PATHOGENICITY OF THE M AND E VARIANTS OF THE ENCEPHALOMYOCARDITIS (EMC) VIRUS

II. Lesions of the Pancreas, Parotid and Lacrimal Glands

John E. Craighead, M.D.

From the Departments of Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

Two variants of the encephalomyocarditis (EMC) virus (E and M) which differ in pathogenicity for mice have been described in a previous report.[1] The E variant is highly neurotropic and produces a rapidly fatal infection in 12-week-old mice. The M variant usually causes a mild, non-fatal illness and widespread myocytolysis in the heart but with few signs of central nervous system involvement.

In the course of studies to determine the properties of these 2 variants, necrosis of the retroperitoneal adipose tissue and the epididymal fat tags was observed in mice infected with the E variant (Fig. 1). Since it seemed likely that these changes were due to retroperitoneal seepage of digestive enzymes, the pancreatic glands of animals inoculated with the 2 variants were examined histologically. Extensive coagulation necrosis of pancreatic acinar cells was found consistently in E-infected mice. In contrast, pancreatic lesions were not present in animals receiving the M variant. When cortisone was administered to M-infected mice, however, widespread necrosis of the islets of Langerhans and occasionally, focal necrosis of the acinar tissue were observed. The parotid and lacrimal glands of animals infected with both variants also were examined because of their morphologic and functional similarities to the pancreas. Alterations were present in these organs under circumstances to be described in detail below.

Experiments undertaken to elucidate the pathogenesis of these virus-induced lesions are reported here. In addition, the histologic features of the lesions are described.

Material and Methods

The origin and some of the properties of the E and M variants of EMC virus have been described in an earlier report.[1] E was prepared as a mouse brain suspension; M consisted of a homogenate of heart tissue. The two variants were adapted to grow in mouse embryo cells by 5 serial passages in monolayer cultures; the cell

This work was supported by Grants HI-06481 and HE-06370 from the United States Public Health Service.

Accepted for publication, August 27, 1965.

culture-adapted variants are designated E-me and M-me, respectively. Details of the methods for preparation and handling of virus pools and determinations of infectious titers in mice (LD_{50}) and cell cultures (ID_{50}) have been recorded.[1]

Twelve-week-old male Swiss mice supplied by Charles River Breeding Laboratories, Inc., (Brookline, Mass.) were used. Three-week-old mice and recently weaned hamsters from a local breeder were employed in a few studies. Except as noted in the text, animals were given subcutaneous inoculations with 0.1 ml aliquots of the virus preparations. The details of individual experiments are described below.

Two different types of studies were carried out using cortisone-treated, infected mice. Selected organs from these animals were: 1) examined histologically after death or sacrifice, and 2) titered at 24-hour intervals after inoculation to determine their virus content. These studies were carried out in the following manner. One-tenth ml cortisone acetate (Merck, Sharp and Dohme, Inc., West Point, Pa.) in 0.85 per cent saline at the appropriate dilution or saline alone was administered intraperitoneally to mice, 24 hours and approximately 1 hour before, and at 24-hour intervals after, inoculation of virus. The cortisone and virus doses are recorded in the text and tables below.

Four to 10 mice comprised the treatment and control groups for histologic studies. Organs were removed and fixed as soon as possible after death or at the time the experiments were terminated on day 14. Paraffin sections were cut at 5 μ for microscopic examination using hematoxylin and eosin stains.

The virus content of the tissues was determined by sacrificing a single animal from the control and treatment groups at 24-hour intervals. Blood was obtained from the retro-orbital sinus and the animal was killed by cervical disarticulation. Clean instruments were used to remove each organ. A 10 per cent suspension of serum or tissue was prepared in Hanks' solution containing 5 per cent chicken serum and antibiotic agents. The preparations were clarified by light centrifugation, and the virus content determined in monolayer cultures of L cells.

Results

E Variant

Necrosis of the pancreas, parotid, exorbital lacrimal and endorbital lacrimal glands was found consistently in 12-week-old mice infected with the E variant. The lesions were present in animals inoculated over a wide range of virus doses by the subcutaneous, intraperitoneal and intracerebral routes. Neutralization tests using antiserum prepared against the antigenically similar Mengo strain of EMC virus showed that the lesions were caused by E variant and not some extraneous substance or contaminating infectious agent.

The sequential histologic changes in the tissues were followed by sacrificing mice at 24-hour intervals after the inoculation of 10^4 LD_{50} of E. Alterations were first recognized in the pancreatic acinar cells approximately 48 to 72 hours later. At this time the animals appeared healthy and exhibited no signs of central nervous system involvement. The initial histologic changes consisted of swelling of individual acinar cells, associated with vacuolation and loss of cytoplasmic basophilia. Confluent areas of coagulation necrosis appeared subsequently; by the fifth day the acinar tissue was largely destroyed (Fig. 2).

Although the overall architecture of the gland was retained, interstitial edema was prominent. Rarely, scattered granulocytes and lymphocytes were found. The islets of Langerhans and pancreatic ducts were usually well preserved; in a few animals, however, individual islets exhibited focal necrosis. Death as a result of polioencephalomyelitis generally occurred 5 to 7 days after the inoculation of virus. At this time necrosis of the pancreas and retroperitoneal adipose tissue was evident grossly (Fig. 1). Phagocytosis of necrotic debris was apparent in the pancreas of the few mice which survived for 10 to 12 days. Evidence of fibroblastic and possibly regenerative activity was observed rarely.

The sequential histologic changes in the lacrimal glands paralleled those in the pancreas. At the time of death this organ consistently exhibited widespread necrosis. Similar alterations were usually found in the parotid glands but they were focal and never extensive.

Lesions of the pancreas also developed in 3-week-old mice and weanling hamsters infected with E variant. Necrosis of retroperitoneal adipose tissue was not observed in these animals possibly because of the short intervals between inoculation and death (3 to 4 days). The ability of E to cause necrosis of the glands was not altered after 5 serial passages in cell cultures (E-me). The infectivity titer of E in 12-week-old mice was not changed significantly by daily administration of cortisone (2.5 mg per day).

M Variant

Repeated attempts to demonstrate lesions in the pancreas, parotid and lacrimal glands of M-infected, 3- and 12-week-old mice were unsuccessful. Several different doses of virus were tried, using the subcutaneous route of inoculation. Similar results were obtained with M variant after it had been passaged in cell cultures (M-me). However, necrosis was found consistently in the lacrimals (but not in the pancreas or parotids) of the occasional animal which died as a result of M-me infection. Lesions were not present in the glands of surviving M-me-inoculated mice at the termination of the experiment. Nonlethal infection of virus inoculated animals was regularly confirmed in these studies by the histologic demonstration of myocardial lesions at the time of sacrifice.

Cortisone was administered to M and M-me-infected mice in an attempt to alter the pathogenicity of the virus. When the steroid was given daily over a wide range of doses, the results differed strikingly from those described above (Table I). Many of the virus inoculated 12-week-old mice developed a rapidly fatal infection; these animals exhibited few

if any signs of central nervous system involvement but showed extensive changes in the heart at the time of their death on the fourth to sixth day. Lesions in the pancreas and lacrimal and rarely the parotid glands were found in fatally infected mice receiving a daily dose as small as 25 μg. The alterations of the acinar cells of the pancreas were similar

TABLE I

EFFECT OF CORTISONE ON OCCURRENCE OF LESIONS IN 12-WEEK-OLD MICE INOCULATED WITH $10^{1.5}$ ID_{50} M-ME

	Cortisone dose (mg/day)				
	2.5	1.25	0.25	0.025	Saline
Mortality *	4/4	6/6	9/9	4/5	6/10
Survival time (days)	4	4–6	3–4	3–6	4–7
Lesions †					
Pancreas:					
Acinar cells	2/4	6/6	5/7	1/5	0/9
Islets of Langerhans	4/4	6/6	6/7	1/5	0/9
Lacrimal	3/4	6/6	7/7	4/4	6/10
Parotid	0/4	0/6	1/6	0/4	0/7
Heart	4/4	6/6	2/2	3/3	10/10

* Number dead/number tested.

† Number of animals with organs showing lesions/number organs examined histologically.

morphologically to those observed in animals receiving E. The changes were never extensive and usually consisted of lysis of individual cells or rarely, clusters of acinar cells. Necrosis of peripancreatic adipose tissue was not found, grossly or microscopically. The lacrimal glands consistently exhibited extensive coagulation necrosis (Fig. 3).

Unique changes were found in the islets of Langerhans of M and M-me-infected mice receiving cortisone. All or the majority of the cells of most islets were necrotic (Figs. 4 and 5). Usually these structures were surrounded by normal appearing acinar tissue, although occasionally there was necrosis of some of the adjacent acinar cells.

The mortality among M-me-infected mice receiving daily inoculations of saline (Table I) was variable and often exceeded that observed in experiments in which the animals were not handled regularly.[1] The reasons for this increased mortality are not known; it is possible that trauma or stress played a role. Alterations were not observed in the glands of non-infected, cortisone-treated mice and infected animals receiving only saline.

Titrations of organs from mice inoculated with $10^{1.2}$ LD_{50} of M-me were carried out in an attempt to determine the relationship of the virus content of an organ to histologically recognizable lesions. Virus was not recovered from the tissues or blood 24 hours after inoculation. At 48 and 72 hours large amounts of virus were present in all of the specimens

examined. The quantities of virus in animals receiving cortisone were usually greater than in mice inoculated only with saline. As can be seen in Table II, the infectious titers of the heart, pancreas and lacrimal glands were higher than the blood and other tissues. Although interpretation of these results is complicated by the presence of virus in blood, they show that substantial quantities of virus were present in organs exhibiting morphologic changes attributable to infection. The results of studies on the parotid glands are not included in the table because of the difficulties encountered in performing a clean dissection of this organ.

Discussion

The lesions described in this report have not been recognized previously in EMC-infected animals. They are not peculiar to the E and M variants since necrosis of the acinar cells of the pancreas occurs in mice infected with several different strains of EMC virus.[2] The acinar cell lesions are virus-induced and are not caused by some extraneous substance or unrecognized infectious agent. In brief, the evidence is as follows: 1) they are produced by virus strains which have been passaged in several different laboratories using cell cultures or animals, or both; 2) they are caused by viruses purified by the plaque technique; 3) they

Table II

EFFECT OF CORTISONE ON VIRUS CONTENT OF TISSUES FROM 12-WEEK-OLD MICE 48 AND 72 HOURS AFTER INOCULATION WITH $10^{1.2}$ ID_{50} M-ME

	Experiment 1				Experiment 2			
	Cortisone *		Saline		Cortisone		Saline	
	48 hours	72 hours	48 hours	72 hours	48 hours	72 hours	48 hours	72 hours
Serum	4.5 †	4.5	1.5	2.0	5.0	4.5	2.5	4.0
Brain	2.3	4.3	<2.0	4.3	3.3	4.8	<2.0	<2.0
Heart	4.8	6.8	<2.0	3.8	5.3	≥7.8	3.3	3.8
Muscle	2.8	2.3	<2.0	2.3	3.3	2.8	2.3	<2.0
Spleen	3.8	4.3	3.8	4.3	— §	5.3	—	4.8
Lacrimal	2.8	5.3	<2.0	5.8	5.3	≥7.8	6.8	6.8
Pancreas	3.8	5.8	<2.0	3.8	5.3	6.3	6.8	5.8

* 2.5 mg per day, intraperitoneally.
† Log_{10} virus titer per gram tissue.
§ Not done.

occur with equal severity over a wide range of virus dosage; 4) they do not develop when the E variant is neutralized by antiserum prepared against a strain which does not cause the lesions (Mengo); and 5) they are not found in control mice inoculated with tissues from animals and cell cultures.

The early appearance of lesions and the recovery of large quantities of virus from the pancreas and lacrimal glands of M-me-infected animals suggest that the 2 organs and possibly the parotids may serve as important sites of virus replication. These cells are endowed with an abundant ergastoplasm and would appear equipped to support the production of viral RNA and protein. The manner by which the cells are destroyed is not known; possible mechanisms were briefly considered in a previous report.[1] Although the virus undoubtedly interferes directly with cell function, coagulation necrosis of organs may be due, in part, to autodigestion by their enzymes. Substantial amounts of amylase have been demonstrated in the blood of mice 48 hours after E-inoculation[3] and the retroperitoneal fat necrosis would appear to result from release of pancreatic lipases.

In this laboratory non-purulent conjunctivitis has been noted in many EMC-infected mice. It is not known whether the lesions of the exorbital and endorbital lacrimal glands account for the conjunctivitis. Histologic studies have failed to reveal other significant alterations in the eyes.

Corticosteroid hormones have been shown to enhance the pathogenicity of other small RNA viruses (polioviruses and group B Coxsackie viruses) which are biologically similar to EMC.[4–6] In the studies reported here the pathogenicity of M, but not E, was substantially altered by cortisone. In addition to increasing mortality, the steroid resulted in the appearance of changes in the lacrimal and pancreatic acinar cells and a unique lesion of the islets of Langerhans. Lesions of the islets of Langerhans resembling those described here have not been reported previously in virus-infected animals. The physiologic significance of these lesions is not known. Preliminary studies have shown that some, but not all, M-infected mice develop hyperglycemia (blood glucose 300 to 500 mg per cent) and exhibit abnormal "tolerance" to intraperitoneally inoculated glucose.[3]

Lesions of the pancreatic and lacrimal acinar tissue (but not the islets of Langerhans) which are similar, if not identical to, those found in E-inoculated animals have been described in adult mice infected with certain strains of group B Coxsackie viruses and the virus of foot-and-mouth disease.[7–9] In addition, large amounts of virus have been recovered from these organs in mice receiving group B Coxsackie viruses.[4,8,10] A common mechanism for the development of lesions caused by these 3 viruses would appear likely.

Changes in the acinar cells and ducts of the pancreas in cortisone-treated rabbits have been reported.[11,12] These animals were given relatively large amounts of steroid for prolonged periods of time. In this study, histologically recognizable alterations were never found in con-

trol mice receiving cortisone. Moreover, all experiments with this drug were terminated by the seventh day after virus inoculation.

Summary

The E and M variants of the encephalomyocarditis (EMC) virus produce lesions of the pancreas, parotid and lacrimal glands in 12-week-old mice. E is highly neurotropic and causes a rapidly fatal infection; M usually results in a mild, non-fatal illness and widespread myocytolysis in the heart. Coagulation necrosis of the acinar cells of the 3 glands is the characteristic lesion found in E-infected mice. At the time of death, these animals exhibit widespread necrosis of the retroperitoneal adipose tissue, presumably resulting from the release of digestive enzymes from the pancreas. In contrast, M-infected mice regularly develop lesions of the lacrimal glands and pancreas only when cortisone is administered. Necrosis of the islets of Langerhans, however, is the most prominent change in the pancreas of steroid-treated animals. The evidence thus far accumulated suggests that "zymogen" glands may serve as important sites of virus replication.

References

1. Craighead, J. E. Pathogenicity of the M and E variants of the encephalomyocarditis (EMC) virus. I. Myocardiotropic and neurotropic properties. *Amer. J. Path.*, 1966, **48**, 333–345.
2. Craighead, J. E. Necrosis of the pancreas, lacrimal and parotid glands associated with encephalomyocarditis virus infection. *Nature (London)*, 1965, **207**, 1268–1269.
3. Craighead, J. E. Unpublished data.
4. Boring, W. D.; Angevine, D. M., and Walker, D. L. Factors influencing host-virus interactions. I. A comparison of viral multiplication and histopathology in infant, adult and cortisone-treated adult mice infected with the Conn.-5 strain of Coxsackie virus. *J. Exp. Med.*, 1955, **102**, 753–766.
5. Kilbourne, E. D.; Wilson, C. B., and Perrier, D. The induction of gross myocardial lesions by a Coxsackie (pleurodynia) virus and cortisone. *J. Clin. Invest.*, 1956, **35**, 362–370.
6. Bodian, D. The enhancement of susceptibility of monkeys to polioviruses of high and low virulence. *Amer. J. Hyg.*, 1956, **64**, 92–103.
7. Pappenheimer, A. M.; Kunz, L. J., and Richardson, S. Passage of Coxsackie virus (Connecticut-5 strain) in adult mice with production of pancreatic disease. *J. Exp. Med.*, 1951, **94**, 45–64.
8. Wilson, W. B., and Cheatham, W. J. Parotitis in weanling mice produced by Coxsackie B-1 (Conn-5) virus. *Amer. J. Path.*, 1962, **41**, 415–424.
9. Platt, H. The occurrence of pancreatic lesions in adult mice infected with the virus of foot-and-mouth disease. *Virology*, 1959, **9**, 484–486.
10. Dalldorf, G., and Gifford, R. Adaptation of group B Coxsackie virus to adult mouse pancreas. *J. Exp. Med.*, 1952, **96**, 491–497.

11. BENCOSME, S. A., and LAZARUS, S. S. The pancreas of cortisone-treated rabbits. *Arch. Path. (Chicago)*, 1956, **62**, 285–295.

12. STUMPF, H. H.; WILENS, S. L., and SOMOZA, C. Pancreatic lesions and peripancreatic fat necrosis in cortisone-treated rabbits. *Lab. Invest*, 1956, **5**, 224–235.

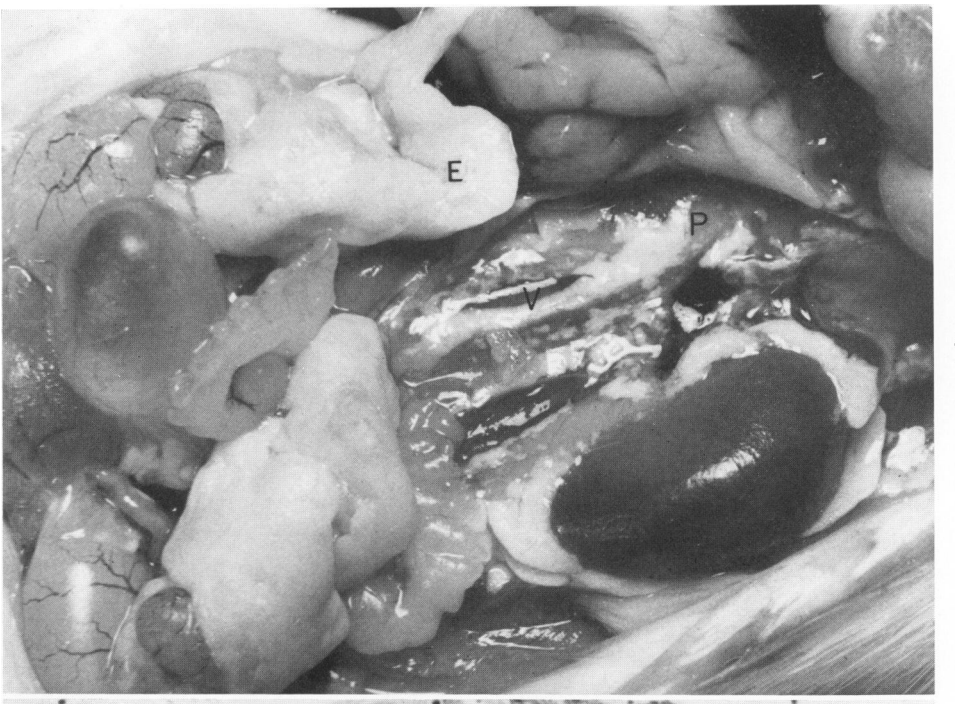
The advice and encouragement of Drs. A. Hertig and G. Dammin are gratefully acknowledged.

LEGENDS FOR FIGURES

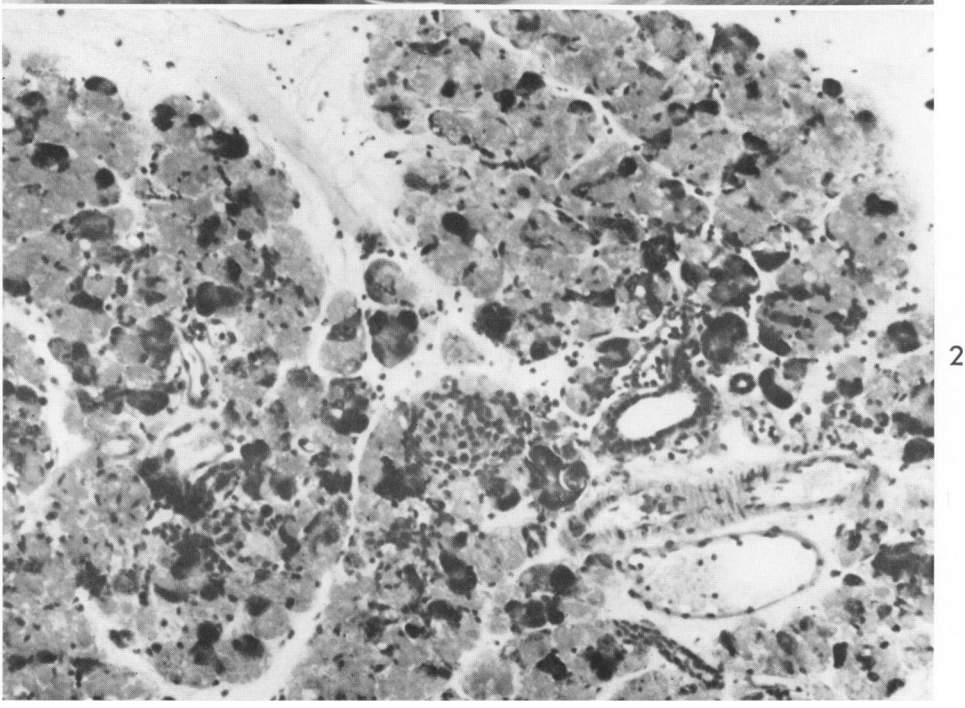
Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Abdominal cavity, 12-week-old mouse sacrificed 5 days after the inoculation of 10^4 LD_{50} of E variant. The peritoneal contents are reflected in order to show the necrotic retroperitoneal adipose tissue adjacent to pancreas (P), vena cava (V), kidney and epididymal fat tags (E).

FIG. 2. A representative histologic section of a pancreas from a 12-week-old mouse sacrificed 5 days after inoculation of 10^4 LD_{50} of E variant. Necrosis of acinar cells, interstitial edema and absence of an inflammatory cell infiltrate are featured. Scattered clusters of intact acinar cells, an islet of Langerhans and ducts can be seen. × 50.



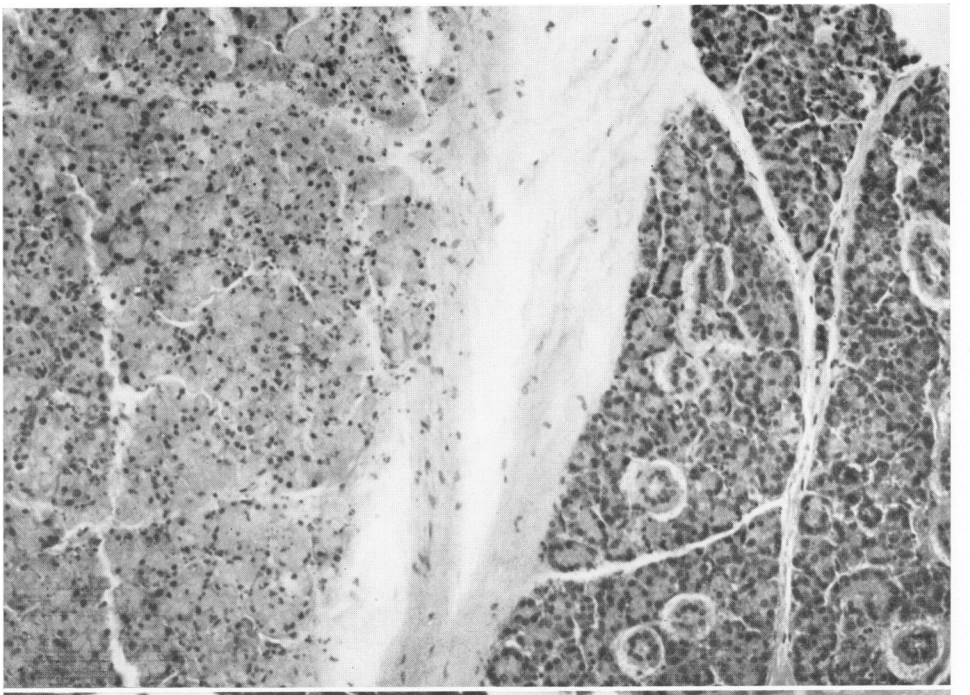
1



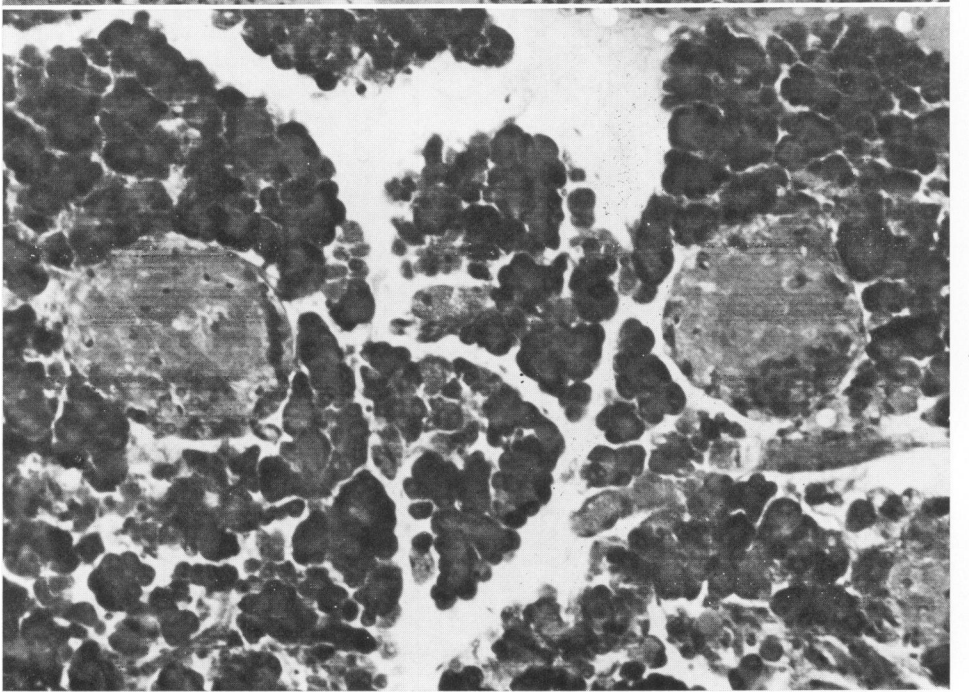
2

FIG. 3. Exorbital lacrimal (left) and parotid gland (right), 12-week-old mouse which died 5 days after inoculation of $10^{1.5}$ LD_{50} of M-me. The parotid tissue is intact. The lacrimal gland is edematous and the cells exhibit marked nuclear pyknosis as well as swelling and lysis of the cytoplasm. × 50.

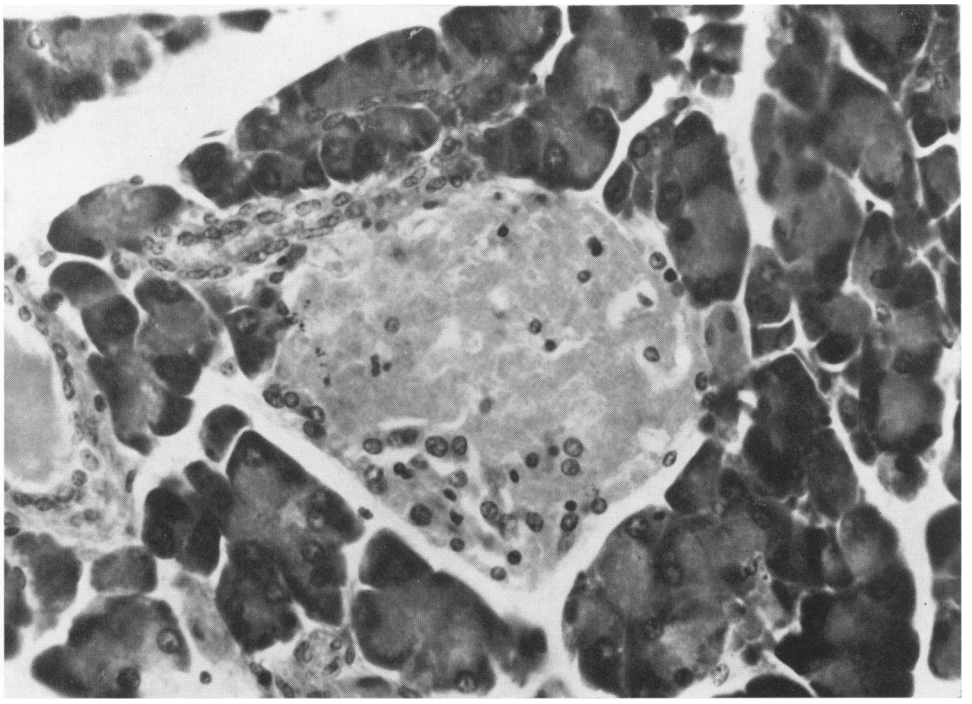
FIG. 4. Pancreas, 12-week-old, cortisone-treated (2.5 mg per day) mouse which died 4 days after the inoculation of $10^{1.5}$ LD_{50} of M-me variant. The islets of Langerhans are necrotic. A few scattered clusters of acinar cells have also undergone necrosis. There is interstitial edema but no inflammatory response. × 100.



3



4



5

FIG. 5. Islet of Langerhans and adjacent ducts and acinar tissue in pancreas of a 12-week-old, cortisone-treated (2.5 mg per day) mouse which died 4 days after the inoculation of $10^{1.5}$ LD_{50} of M-me variant. "Ghosts" of islet cells can be seen; there is no evidence of selective destruction of a specific cell type. Epithelium of ducts elsewhere in this gland was intact. × 200.