxent recipients died of respiratory infections prior to sacrifice. However, both groups of rabbits handled the tumor well; the tumor began regressing 15–20 days after inoculation. Histologically, the tumors produced by those viruses were identical. Patuxent strain recipients were otherwise normal. Boerlage strain recipients showed increased persistence of extramedullary hematopoiesis and scattered foci of parenchymal necrosis in their livers. They also showed considerable cell death in thymic lobules. In rabbits given Patuxent strain SFV, virus antigens were detected only in the tumor by immunohistologic examination. Boerlage viral antigens were found in the tumor and overlying skin. We also detected virus systemically in Boerlage recipients: it was present in fixed tissue phagocytes in the spleen and liver and also in parenchymal cells of the lungs, liver, and kidney. Boerlage strain SFV recipients also showed detectable virus in their thymus, both at the periphery of the thymic lobules and in the connective tissue separating thymic lobules from each other. Despite the disseminated nature of the infection, rabbits that received the latter strain fared as well as those receiving Patuxent strain SFV. (Am J Pathol 1984, 116:342–358)

SHOPE FIBROMA VIRUS (SFV) is reportedly responsible for two different tumor syndromes. In adult rabbits, it induces a localized tumor consisting of a proliferation of infected fibroblasts. This tumor resolves in 2–3 weeks in almost all rabbits.[1] On the other hand, in adult animals receiving immunosuppressive treatments of various kinds and neonatal rabbits progressive tumors develop which may kill the host or regress slowly with a protracted clinical course.[2,3]

In the course of our investigation on the Patuxent strain of SFV, we reported a comparative immunohistologic examination of neonatal and adult infections.[4] Our results paralleled previous observations regarding the course and complications of neonatal SFV infection. We found that neonatal SFV infection either regressed slowly or not at all, and that SFV antigens were detectable systemically.

Subsequently, we discovered a contaminant virus in our SFV preparation. This virus, which we call malignant rabbit fibroma virus (MV), is essentially a recombinant between SFV and rabbit myxoma virus (MYX), and was a minor contaminant in our original preparation of Patuxent strain SFV.[5] Tumors induced by MV follow an aggressive clinical course in adult rabbits. MV-induced tumors disseminate widely and

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are complicated by severe mucosal gram-negative infection. Death occurs within 12 days. The immunohistologic picture of adult MV infection resembled that we reported for neonatal infection with our uncloned stock of Patuxent strain SFV.[4,6]

Accordingly, to determine whether the presence of a small amount of MV explained the clinical and immunopathologic pictures reported for neonatal infection with uncloned SFV, we compared the immunohistologic syndromes induced by two different strains of plaque-purified SFV with that produced by our uncloned stock of Patuxent strain SFV.

Materials and Methods

Rabbits

Adult 2.5–3-kg female New Zealand white rabbits were obtained from local suppliers. Pregnant New Zealand white rabbits were obtained from the same suppliers. These were all at least 1 week from delivery at the time of receipt. Newborn rabbits were handled with gloves.

Virus

Patuxent strain SFV was the kind gift of Dr. W. A. F. Tompkins (University of Illinois, Urbana, Ill). Boerlage strain SFV was the gift of Dr. H. Hinze (University of Wisconsin, Madison, Wis). Both viruses were grown in RK-13 cells as described elsewhere[7,8] and were plaque-purified according to previously detailed procedures.[5] Both strains of virus were administered intradermally in 1-ml suspensions at 10^7 ffu/animal. Neonates and adults received the same doses. All animals were clinically monitored daily following administration of virus. They were sacrificed at various intervals with intracardiac Euthanol.

Histologic Observations

Sections from all rabbits were fixed in neutral-buffered formalin and processed routinely for embedding in paraffin and staining with hematoxylin and eosin (H&E). Material for immunohistologic examination was fixed in cold acid alcohol and processed as described.[6] Those later sections were embedded in paraffin. For immunofluorescence studies, they were deparaffinized and stained with FITC-rabbit anti-SFV[6,9] without counterstain. Immunohistologic observations were made on a Zeiss fluorescence photomicroscope with Ploem optics and recorded on Tri-X film pushed to ASA1600.

Steroid Treatment

Adult rabbits received Solu-medrol (Upjohn) subcutaneously daily on one of two dosage schedules. Some received 10 mg/kg for 2 weeks before receiving SFV, followed by an additional week of treatment after virus inoculation. Others received the same dosage daily for 2 weeks following Patuxent strain SFV inoculation. Clinical and experimental observations were made in these rabbits as on the others.

Proliferative Assays

Our assays of proliferative activity to SFV are described in detail elsewhere.[10] Briefly, lymphocytes obtained from the spleen, peripheral blood, or draining lymph nodes from tumor-bearing rabbits were incubated in RPMI-1640 with 5% fetal bovine calf serum in microtiter plates (Linbro Plastics) with SFV at a ratio of 10 lymphoid cells to 1 ffu SFV. This ratio was previously determined to be optimal for stimulation.[10] Incorporation of ^{125}IUdR was measured following 24-hour incubation with the isotope and harvesting in an automatic cell harvester (Otto Hiller Co., Madison, Wis). We incubated control lymphocyte cultures with concanavilin A (Con A, Miles-Yeda, Rehovot, Israel) and anti-Ig serum[11] to ascertain T and B lymphocyte responsiveness, respectively.

Results

Patuxent Strain SFV

The clinical observations pertaining to adult recipients of plaque-purified Patuxent strain SFV have been reported.[5] Briefly, these rabbits developed a small tumor, 1–2 cm in maximum size, at the site of injection. The tumor peaked on about Days 9–11, crusted, and regressed shortly thereafter. The rabbits remained well and developed neither metastatic disease nor evidence of viral dissemination.

Histologically, the tumor at its peak appeared as a proliferation of spindle cells in a variably myxoid matrix (Figure 1a). The overlying skin showed reactive changes, and diffuse (T-lymphocyte zone) hyperplasia was noted in draining lymph nodes. The histologic course of regression of such tumors has been reported.[12] Immunohistologic examination of the peak tumors from Patuxent strain SFV recipients showed considerable viral antigen present within the proliferating tumor cells.[6] Overlying skin and the systemic reticuloendothelial system were consistently negative for viral antigen.

In vitro proliferative responses of these rabbits' lymphocytes to SFV antigens were analyzed and are reported elsewhere.[10] Briefly, by 7 days after inoculation all lymphocyte populations proliferated considerably in response to SFV. Responses to control mitogens (Con A and anti-Ig) were not affected.

Neonatal rabbits received plaque-purified SFV as well. These rabbits developed a localized tumor which peaked over the course of 10–14 days and regressed over a comparable period of time. They did not develop metastatic disease. In all rabbits studied, the tumor eventually regressed within 1 month of inoculation. Extensive local spread was not observed.

Histologically, Patuxent strain tumors in neonates, sampled at peak tumor size, appeared more cellular than those found in adults (Figure 1 B and C). Mitotic activity and large tumor cells were more commonly observed. Atypical mitoses were absent. These tumors, like those seen in adults, were composed of a proliferation of round and spindle cells. Skin overlying those tumors showed only mild reactive changes. Draining lymph nodes showed diffuse cortical hyperplasia, variable sinus histiocytosis, and some germinal center activity. No systemic changes were noted.

At 15 days after virus inoculation, regression had already begun histologically. These tumors were infiltrated (Figure 2) by a mixed mononuclear/polymorphonuclear inflammatory infiltrate. The degree to which different animals' tumors were involved in this inflammatory reaction varied. By 28 days, no tumor remained, and only occasional clusters of mononuclear cells were appreciated.

Other tissues from neonatal recipients of plaque-purified Patuxent strain SFV were unremarkable. Extramedullary hematopoietic activity was observed in both spleen and liver. The former also showed a somewhat dilated sinusoidal pattern. Thymus, lungs, and gastrointestinal tract were normal.

Tissues from these neonatal rabbits were stained with FITC-rabbit anti-SFV, and we examined them to determine the extent to which virus antigens were detectable locally and systemically. Tumor cells stained strongly positively for SFV antigens (Figure 3). Overlying skin was negative. No SFV antigens were detected anywhere else: in thymus, kidney, spleen, liver, gastrointestinal tract, or lung.

Proliferative responses to SFV were not detectable in spleen cells from these SFV-exposed neonates. Responses to control mitogens were unaffected by virus inoculation (data not shown). The proliferative responses of long-term survivors were not examined, but such animals were resistant to further challenge with SFV or MV.

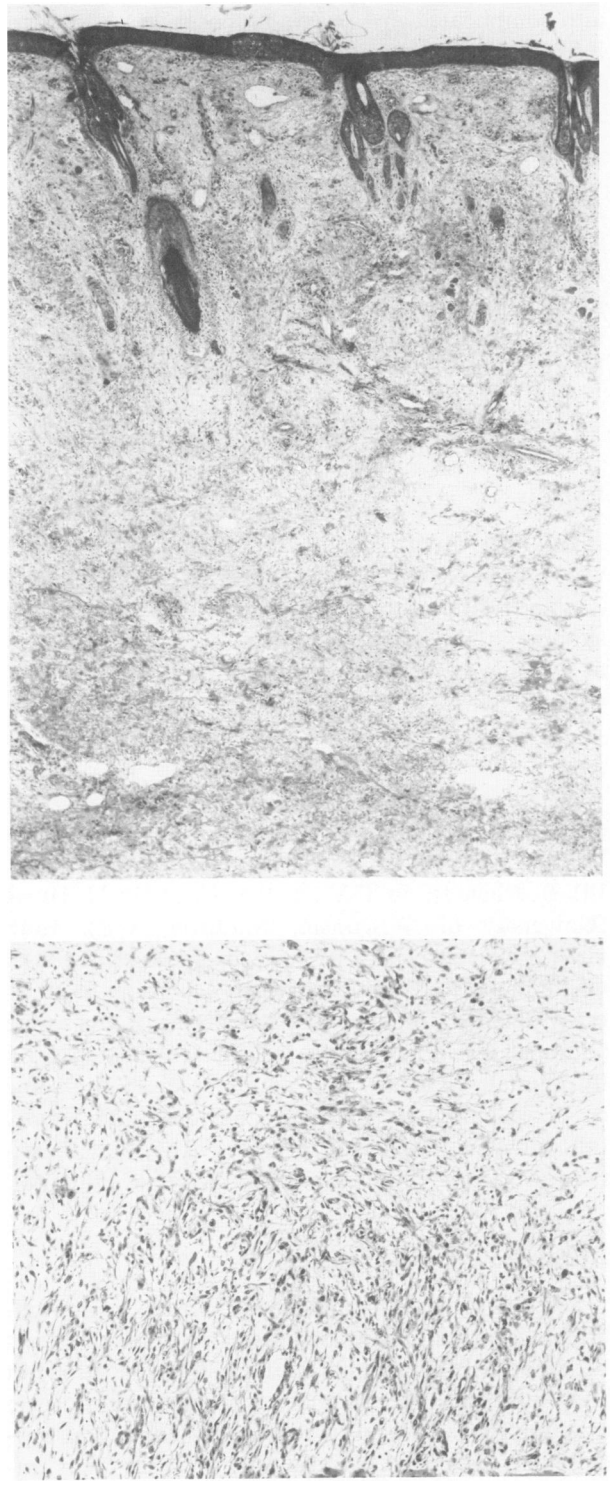
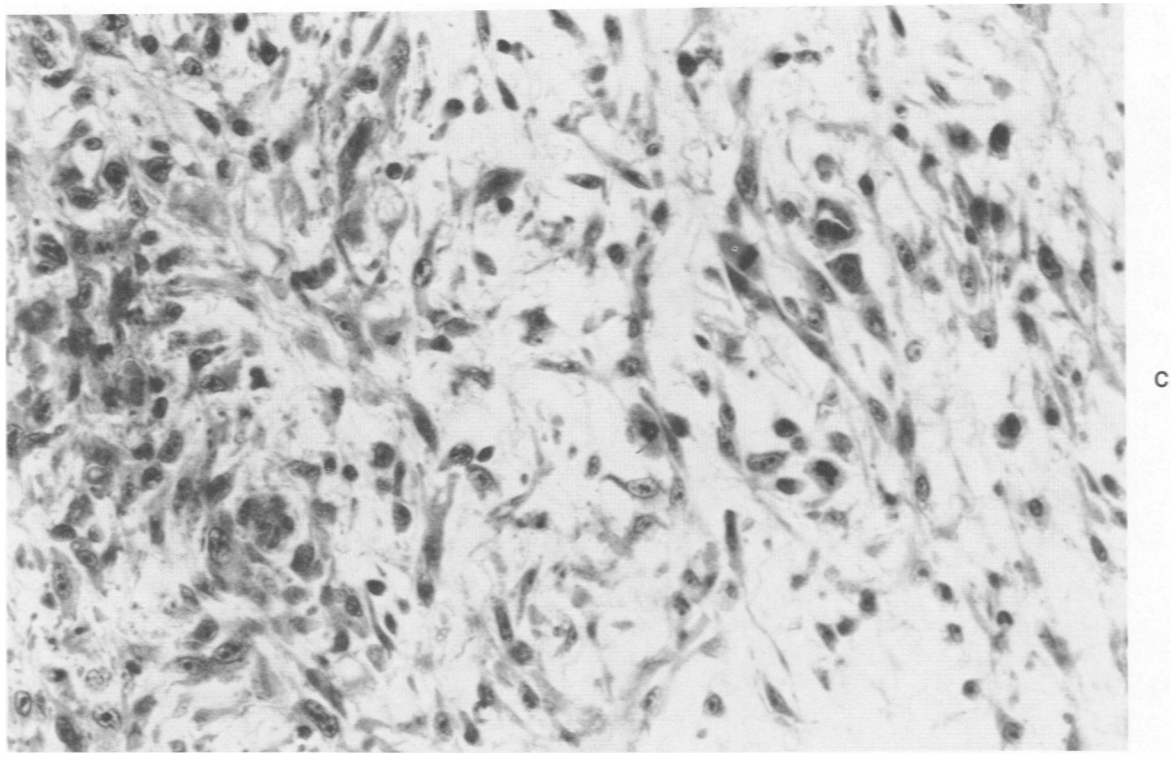


Figure 1A – A section from the tumor of an adult rabbit receiving 10^7 ffu Patuxent strain SFV. Section taken at 7 days after inoculation. **B** – Representative section of the tumor from an 8-day-old rabbit that received 10^7 ffu plaque-purified Patuxent strain SFV on Day 1. The tumor is considerably more cellular than the comparable tumor in adults (see **A**) **C** – Higher-power micrograph of another comparable tumor illustrating the cytologic characteristics and mitotic activity of the tumor cells.



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Boerlage Strain SFV

Adult female rabbits given Boerlage strain SFV followed a clinical course similar to that observed in Patuxent strain recipients. Tumors appeared to reach 1 or 2 cm greater size in these Boerlage recipients than in Patuxent strain recipients but regressed in a comparable time frame. Histologic and immunohistologic studies in adults receiving Boerlage strain SFV yielded results comparable to those observed in Patuxent recipients. Proliferative responses *in vitro* of Boerlage strain recipients in response to SFV were not measured. However, rabbits receiving Boerlage strain SFV were resistant to secondary challenge with Boerlage strain SFV or MV.

Neonatal rabbits, 1 or 2 days of age, received 10^7 ffu Boerlage strain SFV intradermally in the thigh. In these rabbits local tumors appeared, peaked at 10–15 days, and regressed completely during the following 2 weeks. These tumors reached a maximum size of 2–3 cm. None developed secondary or satellite tumor nodules, and all remained healthy. All rabbits not sacrificed for histologic studies survived and were resistant to secondary challenge with SFV or MV.

Histologically, the tumors from neonates given Boerlage strain SFV were similar to those found in neonates receiving Patuxent strain virus. Thus, we saw a florid proliferation of spindle cells and round cells of various sizes, sometimes closely packed and sometimes embedded in a myxoid matrix (Figure 4). Overlying skin was unremarkable save for mild reactive changes. By Day 15, pronounced lymphocytic infiltration had begun (Figure 4). Tumors were, for the most part, not detectable by Day 28.

The liver from 7-day-old rabbits given Boerlage strain SFV at birth showed greater than normal extramedullary hematopoiesis (Figure 5). Numerous foci of hepatocellular necrosis were appreciated in 7-day-old rabbits (Figure 5). These were randomly situated with regard to the portal tracts and incited scant inflammatory reaction. By Day 15 after virus inoculation these necrotic foci had disappeared (Figure 6).

The spleens from 7-day-old neonates given Boerlage strain SFV at birth showed delayed disappearance of extramedullary hematopoiesis, and focal depletion of lymphocytes from white pulp. By Day 15 these, too, had returned to normal (Figure 7). Lung specimens showed acute bronchopneumonia in some rabbits, but were otherwise unremarkable. The kidneys were normal.

Sections from the thymus taken 7 days after neonatal virus inoculation showed pronounced necrosis in thymic parenchyma (Figure 8). This process appeared to affect primarily the interiors of thymic lobules, but in more severely involved areas the entire thymus was affected. The periphery was variably

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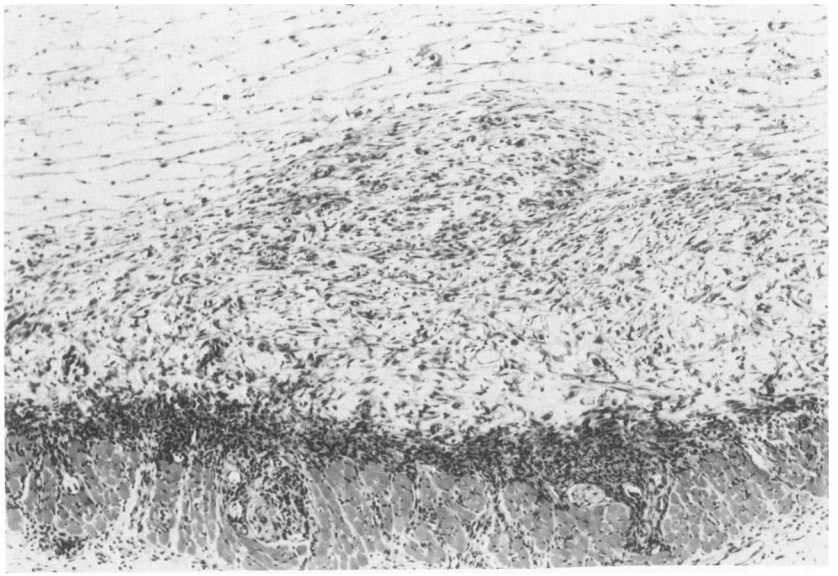

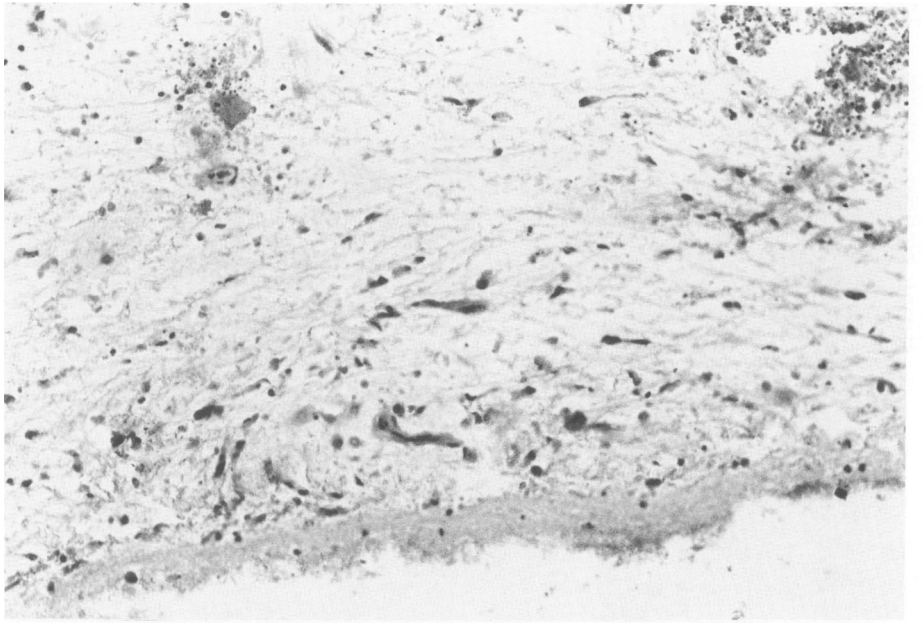

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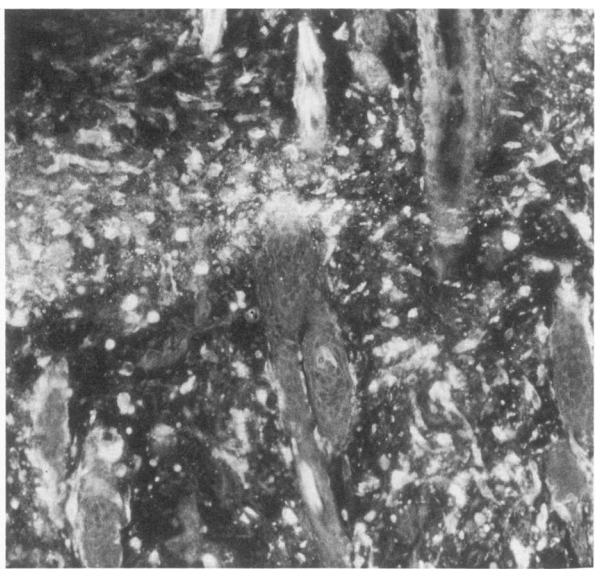
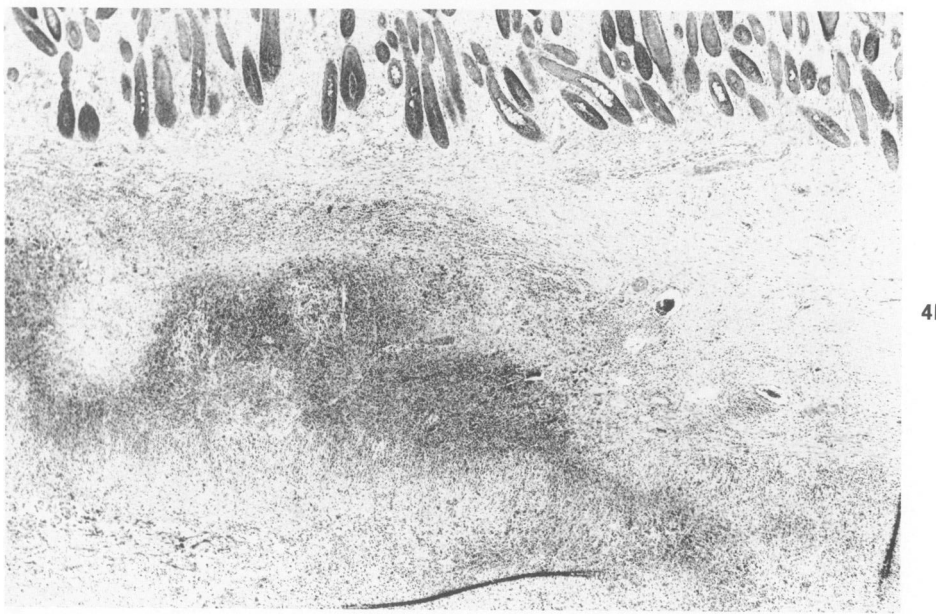




4B

affected. Interlobular soft tissues appeared reactive, with a number of reactive fibroblasts. The thymic necrosis was only observed in rabbits examined at 7 days after virus inoculation and had healed by 15 days. All of these rabbits appeared clinically healthy.

Immunohistologic examination for SFV antigens showed different results in Boerlage recipients from those observed in rabbits given the Patuxent strain. As expected, tumor cells contained virus antigen (Figure 9). Skin overlying these tumors also stained positively for SFV. The Kupffer cells of the liver and the phagocytes in the spleen and lung contained detectable viral antigen (Figure 10). The comparison of light and fluorescence studies on livers of Boerlage recipients suggests strongly that virus antigen is present in the hematopoietic cells in the sinusoids (Figure 10). In addition, thymus showed SFV in the areas of necrosis, at the edges of the necrotic areas, and in the stromal fibroblasts between the thymic lobules (Figure 10).

Epithelial staining was also observed in Boerlage recipients using FITC anti-SFV (Figure 11). Occasional hepatocytes and bile duct cells contained detectable virus. The renal parenchyma also stained strongly positively for SFV. We found virus in occasional glomeruli, apparently in mesangial cells. Mostly, renal tubular epithelium was positive: both the convoluted tubules and the collecting ducts.

Steroid-Treated Rabbits

Adult female rabbits received corticosteroids subcutaneously and Patuxent strain SFV intradermally according to the regimens described above (see Materials and Methods). These rabbits were observed clinically. The clinical courses of these rabbits were identical despite differences in steroid treatment regimen. Tumor development in these rabbits paralleled that observed in comparable rabbits not given steroids. That is, tumors grew to about 2–3 cm in size over the course of 9–11 days and regressed over a similar period of time. Histologic examination at peak tumor size showed tumors essentially identical to those seen in adult Patuxent strain recipients that had not received steroids (Figure 12). Systemic abnormalities were not observed.

These rabbits were examined for the ability of their cells to proliferate *in vitro* in response to SFV and Con A. The ability of lymphocytes from spleen, draining lymph node, and peripheral blood to respond to these mitogens was not affected by steroid treatment (data not shown).

Recipients of Uncloned SFV

Our original uncloned preparation of SFV contained MV as a contaminant. This problem confounded our analysis of adult SFV infection and prompted a study of adult rabbits given purified SFV or MV.[12] In the present case, we set out to reproduce our original observations with uncloned SFV.[4]

Litters of rabbits receiving this preparation experienced repeated problems with maternal rejection and bacterial respiratory infections. None survived beyond the 15-day examination period. At that time, however, none showed satellite tumor nodules or gross distant metastases.

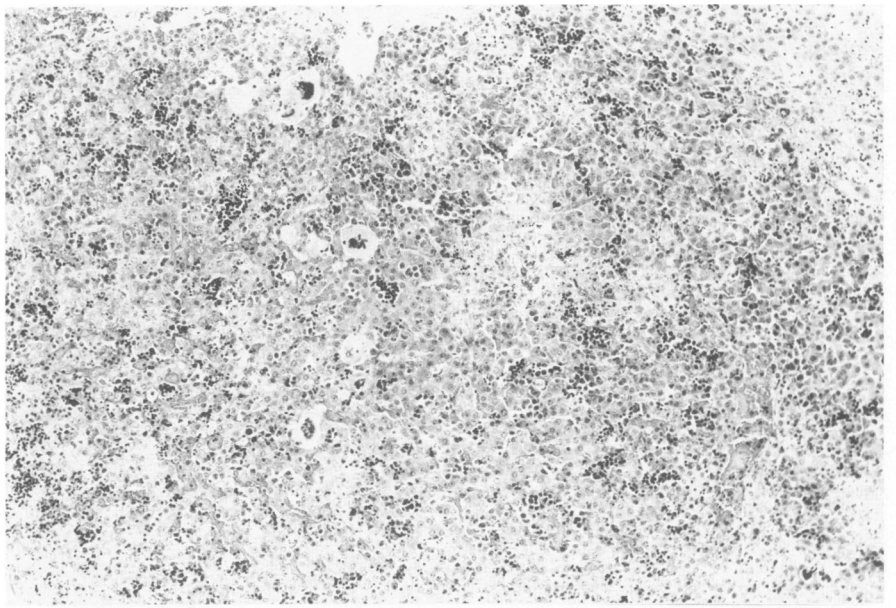
Histologically, the tumors from uncloned SFV recipients were considerably more myxoid in appearance than were those from recipients of purified Patuxent strain SFV (Figure 13). Though some lymphocytic or polymorphonuclear inflammatory infiltrate was focally observed on Day 15, in no animals were these as prominent as those described above for recipients of cloned Patuxent or Boerlage strains of SFV.

Metastatic nodules of tumor, not evident grossly, were seen microscopically at several locations in rabbits Day 7 and Day 15 after-inoculation (Figure 14). Such metastatic tumors were particularly evident in the thymus, kidney, and gastrointestinal tract. Focal parenchymal necrosis, comparable to that seen in Boerlage recipients, was also appreciated in the thymus but not in the liver. Immunohistologic studies performed on these rabbits confirmed observations reported previously.[4]

Discussion

Immune competence is an important factor in host defenses against viral infections, and oncogenic viral infections are no exception.[13–15] SFV is an oncogenic poxvirus of rabbits. Discovered by R. E. Shope in 1932, SFV produces a characteristic clinical syndrome

Figure 2 — Mixed lymphocytic/polymorphonuclear cell infiltrates appear at the periphery of the tumor in rabbits given plaque-purified Patuxent strain SFV. This section is from a 15-day-old rabbit that received 10^7 ffu SFV on Day 1 of life. **Figure 3** — Direct fluorescence examination of a 7-day tumor and cutaneous adnexal structures from a rabbit given plaque-purified Patuxent strain SFV on Day 1 of life. FITC-rabbit anti-SFV was used. Tumor cells, but virtually no squamous cells, stain positively. **Figure 4A** — Boerlage strain SFV induces a more myxoid tumor, seen here on the seventh day of growth following 10^7 ffu given locally on Day 1 of life. **B** — By 2 weeks, such tumors are heavily infiltrated by a mixture of lymphocytes, histiocytes, and polymorphonuclear leukocytes.



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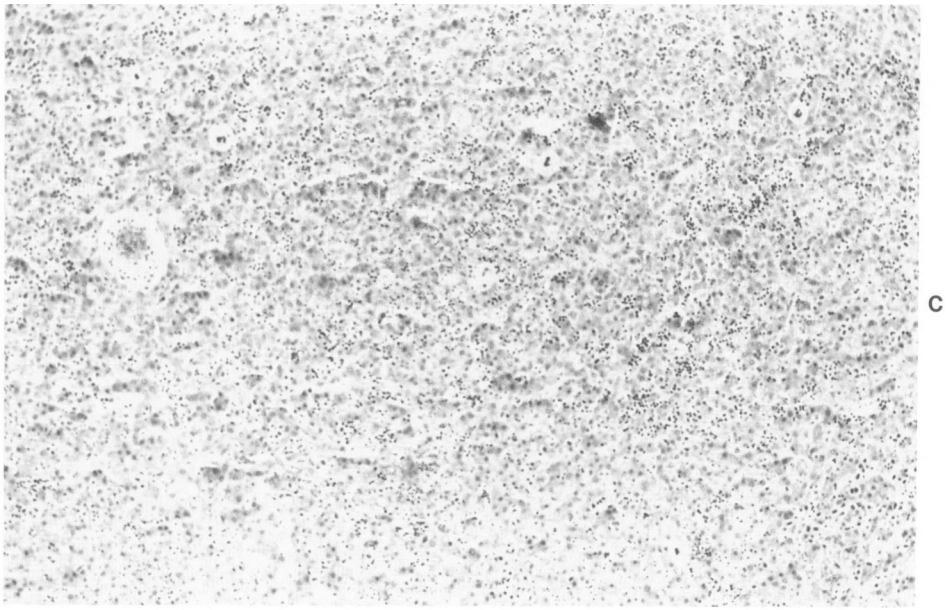

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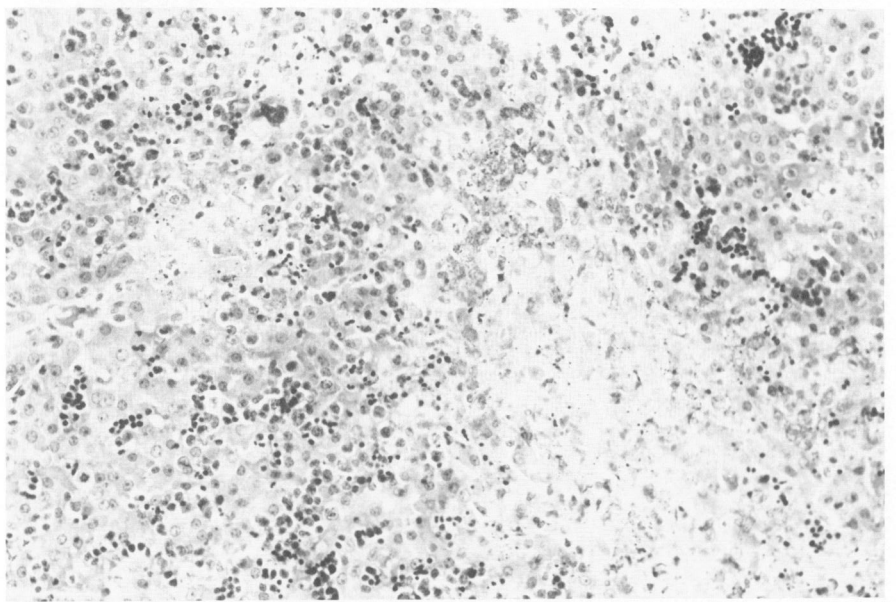

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Figure 5A — Boerlage recipients showed augmented extramedullary hepatic hematopoiesis at 7 days after virus inoculation. Notice the focus of hepatocellular necrosis at the left. **B** — Livers from Boerlage strain SFV recipients contained numerous foci of hepatocellular dropout, distributed randomly in the hepatic lobules. This is from an 8-day-old rabbit, 7 days following intradermal injection of 10^7 ffu Boerlage strain SFV. **C** — Liver from a comparable Patuxent strain recipient for comparison.

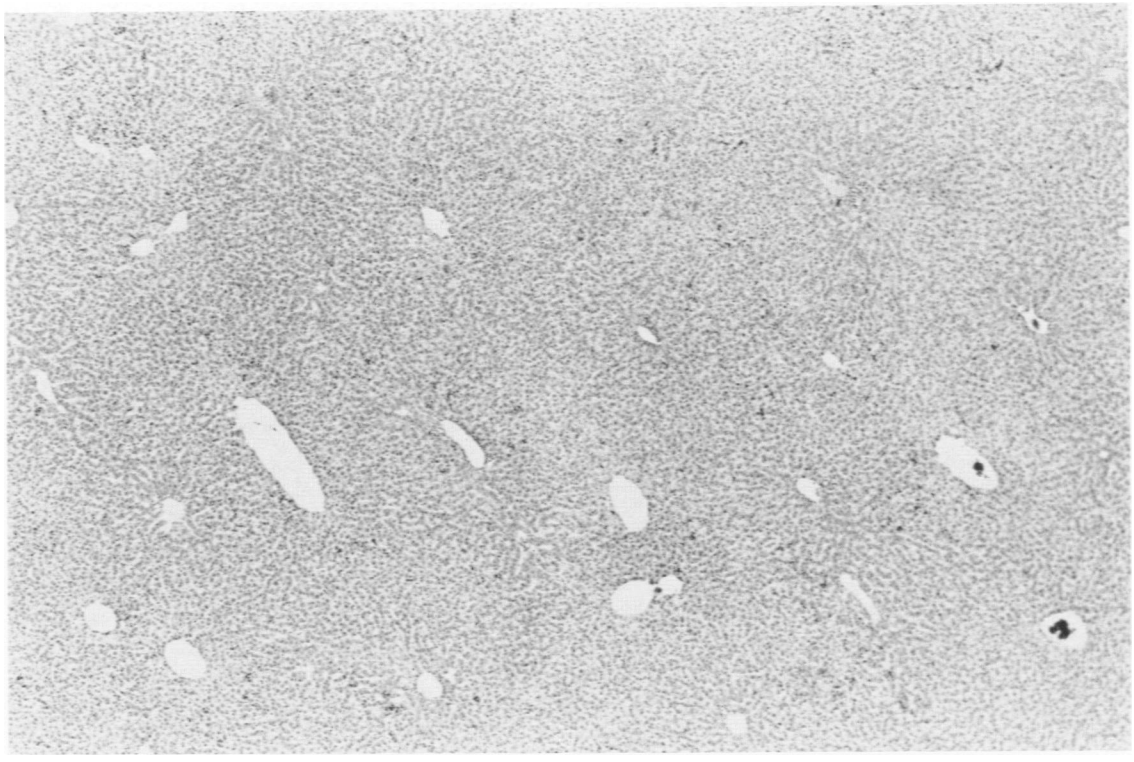

Figure 6 — By 15 days of age, the necrotizing lesions in the livers of Boerlage strain recipients had cleared, and extramedullary hematopoiesis had diminished.

in infected adult rabbits: a benign, self-limited course of tumor development and regression followed by lasting resistance to subsequent challenge.[16,17]

After the discovery of SFV, a number of investigators sought to examine the effects of altered host defenses on the course of SFV-induced tumors. These efforts are summarized in Tables 1 and 2. The basic tenor of these experimental observations is that SFV infection of immunologically compromised adult rabbits or of neonates produces a progressive, often fatal, tumor with widespread metastases. In our own laboratory, similar observations were made, together with a detailed immunohistologic analysis.[4] We subsequently found that our original stock of Patuxent strain SFV contained a minor contaminant which was capable of producing a highly malignant tumor syndrome and profound immunologic impairment.[5,18] This contaminating virus, malignant rabbit fibroma virus (MV) is for the most part a recombinant between SFV and MYX, and we have reported analyses of the histologic and immunohistologic aspects of MV-induced tumors.[6,12]

In the series of experiments detailed here, we report the first attempt at reproducing with plaque-purified SFV the results other authors obtained using uncloned virus stocks. To our surprise, neonatal infection with neither Patuxent or Boerlage strain of SFV was capable of inducing the disseminated tumor seen when uncloned SFV preparations were employed. Furthermore, administering large doses of corticosteroids inhibited neither the development of virus-specific proliferation nor the benign course of the tumor.

Our analysis of Patuxent strain SFV tumor-bearing neonates indicates that in these rabbits this strain of SFV does not produce disseminated infection, as determined by immunohistologic methods. The localization of this virus to the site of inoculation may thus permit a variety of host defense mechanisms to induce tumor regression. There is evidence that neonatal animals of various species may be relatively deficient in some of these functions, though,[19-21] and the mechanisms whereby neonatal rabbits rejected Patuxent strain SFV-induced tumors in our studies must remain speculative.

We also noted that purified Boerlage strain SFV causes a disseminated infection that follows an essentially identical clinical course. In neonates, the Boerlage strain causes extensive necrosis in several organs 7 days after virus injection. Virus antigens are detectable systemically. One week later, these lesions appear almost to have healed. Throughout, the young rabbits

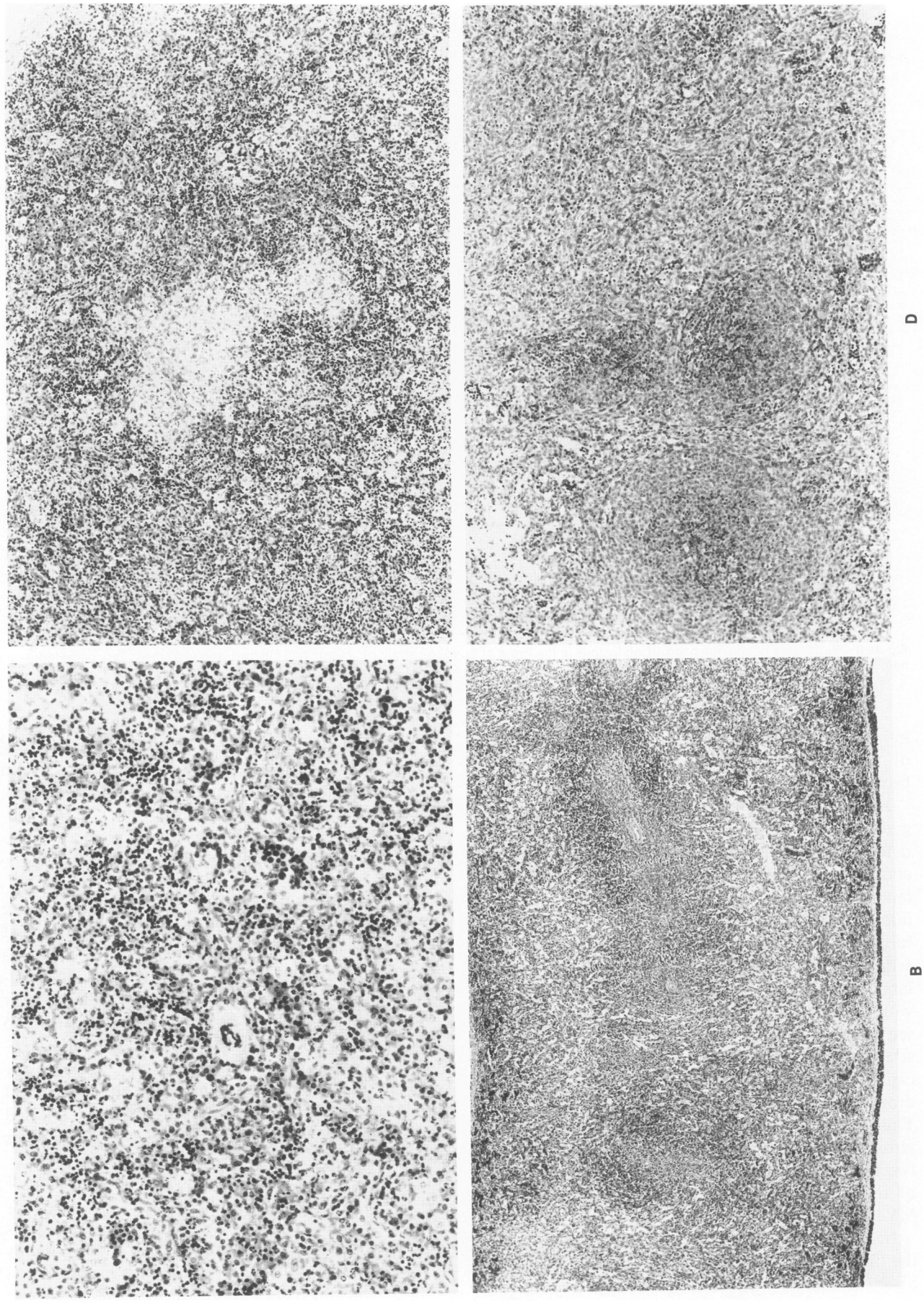

Figure 7A — Extramedullary hematopoietic activity persistent in spleens of 8-day-old rabbits receiving Boerlage strain SFV on Day 1 of life. **B** — For comparison, 8-day-old recipients of Patuxent strain SFV showed little residual hematopoietic activity in their spleens. **C** — Necrotizing lesions resembling granulomas are scattered throughout the spleen of Boerlage recipients at 8 days of age. **D** — By 15 days of age, no areas of necrosis remain, and hematopoietic activity is minimal in Boerlage recipients.

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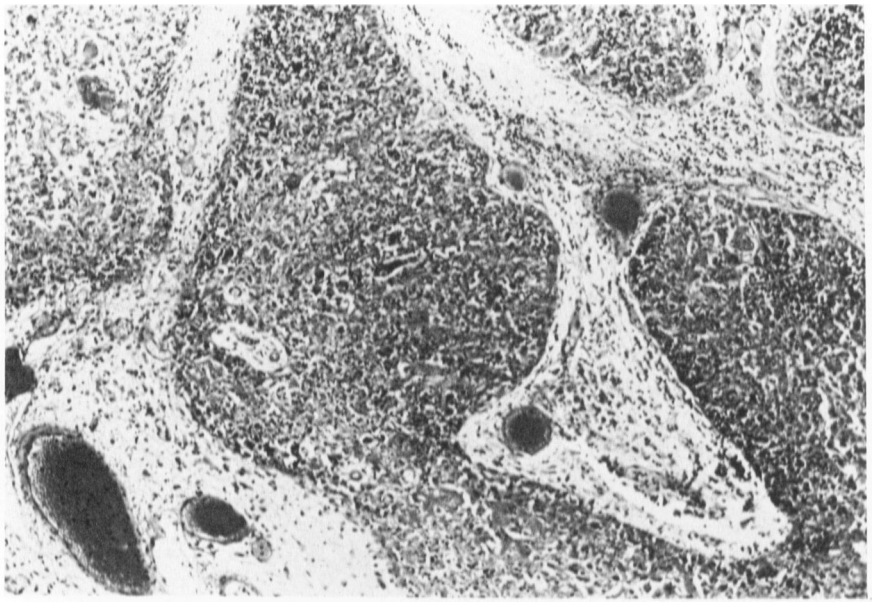

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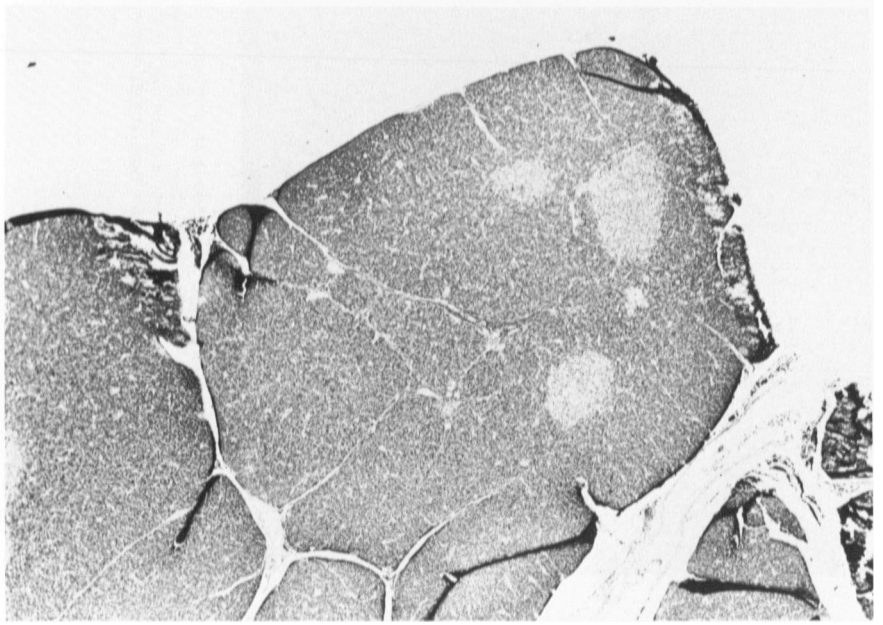

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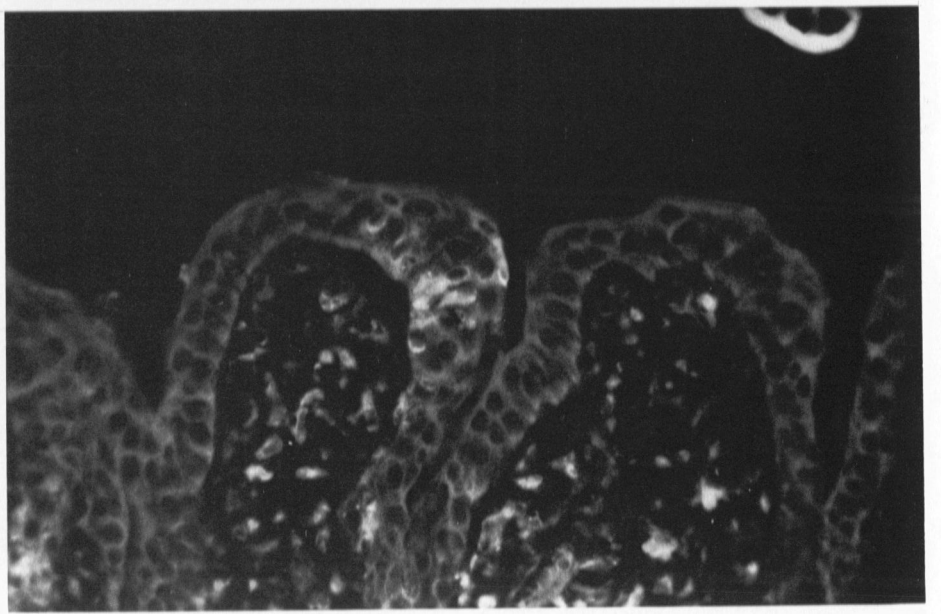


Figure 8A – Thymus 7 days following postnatal inoculation of Boerlage strain SFV. Extensive necrosis is seen, particularly in the interior of the thymic lobules. A moderate lymphocytic infiltrate in the fibrous septa is also noted. **B** – By Day 15, this process has completely resolved. **Figure 9A** – Tumor cells from Boerlage recipients stained positively for SFV antigens with the use of direct immunofluorescence techniques with FITC-rabbit anti-SFV. **B** – Epidermis overlying tumors often stained positively for SFV antigens as well in Boerlage recipients.

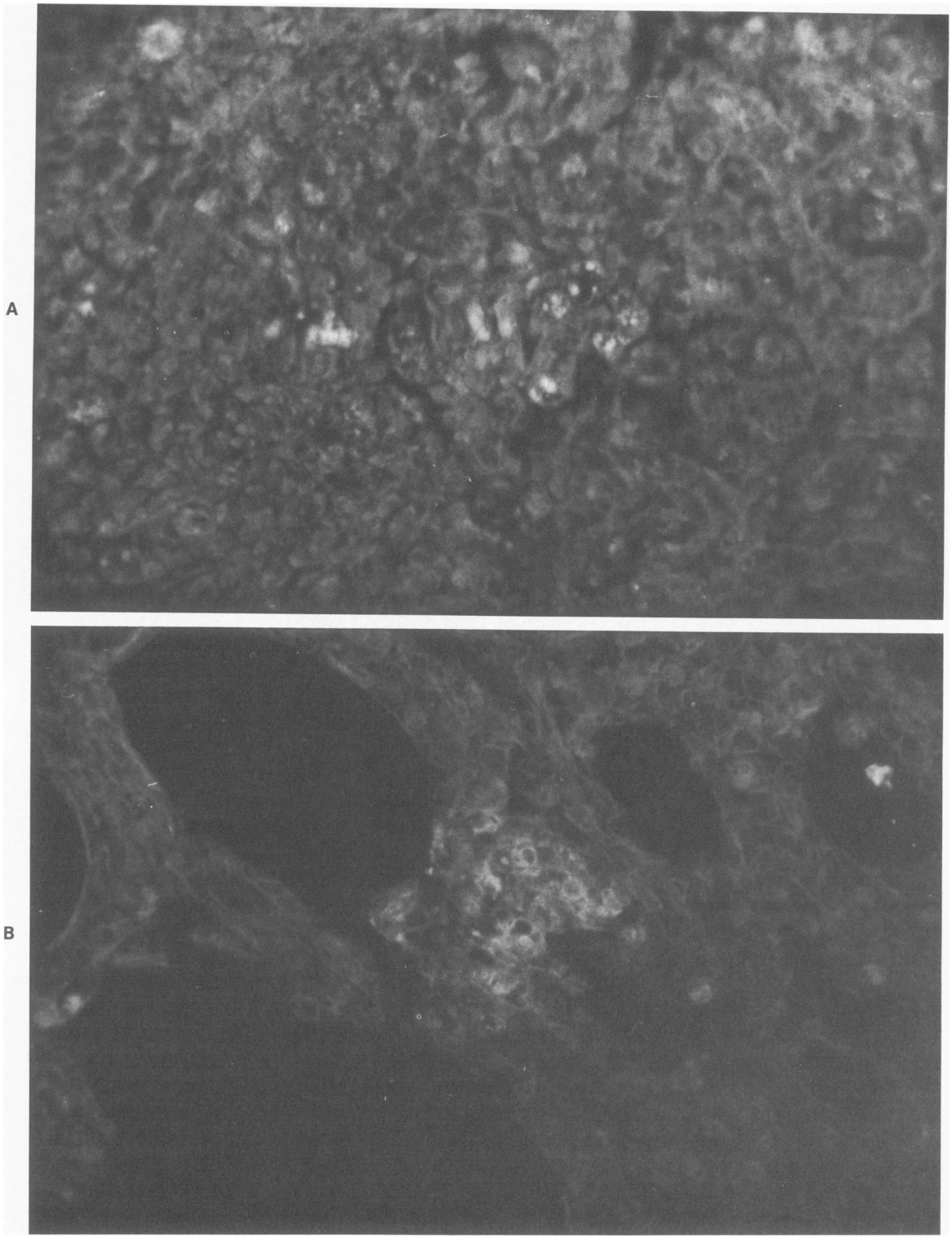
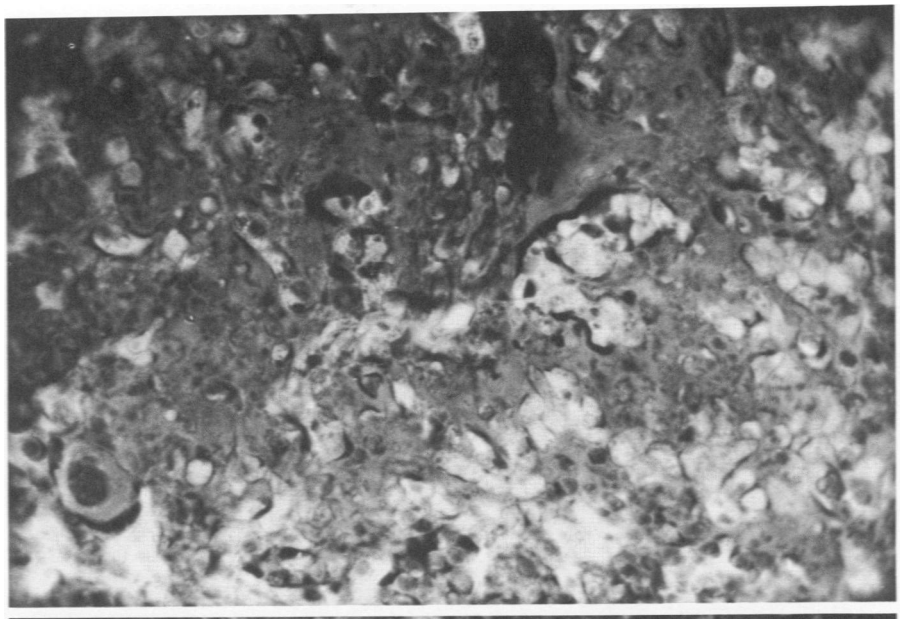


Figure 10—Sections from different organs from neonatal recipients of Boerlage strain SFV, at 7 days after inoculation. These sections were stained with FITC rabbit anti-SFV. Boerlage strain SFV thus causes a disseminated infection in neonates. **A**—Spleen. **B**—Lung. **C**—Liver, showing virus antigen in intrasinusoidal cells, most likely both hematopoietic and phagocytic. Parenchyma is not positive in this illustration. **D** and **E**—Thymus, showing both interstitium and lobular lymphocytes staining positively for SFV viral antigen.



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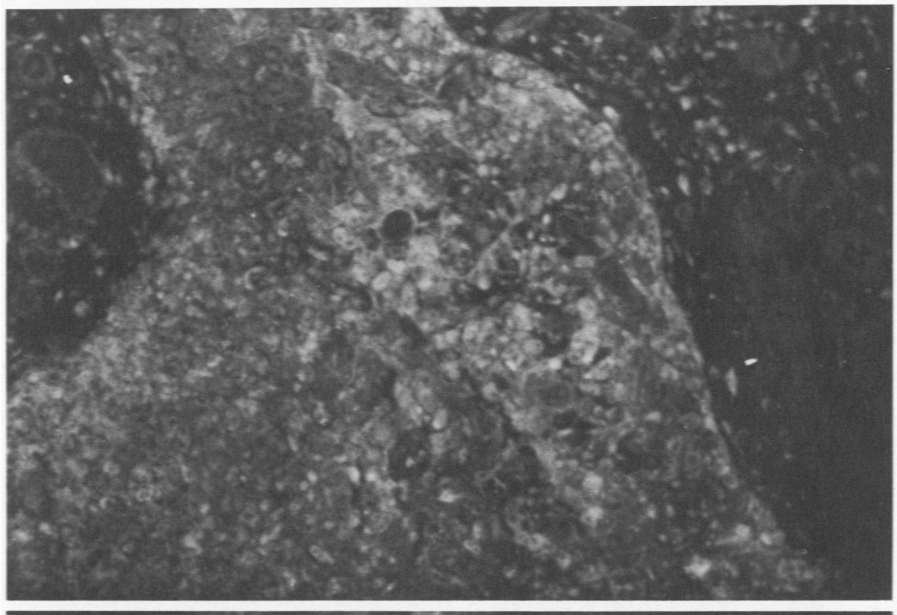

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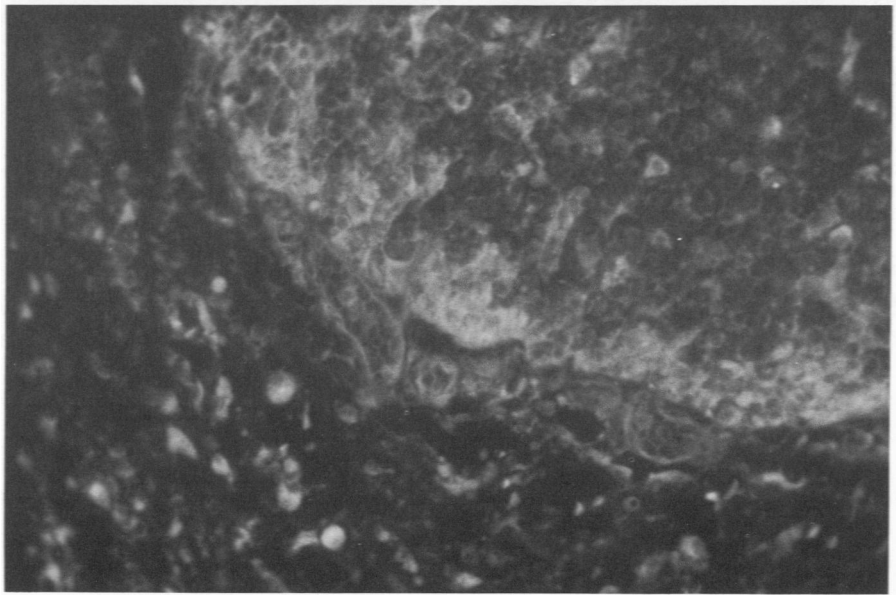

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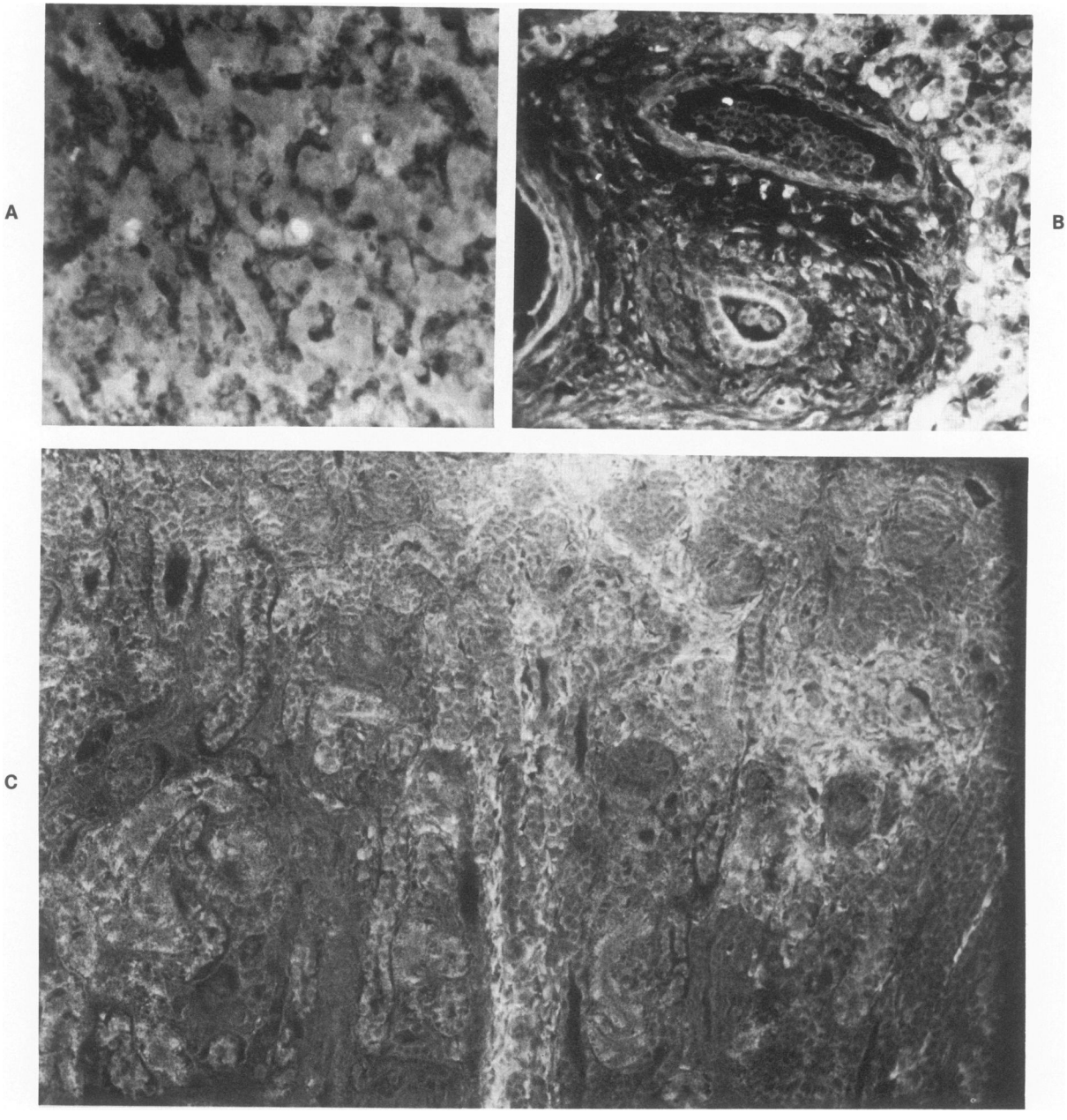

Figure 11 — Parenchymal organ staining was observed as well in neonatal recipients of Boerlage strain SFV. Here, 8-day-old rabbits are examined as described 7 days following infection. **A** — Liver, showing occasional hepatocellular positivity for SFV antigen. **B** — Bile duct epithelium and hepatocytes stain positively, as well as occasional cells in the circulating blood and portal tract stroma. **C** — Kidney, with positive staining for virus antigen in collecting ducts, proximal nephron, and stroma and occasionally in glomeruli.

are clinically robust. In our hands, Boerlage strain infection in adults does not differ substantially from Patuxent strain infection. However, restriction endonuclease analysis of DNA from a variety of SFV strains shows considerable consistency among strains except for the Boerlage strain. This strain differed substantially from other strains and resembled MYX and MV more closely than did the others (Cabirac et al, unpublished data).

It is tempting to ascribe the results of other investigators to a possible MV- or MYX-like contaminant in those Patuxent or Boerlage preparations they used. The possibility of a nonpoxvirus passenger virus in older nonpurified preparations must also be considered. Still, these explanations remain conjectural. Other possibilities such as rabbit strain differences in a variety of host resistance mechanisms, viral genetic drift with time, and virulence attenuation with repeated *in vitro* passage must also be considered.

We cannot offer definitive explanations for our

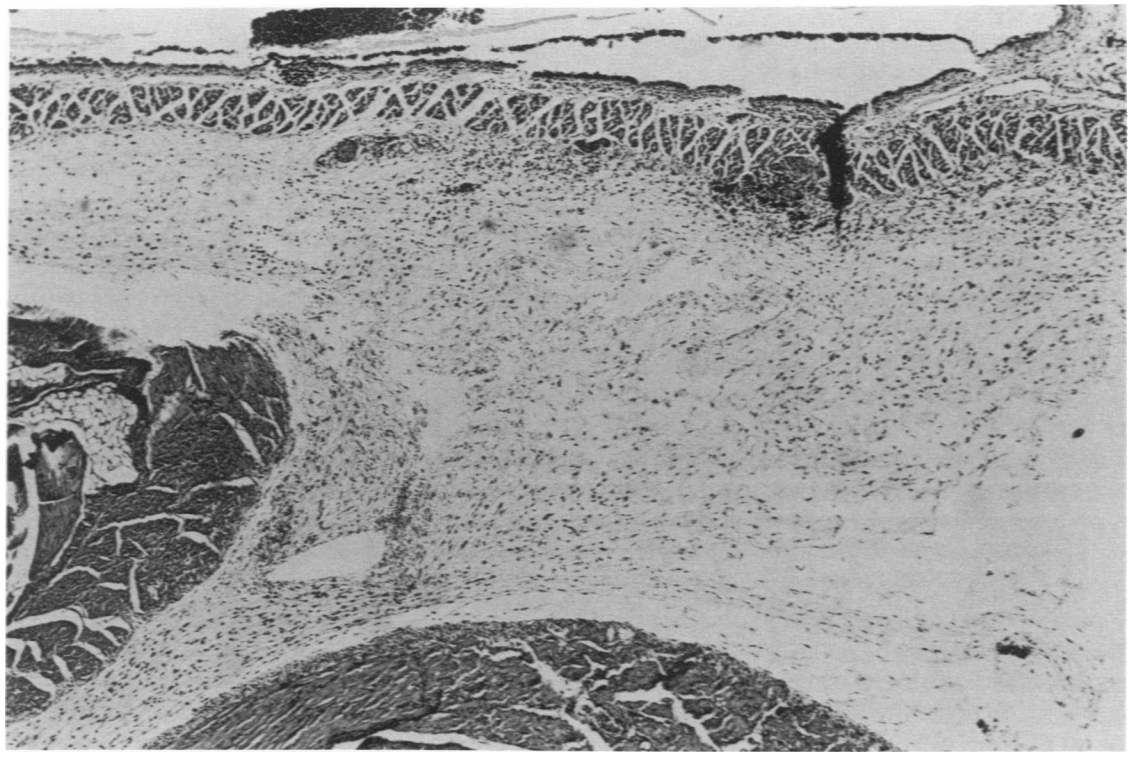

Figure 12 – Rabbits given Solu-medrol in two different regimens received 10^7 ffu plaque-purified Patuxent strain SFV. Their tumors, examined at peak size, did not differ from those seen in untreated adult recipients of plaque-purified Patuxent strain SFV.

Table 1 – SFV Behavior in Compromised Adults*

Reference	SFV strain	Therapy	Age	Findings compromised/neonates versus control
3	Boerlage	Cortisone	Adult	Larger tumors. Occasional satellite nodules.
		6-MP	Adult	Larger tumors. Distant metastases.
22	OA	Coal tar	Adult	Delayed tumor regression. Disseminated tumor (IV).
23	Boerlage	MTX	Adult	Delayed tumor regression, extensive local disease. Disseminated tumor (IV or IP).
		Cortisone	Adult	Delayed tumor regression.
24	OA	300–700 R	Adult	Delayed tumor regression. Disseminated tumor (IV).
31	Patuxent	Prednisolone	2–3 kg	Delayed skin test and Ab reactions. Delayed tumor rejection and lymphocyte infiltration.
		Coal tar	2–3 kg	Delayed skin test and Ab reactions. Delayed tumor rejection and lymphocyte infiltration.
		DMBA + prednisolone	2–3 kg	Delayed skin test and Ab reactions. Delayed tumor rejection and lymphocyte infiltration.

* A compendium of most findings with different strains of SFV, ages of rabbits, and immunosuppressive agents. Abbreviations: IV, intravenous; IP, intraperitoneal; MIF, migration inhibitory factor; MTX, methotexate; 6-MP, 6-mercoptopurine; DMBA, dimethylbenzanthracene; DTH, skin test reactivity (delayed type hypersensitivity). The original strain of SFV is OA. Occasional authors report using OI strain. These are recombinants between SFV and MYX, generated artificially, and are not included here.[33,34]

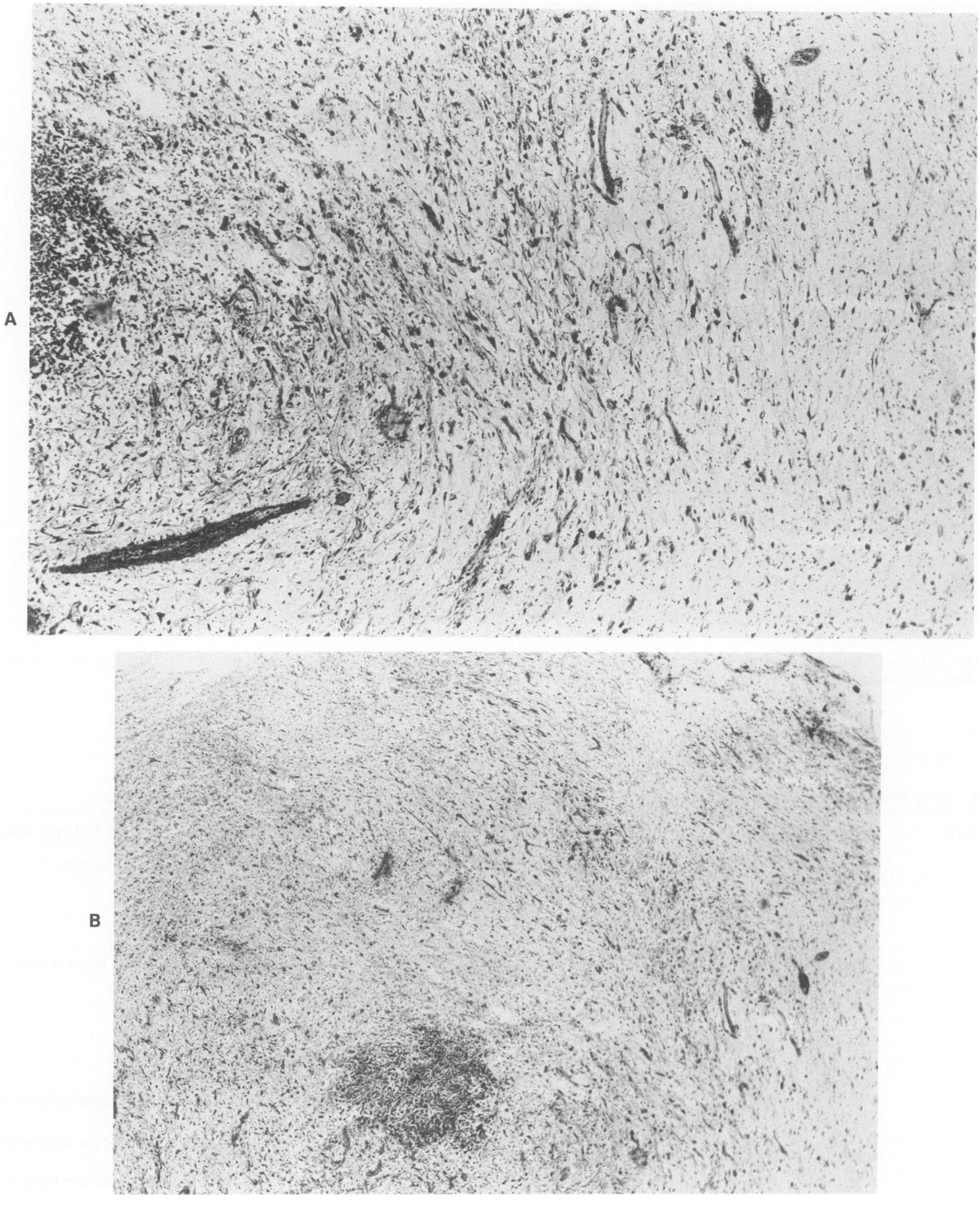

Figure 13 — One-day-old rabbits received 10^7 ffu of our original non-plaque-purified Patuxent strain SFV, so that we could repeat previous work.[4] This preparation contains small amounts of MV.[12] **A** — These tumors were intermediate in appearance between the highly myxoid tumors seen in adults receiving plaque-purified Patuxent strain SFV and the more cellular histology of tumors from neonatal recipients of the same virus. This specimen was taken at 15 days of age. **B** — Focal lymphocytic/polymorphonuclear infiltration was noted but was not extensive.

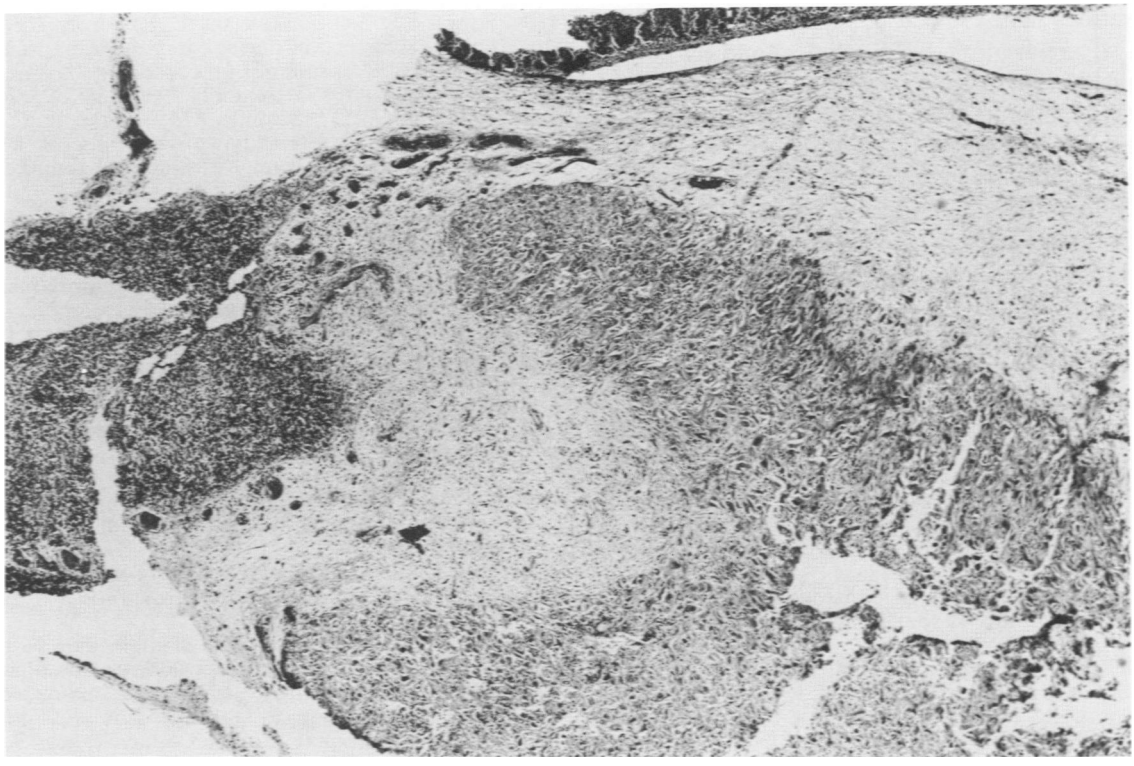

Figure 14—Necrosis and metastases were observed in the thymus in these rabbits. Metastases were seen elsewhere as well.

Table 2—SFV Behavior in Neonates

Reference	SFV strain	Therapy	Age	Findings compromised/neonates versus control
2	Original (OA)	–	2-3 days	Extensive tumor causing death. Delayed both antibody response and resistance to secondary challenge. Persistent viremia.
4	Patuxent	–	3-4 days	Satellite lesions and extensive local and distant disease.
19	Patuxent	–	1-3 days	Metastatic disease. Decreased MIF. No DTH. Antibody formation intact.
21	Patuxent	–	1-2 days	Defective interferon production in neonates.
25	Madison	–	Suckling (6 days)	Disseminated tumor and death.
26	OA	–	8 days	Invasive tumor and death.
			10 days	Half developed fatal tumor. Half rejected tumor within 2 months.
			15 days	All developed tumors which regressed.
		Cortisone	13–15 days	All 12 treated rabbits developed fatal tumors, compared with 2 of 18 controls.
27	Boerlage	–	2-5 days	Progressive tumors with occasional metastases. All died.
28	Patuxent	–	1-2 days	Tumor grew for 4-5 weeks and disappeared by 8 weeks.
29	Patuxent	–	4 days	Half survived with regression at 2-3 weeks. Half died. Antibody formation intact.
30	Original (OA)	–	1-5 days	Death with large systemic tumor burdens (IV).
32	OA	–	12 hours to 15 days	Extensive invasion and metastasis causing death.
		Benzpyrene	Adult	IV virus induced no visible tumor.
		Cortisone	Adult	Some died with extensive disease. Others survived with protracted course.

observations here. However, this is the first report of which we are aware in which clinical and pathologic differences in SFV strain behavior are examined with the use of plaque-purified virus preparations. The contrast between our results and the results of others underscores the importance of using homogeneous virus preparations in such studies. In addition, this report suggests that the *in vivo* behavior of different SFV strains is not necessarily uniform.

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