A NEW MOUSE VIRUS CAUSING NECROSIS OF THE THYMUS IN NEWBORN MICE

By WALLACE P. ROWE, M.D., and WORTH I. CAPPS

(From the Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Infectious Diseases, Bethesda, Maryland)

Plates 80 to 84

(Received for publication, December 7, 1960)

This report describes preliminary efforts to characterize a previously undescribed mouse virus which produces necrosis of the thymus in newborn mice. Because of the marked tropism of the agent for this tissue, the name "mouse thymic virus" is proposed as a tentative designation.

Materials and Methods

Mice.—In the initial studies, suckling mice less than 24 hours old of the National Institutes of Health, General Purpose (G.P.) strain were used; this mouse strain is a Webster Swiss maintained by random mating. More recently, the N.I.H. mouse strain was used; this strain was derived from the G.P. strain in 1936, and has been maintained by brother-sister mating. Both strains were supplied by the Animal Production Section of the National Institutes of Health. After receipt in the experimental laboratory, the mother mice were fed on apples and Purina small animal pellets.

Virus.—Most studies reported here were done with virus strain B, the origin of which is described in the text. For routine studies, the virus source consisted of a freshly prepared 10 per cent or 20 per cent suspension of thymus, spleen, kidney, and pancreas of G.P. mice harvested on the 7th day after inoculation with serial passage virus; the suspensions were prepared by grinding the tissues in a glass tissue grinder, suspending in Eagle's basal medium containing penicillin (250 units/ml.) and streptomycin (250 μg./ml.), and clarifying by centrifugation at 2000 R.P.M. for 15 minutes. These suspensions will be referred to as "standard fresh virus suspensions."

Tissues and suspensions were held in an ice bath at all times.

Virus Assay.—Tests for virus infectivity were carried out by inoculating two to four litters of mice, usually with eight mice per litter. In early experiments combined intracerebral and intraperitoneal inoculation was used, the dosage being 0.02 and 0.05 ml., respectively. In later studies the intraperitoneal route alone was used. 12 to 14 days after inoculation the mice were sacrificed, and the thymus examined macroscopically for the characteristic necrotic appearance. For more sensitive tests for virus, as in isolation attempts, a blind passage was made on the 7th day; half of the mice in each litter were sacrificed, and a suspension of their pooled thymuses, livers, spleens, and kidneys was passed to two litters of newborn mice. The remaining first passage mice and all of the blind passage mice were examined for thymic lesions on the 14th day after inoculation. Positive thymuses were harvested for passage and histologic examination.

The criterion for a positive isolation attempt was induction of typical macroscopic necrosis

of the thymus in one or more mice in each of two consecutive passages, confirmed by histologic examination on at least one mouse.

Neutralization Tests.—Seed virus consisted of standard fresh passage suspensions of the B strain. Serum was diluted 1:10 in saline solution, and inactivated by heating at 56°C. for 30 minutes. Equal volumes of the serum dilution and virus dilution were mixed, and the mixtures held in an ice bath for 30 minutes. Each mixture was then inoculated intraperitoneally into multiple litters of newborn mice. The virus control consisted of a mixture of equal volumes of virus dilution and saline solution. The mice were sacrificed at an appropriate time, usually 12 to 14 days after inoculation, and the thymuses examined by gross inspection.

Histologic Examination.—Tissues were fixed in a solution of nine parts saturated solution of mercuric bichloride and one part formalin, the two components being mixed immediately before use. Staining was done with hematoxylin-eosin.

EXPERIMENTAL

Isolation of Strains.—The initial isolation of the thymic agent was encountered as an incidental finding during a blind passage series; a suspension of pooled lactating breast tissue, mammary tumors, and stomach contents of suckling mice, all from mice of strains considered to be carriers of the mammary tumor agent, was inoculated into newborn mice of the G.P. strain, and a blind passage series initiated. The passages were made at 2 week intervals by pooling organ suspensions from mice harvested at 7 and 14 days, and inoculating into two litters of newborn G.P. mice. No illness was observed until in routine histologic sections of one of two fifth passage mice sacrificed on the 14th day after inoculation, it was observed that a small portion of the thymus was necrotic. Thymus was not included in the sections made in the following passage, but each of two mice examined histologically at both the seventh and eighth passages showed massive thymus necrosis. It was then found that the thymuses of the recipient mice in the subsequent passages were markedly abnormal on gross inspection. The agent causing the thymic necrosis has been carried through 39 serial newborn mouse passages since the fifth passage of the initial series, with induction of macroscopic thymic necrosis in 744 (96 per cent) of 807 passage mice examined at 6 to 8 days, and in 72 of 75 examined at 14 to 20 days.

Twenty-one additional isolations of apparently identical agents have been made from tissues and mouth swabs of retired breeder mice of the G.P. mouse strain. In addition, a number of apparent isolations have been made from wild house mice; however, due to the almost ubiquitous occurrence of mouse salivary gland virus among these mice (1) it has not yet been possible to obtain a strain of thymic agent from wild mice which is not contaminated with the salivary gland virus, and characterization of these strains is not yet feasible.

Description of the Disease in Mice.—Infant mice inoculated with freshly prepared suspensions of infected tissues show no evidence of overt disease or excess mortality. At 3 to 5 days after inoculation, sections of thymus show beginning necrosis in the medulla, with large numbers of basophilic bodies of

varying size in the cytoplasm of the epithelial cells; this material presumably represents karyorrhectic debris. At this time the nuclei of many thymocytes are markedly altered, containing a pale, amphophilic inclusion body surrounded by a clear halo and peripherally marginated chromatin (Figs. 1 and 2). Cytochemical studies by Dr. Robert Love, of the National Cancer Institute, indicated that the intranuclear inclusions stain positively by the Feulgen procedure. The thymic necrosis proceeds rapidly, progressing centrifugally; in the majority of animals the thymocytes are completely destroyed, as well as much of the medulla, so that by the 7th day, histologic examination shows the thymus to consist of massive areas of eosinophilic necrotic debris surrounded by a somewhat thickened capsule, with cords of thymic epithelium and connective tissue growing in from the capsule and along the blood vessels (Fig. 3). Repair processes and clearing of necrotic debris occur during the 2nd week, with proliferation of thymic epithelium, infiltration with macrophages, formation of foreign body giant cells, and some deposition of calcium (Figs. 4 and 5). After the 2nd or 3rd week repopulation with thymocytes occurs, and by 4 to 6 weeks the normal architecture is generally restored, except for scarred areas consisting of calcifying amorphous material enclosed in a capsule composed of mesenchymal and thymic epithelial elements (Fig. 6).

The macroscopic appearance of the thymus shows a corresponding sequence of changes. First changes are seen at 5 or 6 days, with opacity and decrease in size of the thymus; occasionally the earliest changes are yellow or white streaks on the anterior surface. At 7 or 8 days, the thymus is decreased in size, block-shaped with loss of normal contours, and opaque yellow-white in color (Fig. 7). The gross changes are most readily observable at 12 to 14 days, since the thymuses in control animals are much larger; the average weight of normal thymuses on the 14th day is 40 to 45 mg., while that of the infected thymuses is 11 to 16 mg. By 4 to 6 weeks there is generally return to normal gross appearance of the thymus.

Variation in the pattern of response, seen with lower doses of virus or in the early passages of freshly isolated strains, consists of delay in the sequence of changes, or involvement of only part of the thymus.

Infrequently, nuclear inclusion bodies or partial necrosis has been observed in histological sections of lymph nodes of inoculated mice, but there were no other lesions which could be attributed to the agent. Serial total and differential white blood cell counts through the first 14 days after inoculation showed no deviation from the values in uninoculated controls.

Host Range and Problems of Virus Assay.—Precise characterization of the thymic agent has been greatly hindered by the limitations in the available virus assay system. Initially, the agent was studied in mice of the G.P. strain, in which it was originally isolated; newborn mice of this strain were almost uniformly susceptible to induction of thymic necrosis following inoculation

with undiluted fresh virus suspensions, but with 10-fold or 100-fold dilutions of such suspensions the response was highly variable between different litters. Also, it was found that the agent is enzootic in this stock.

In order to supplant the G.P. strain as the indicator animal, a number of mouse strains were tested for susceptibility. Newborn mice of various strains

TABLE I

Tests of Mouse Strains for Susceptibility of Newborn Mice to Thymic Agent, Compared with General Purpose Mice Inoculated with the Same Material

Mice		Thymus response* by litter	
Strain	Colony	Tested strain	G.P. mouse controls
Swiss "Specific pathogen free"	Microbiological Assoc.	0/3‡; 0/11	7/7; 9/9
" "	" "	8/9; 0/3	8/8; 6/6; 9/9; 7/7
Balb/c, "Specific pathogen free"	" "	3/9	Same as above
C3Hf	" "	4/5; 2/3	1/2; 6/6
"	" "	0/3; 0/3‡	8/8; 8/8
DBA/2	" "	0/4; 0/5; 1?/5; 0/5§	6/6; 8/8; 8/8; 7/7; 8/8
CFW	Carworth Farms	5/9	6/6; 6/6
AKR/LwN	N.I.H.	3/5	not done
AKR/Lw	Dr. L. W. Law	0/6	7/7; 6/7; 8/8
C57Bl/Lw	"	0/7	Same as above
N.I.H. Swiss	N.I.H.	6/6; 6/6	6/6; 5/6
" "	"	6/6; 6/6; 6/6	6/6; 6/6
NMRI Swiss ‖	Naval Medical Research Institute	7/7; 8/8; 6/6	7/7; 2/2; 7/7

* Numerator = number of mice showing macroscopic thymic necrosis; denominator = number of mice observed. All mice were examined 12 to 14 days after inoculation with undiluted standard fresh virus suspensions.

‡ A blind passage of tissues of littermates harvested at 7 or 8 days was positive in G.P. mice.

§ Blind passage of the sacrificed mice was positive in G.P. mice.

‖ This strain of mice was derived from the G.P. strain in 1951, and maintained by brother-sister mating.

were inoculated in the usual manner with undiluted fresh passage virus suspensions, and a number of G.P. litters were inoculated at the same time to control the potency of the inoculum. Table I shows the results of these tests. The majority of the tested strains were less responsive than the G.P. mice, but two strains of Swiss mice, both of which are inbred derivatives of the G.P. strain, did show uniform response. The virus apparently propagated in the resistant strains, since passage of their tissues to G.P. mice produced the disease.

In view of the susceptibility of the N.I.H. mouse strain, this strain has been used for additional studies. Tests to date indicate that this strain is not spontaneously infected with the agent; ten pools of tissues of ten or twenty retired breeders each have been tested by the blind passage procedure, with negative results. Also, virus titrations in newborn mice of this strain did not show the litter variation encountered in the G.P. mice. Although there was little litter variation, erratic end-points in virus titrations indicated that there was significant variation in susceptibility of individual animals.

Table II shows the influence on thymic response of age at time of inoculation with standard fresh virus suspensions; only the newborn animals were uniformly responsive, but occasional animals developed gross thymic necrosis when inoculated as late as 10 days of age.

TABLE II

Influence of Age of Mice on Response to Thymic Agent

Age of mice at time of inoculation	Incidence of thymus necrosis, by litter, at 14 days after inoculation	
	Experiment 1—General Purpose mice	Experiment 2—NIH mice
<24 hrs.	7/7; 11/11; 6/6	8/8; 6/6; 3/3
2 days	0/8; 8/8; 0/7	4/7; 4/8; 5/8; 3/6
4 "	1/8; 0/8; 2/8	
7 "	0/8; 0/8; 0/8	
10 "	0/8; 0/8; 1/8	
14 "	0/8; 0/8; 0/8	

No evidence of illness was observed following inoculation of weanling G.P. or N.I.H. strain mice by the intraperitoneal, intracerebral, or intraspinal routes, during an observation period of 30 days. Also, inoculation of virus into weanling G.P. mice simultaneously with *Eperythrozoön coccoides* and into "specific pathogen-free" Swiss mice pretreated with *E. coccoides* 2 days previously did not elicit signs of thymic or hepatic disease.

Route of inoculation of newborn mice was not of major importance. High percentage response was obtained following inoculation by the intraperitoneal, intracerebral, or intrathoracic routes, somewhat lower frequency of response following subcutaneous or thigh muscle injection, and very poor response (1 of 21) after intranasal instillation.

No thymic lesion detectable in the gross or by histologic examination was induced in newborn rats or hamsters, and passage of their organs at 14 days to N.I.H. mice was negative for the agent. No overt disease was produced in guinea pigs or rabbits inoculated intracerebrally and intraperitoneally.

Attempts to cultivate the agent in tissue culture have been unsuccessful. Virus was inoculated into primary trypsin-dispersed monolayer cultures of mouse embryo and mouse kidney, explant cultures of mouse thymus and

spleen, and continuous passage lines of mouse melanoma S-91, mouse lung carcinoma CA-755, mouse sarcoma S-180, and mouse leukemia P-1534; no cytopathic effects were seen, and assays of culture fluids in newborn mice were consistently negative.

Since many of the initial studies of the agent were made in mice of the G.P. strain, which is spontaneously infected, it is worthwhile recording the results obtained with various controls. Four types of control groups have been used: uninoculated litters; litters inoculated with sterile saline solution; litters inoculated with fresh tissue suspensions of 7-day-old mice; and a blind passage series of normal suckling mouse tissue.

Table III shows the results of examination of the thymuses of the first two

TABLE III

Incidence of Thymic Necrosis in Saline-Inoculated and Uninoculated Control Mice

Day of sacrifice	Strain		Total
	General Purpose	N.I.H.	
5–10	0/326	0/33	0/359
11–16	0/1149	0/309	0/1458
17–23	1/165	0/50	1/215
Total.....	1/1640	0/392	1/2032

types of control; the only positive control mouse was an uninoculated G.P. mouse examined on the 20th day.

In the third type of control, 23 suspensions of pooled viscera of normal 7-day-old G.P. mice have been tested in newborn mice of the same strain; 22 of these, containing tissues from a total of 133 mice, gave negative results in a total of 246 recipients. One suspension, composed of tissues of 14 mice, yielded virus, producing thymus necrosis in all seven recipients in one litter, but in none of seven in the other litter inoculated at the same time; passage of a suspension of the positive thymuses induced typical thymic necrosis in subsequent passage mice. It is noteworthy that the positive normal suspension was the only one made from mice freshly received from the animal production center; the others were made from control mice held in the experimental animal room since the day of birth.

Further evidence that the virus may be acquired from the assay system was provided by the fourth control. A fresh tissue suspension of viscera of 7-day-old uninoculated control mice of the G.P. strain was passed to two litters of newborn mice of the same strain; these mice were sacrificed on the 7th day, the

thymuses examined, and a fresh suspension of their tissues passed as before. The 7 day passage series was continued for 16 passages. Through the first 14 passages, which included passage of tissues of 183 mice, no thymic necrosis was observed, but in the 15th and 16th passages the typical lesion was observed in many recipients. It appears probable that the initial isolation of thymic agent, as described in a preceding section, also represented acquisition of virus during a blind passage series in G.P. mice.

General Properties of the Agent.—The properties of the thymic agent are compatible with those of a virus. The agent passes through Selas 02 and 03 candle filters, and is not affected by suspension in a medium containing penicillin (100 units/ml.), streptomycin (100 μg./ml.) achromycin (10 μg./ml.), and mycostatin (25 units/ml.). Also, repeated attempts to cultivate the agent on a variety of bacteriologic media have yielded no evidence of growth; two fresh suspensions of infected thymuses were tested for pleuropneumonia-like organisms by Dr. Michael F. Barile, of the N.I.H., Division of Biologic Standards, with negative results.

The infectivity of the agent is quite unstable; activity of potent suspensions was destroyed by exposure to 20 per cent diethyl ether for 2 hours at 2°C. (three experiments), and by heating at 50°C. for 30 minutes (two experiments). Also, storage at −60°C. for short periods often resulted in a decrease in activity; suspensions with low activity, such as early passage materials of newly isolated strains, often were non-infectious after storage in the frozen state. Shell freezing by immersion in a methylcellulose-dry ice bath with subsequent storage at −60°C., and inclusion of 5 per cent guinea pig serum in the suspending medium has given more consistent preservation of infectivity. Storage at refrigerator temperature for 8 days resulted in complete or nearly complete loss of activity in three experiments.

Serologic Studies.—Development of a neutralization test was greatly hindered by the irregular assay system provided by G.P. mice, as well as by the apparently low antigenicity of the agent. After the finding that the N.I.H. mice were more uniformly susceptible, it has been possible to demonstrate some degree of neutralizing antibody response. Table IV gives the results of four neutralization tests, showing that convalescent serum of mice inoculated as newborns or hyperimmunized after weaning generally produced a significant reduction in frequency of thymic necrosis. It is particularly noteworthy that the serum of normal retired breeders from the G.P. colony gave positive neutralization results, while serum of comparable mice from the N.I.H. colony did not neutralize the agent. The data in this table also demonstrate the degree of litter variation in the G.P. mice, the general lack of litter variation in N.I.H. mice, and the somewhat erratic dose-response relationship in both strains.

Pathogenesis of Infection.—Preliminary studies have been made of the growth curve and distribution of virus in inoculated mice.

TABLE IV

Four Representative Neutralization Tests with B Strain of Thymic Agent

Test No.	Strain of mice	Day of sacrifice	Mouse serum	Virus dilution								No virus
				10^{0} or $10^{-0.5}$*		10^{-1}		10^{-2}		10^{-3}		
				Thymus necrosis	Per cent positive	Thymus necrosis	Per cent positive	Thymus necrosis	Per cent positive	Thymus necrosis	Per cent positive	Thymus necrosis
407	General Purpose	8	No. 171-32 Convalescent serum from inoculated newborns; 32 days	8/8, 7/7, 8/8, 8/8	100							
			Saline	8/8, 7/7, 6/6	100							0/8, 0/8
511	General Purpose	13	No. 343-47 Convalescent serum from inoculated newborns; 47 days			0/8, 0/6, 0/7, 0/8, 0/8, 0/7	0					0/8
			No. 143 Serum from weanlings inoculated two times; 32 days			0/7, 1/8, 0/8, 0/8, 0/8, 0/5	2					
			Normal serum from SPF‡ retired breeders			0/6, 6/8, 0/7, 0/7, 5/8, 1/8	27					
			Saline	8/8, 6/6 2/7	76	0/8, 4/7, 2/6, 5/6, 1/8, 1/8	30	0/7, 6/7, 1/6	35	0/8, 0/6, 0/8	0	0/9, 0/8

700	N.I.H.	13	No. 343-47 See above			3/5, 6/7, 8/8, 3/6	77					
			No. 343–85 Same group as above, bled at 85 days			4/7, 1/8, 3/6, 2/7	36					
			No. 588 Serum from adults inoculated five times; 35 days			5/7, 1/8, 0/7, 4/7	34					
			Normal serum from N.I.H. retired breeders			3/3, 6/8, 8/8, 6/6	92					
			Saline			4/4, 6/6, 7/7, 7/7, 5/6, 5/7	92	0/7, 0/7, 2/7, 1/7	11	1/8, 0/6	7	0/7, 0/7
894	N.I.H.	13	No. 343-47 See above	0/7, 1/7	7	0/8, 0/7	0					
			No. 588 See above	0/8, 0/7	0	0/8, 0/8	0					
			No. 4096 Serum of SPF adult mice inoculated three times; 102 days	0/8, 1/7	7	1/8, 0/7	7					
			Normal serum from G.P. retired breeders	0/8, 0/7	0	0/8, 0/8	0					
			Normal serum from N.I.H. retired breeders	5/8, 4/8	53	5/7, 4/7	64					
			Saline	6/8, 8/8, 5/6, 4/7	79	5/8, 3/7, 5/8, 3/8	52	0/8, 1/7	7			0/8, 0/7

* 10^0 dilution used in Experiments 407, 511, and 700; $10^{-0.5}$ dilution used in Experiment 894.

‡ SPF = Specific pathogen-free Swiss mice.

Growth curves were determined in newborn mice of the G.P. strain. The mice were inoculated intraperitoneally with undiluted fresh passage suspension, and at intervals, groups of three or four mice were sacrificed and the thymus, lungs, heart, liver, kidneys, adrenals, spleen, pancreas, and salivary glands of the four mice pooled and made to 20 per cent suspensions. The suspensions were clarified by centrifugation and titrated in G.P. mice, using two litters per 10-fold dilution. The virus assays were performed on the day of sacrifice. Table V shows the results of the two experiments. In both tests, the infectivity titers reached a peak on the 7th day and subsequently declined. In Experiment 184 the titer declined by the 10th day, and in Experiment 343 the virus was not detectable in either group at 28 days. However, it is striking that virus was again present, in relatively high titer as compared to the earlier time periods, at 84 days and in one of two groups, at 127 days. When this result was found, the frozen 28 and 84 day specimens in Experiment 343 were retested simultaneously to attempt to eliminate variation in host sensitivity; in the repeat test of the undiluted suspensions, the 28 day materials were again negative, while the 84 day suspensions induced thymic necrosis in the majority of recipients. In other experiments in which tissues were tested at late periods after inoculation of newborns, two groups at 34 days yielded virus, the undiluted organ suspensions inducing thymic necrosis in 20 of 21 recipients and 21 of 24 recipients, respectively; a suspension at 61 days was negative (0/16 recipients); and a suspension at 176 days was positive (18/20 recipients). Also, mouth swabs of inoculated mice were positive for virus for prolonged periods. Mouth swabs of groups of mice inoculated as newborns were taken by swabbing the mouth with a fresh cotton swab, and rinsing out the swabs in a single vial containing 5 ml. of Eagle's basal medium; this pooled mouth swab rinse was then inoculated into newborn N.I.H. strain mice. Of four such swabs taken from groups of mice inoculated 160, 172, 190, and 241 days previously, all were positive, inducing thymic necrosis in the great majority of recipients. Another pooled swab fluid, taken from mice inoculated 373 days previously, was negative.

During the initial peak of infectivity, virus is widely distributed; separate suspensions of thymus, blood, brain, liver, kidney, and carcass harvested 7 days after inoculation of newborn G.P. or N.I.H. mice with passage virus were all positive for virus.

The observation of a decline in titer between the 7th and 14th days has been made repeatedly in isolation attempts. As described in the Materials and Methods section, the virus isolation procedure consisted of blind passage of half the recipient mice at 7 days, with sacrifice of the remaining littermates at 14 days for observation of the thymus and passage of tissues of mice showing gross thymic necrosis. It has often been found that the 7 day passage, made from mice with grossly normal thymuses, was positive, while the 14 day passage of the positive thymuses was negative.

Differentiation from Other Mouse Viruses.—The type of disease and of inclusion body, host range, and ether sensitivity of the thymic agent serve effectively to indicate its distinctness from other known mouse viruses. Thymuses of infant mice inoculated with polyoma virus, mouse salivary gland virus, K virus, mouse adenovirus, and mouse strains of Reoviruses types 2 and 3 have been examined in the gross and microscopically, and in no instance have similar lesions been seen. Also, a pooled serum of weanling mice hyper-

immunized by three intraperitoneal injections of fresh thymic agent suspensions did not contain hemagglutination inhibiting antibodies for polyoma virus, pneumonia virus of mice (PVM), or Reovirus types 1, 2, 3, was negative in complement-fixation tests against potent antigens for mouse adenovirus, K virus of mice, mouse salivary gland virus, the hepatoencephalitis virus of Stanley, Sendai virus, lymphocytic choriomeningitis, and psittacosis virus, and was negative in tissue culture neutralization test against mouse salivary gland virus. This serum (No. 4096) contained neutralizing antibody for the B strain of thymic agent as shown previously in Table IV, Experiment 894.

TABLE V

Growth Curve of Thymic Agent

Days after Inoculation	Experiment 184		Experiment 343			
	Thymus necrosis*	Titer‡ of pooled viscera	Thymus necrosis		Titer of pooled viscera	
			Group 1	Group 2	Group 1	Group 2
3	0/3	Trace	0/4	0/4	Trace	Neg.
5	1/3	$10^{0.4}$	0/4	0/4	$10^{0.6}$	$10^{0.6}$
7	1–3/3	$10^{1.9}$	4/4	4/4	$10^{2.7}$	$10^{1.6}$
10	2/3	Trace	4/4	4/4	$10^{1.7}$	$10^{1.1}$
14	2/5	$10^{0.0}$	4/4	4/4	$10^{1.4}$	$10^{0.9}$
28			3/4	3/4	Neg.	Neg.
84			0/3	0/3	$10^{1.6}$	$10^{1.7}$
127			0/3	1/3	Neg.	$10^{1.8}$

* Number of donor mice with macroscopic necrosis of thymus/number sacrificed.

‡ Titer expressed as the number of ID_{50} per 0.05 ml. of 10 per cent tissue suspension. "Trace" means that the undiluted suspension induced thymic necrosis, but in fewer than half of recipients.

Although the original B strain of thymic agent derived from a passage series of material presumably containing mammary tumor agent, there is little reason to suspect a relationship between the two.

Electron Microscopic Observations.—Dr. William G. Banfield, of the National Cancer Institute, has made electron micrographic studies of thin sections from infected mice, and has kindly made available a preliminary report of his findings.

"Thymic tissue from suckling mice 6 days after inoculation with the agent was fixed in buffered osmium tetroxide (2), embedded in methacrylate and examined in the electron microscope after thin sectioning.

"Within the nucleus of many of the infected cells were parallel fibrils 90 A in thickness filling a single area (Fig. 8). In degenerating cells the fibrils were more abundant and more clearly defined (Fig. 11).

"The nucleus also contained oval to round bodies in variable numbers (Fig. 8), sometimes

within an altered area of nucleoplasm. They were from 750 to 1000 A in diameter. A few of the bodies were dense throughout, but usually they were less dense centrally with walls from 100 to 270 A thick. Some contained a 190 to 360 A diameter internal granule of varying density and eccentricity. Often the walls were not limited by a membrane while in a few, the walls were limited by one or two membranes. Within the nucleus relatively large dense granules similar to those reported in other virus infections (3) were occasionally seen.

"Intranuclear filaments 265 A in diameter have been reported in association with the polyoma virus (4). They are larger than those described here and can apparently give rise to individual virus particles by segmentation. Filaments have also been found in association with the Lucké frog tumor agent (4); however they are uncommon and are intracytoplasmic. The Lucké fibrils are larger than the present fibrils, measuring about 250 A, and are made up of smaller filaments from 50 to 80 A in width.

"Particles were present in the cytoplasm larger than those in the nucleus, and more discrete and uniform (Fig. 9). They were round or somewhat elliptical, from 930 to 1,460 A in diameter, with most between 1,190 and 1,320 A. Their wall was usually dense, from 135 to 370 A thick, often with a rough surface as if sectioned through nodules or spines. A dense internal granule 135 to 400 A in diameter was frequently present. The granule was round, elliptical or dumbbell-shaped with variable eccentricity, possibly depending upon the plane of section.

"It is probable that the cytoplasmic particles came from the nucleus and that the thickening, increased density, and rough appearance of the wall are the result of passage through the nuclear membrane.

"A second cytoplasmic particle is not uncommon. This has an additional membrane, possibly of cellular origin, (Figs. 10 and 11)."

DISCUSSION

The thymic agent appears to represent a previously undescribed virus which is enzootic in certain colonies of laboratory and wild mice. The evidence for the viral nature of the agent is its ability to pass through bacteria-tight filters, inability to grow on bacteriologic media, resistance to antibiotics, and the presence of nuclear and cytoplasmic karyoannular particles in electron micrographs of acutely infected thymuses. Also, the production of intranuclear inclusion bodies is strongly indicative of a virus.

A number of technical factors have made it difficult to obtain exact information: instability of the virus on storage; very low infectivity titers; low susceptibility of most mouse strains; enzootic infection of the most susceptible strain available in quantity, with resultant variation in susceptibility of litters and occasional acquisition of virus from the assay system; absence of an endpoint determinable by external inspection; and in early passages an apparent dissociation between time of highest virus titer and time of the grossly recognizable lesion.

The problem of variation in susceptibility between different mouse strains and between different litters of a given strain has been of particular importance in hindering development of a reliable assay system, and the reasons for the variations have not been fully clarified. The occurrence of litter variation in the G.P. colony, which is endemically infected, and the absence of this variation in the N.I.H. colony, which appears to be free of the infection, suggests that

the litter variation may be due to immunological factors; also, preliminary experiments, not reported here, suggest that there may be passive transfer of maternal antibody in the G.P. strain. Whether this can explain the marked resistance of many litters from mice raised under "specific pathogen-free" conditions appears less likely. Since virus was readily recovered from thymuses of inoculated mice of the resistant strains, it is possible that the resistance is concerned with failure of infected cells to undergo necrosis, rather than with inhibition of virus growth.

The present picture of the course of infection after inoculation of newborn mice can be summarized as follows. There is an acute necrosis of the thymus, beginning in the medulla and extending centrifugally, generally resulting in complete destruction of thymocytes. Intranuclear inclusion bodies are present in thymocytes during the acute necrotic stage. The acute necrotic stage is followed by granulomatous reaction, repopulation with thymocytes, and eventual encapsulation of calcified debris. Virus titers are at a peak at the end of the necrotic stage, and are variable thereafter. In some animals virus persists for at least 8 months. Virus is widely distributed throughout the body during the acute stage, but the extent to which it multiplies and persists in various organs has not been determined. Antibody response in convalescent animals appears to be relatively poor.

Whether the virus induces disease in mice in naturally infected colonies is not known. The finding of typical thymic necrosis in one uninoculated control and the induction of thymic necrosis in one mouse by intranasal instillation suggest that spontaneous disease may occur as a rare event. Thymuses from virus-positive spontaneously infected mice in the G.P. colony showed no histologic evidence of disease. Dr. Thelma Dunn, of the National Cancer Institute, who kindly reviewed some of the sections, reported that she has not previously encountered this type necrosis.

There can be little doubt that the thymic necrosis is a direct viral effect and not a secondary response. The presence of inclusion bodies and highly active virus in the thymus of inoculated mice, is strongly in support of this view. Also, the histologic pattern of the thymic alteration is distinct from that induced by cortisone and estrogens.

SUMMARY

A new mouse virus has been recovered from laboratory and wild mice. The agent induces a non-fatal disease in infant mice, characterized by acute massive necrosis of the thymic medulla and cortex, granulomatous reaction, and subsequent restoration of essentially normal architecture with scarring. Intranuclear inclusion bodies are produced. Virus may persist in tissues of convalescent mice for many months. Electron micrographs of acutely infected thymuses showed nuclear and cytoplasmic karyoannular particles and masses of parallel fibrils in nuclei.

BIBLIOGRAPHY

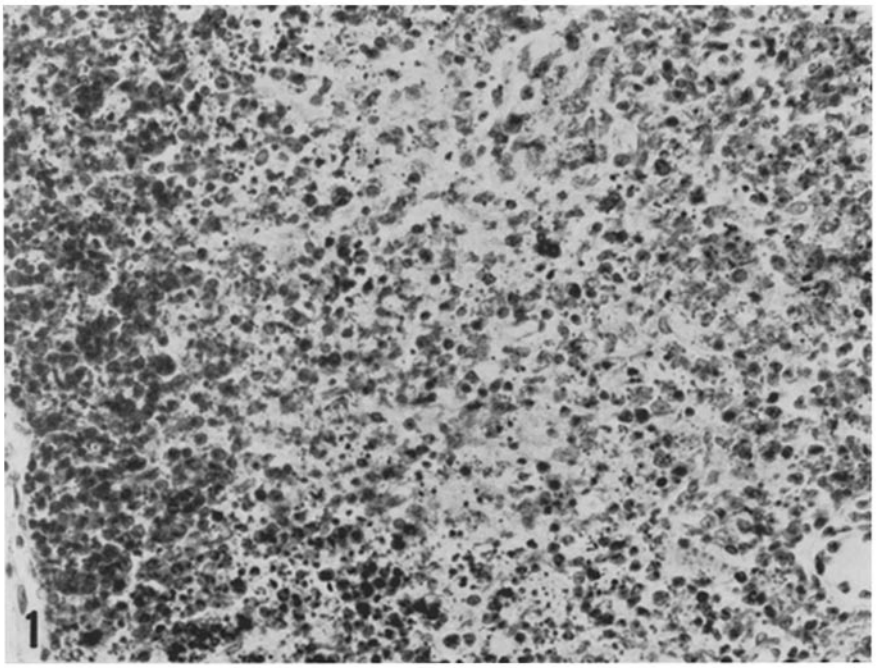
1. Rowe, W. P., The epidemiology of mouse polyoma virus infection, *Bact. Rev.*, in press.
2. Palade, G. F., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95,** 285.
3. Banfield, W. G., Dawe, C. J., and Brindley, D. C., Intracellular and extracellular particles in tissue cultures inoculated with parotid tumor agent (polyoma virus), *J. Nat. Cancer Inst.*, 1959, **23,** 1123.
4. Fawcett, D. W., Electron microscope observations on intracellular virus-like particles associated with cells of the Lucké renal adenocarcinoma, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 725.

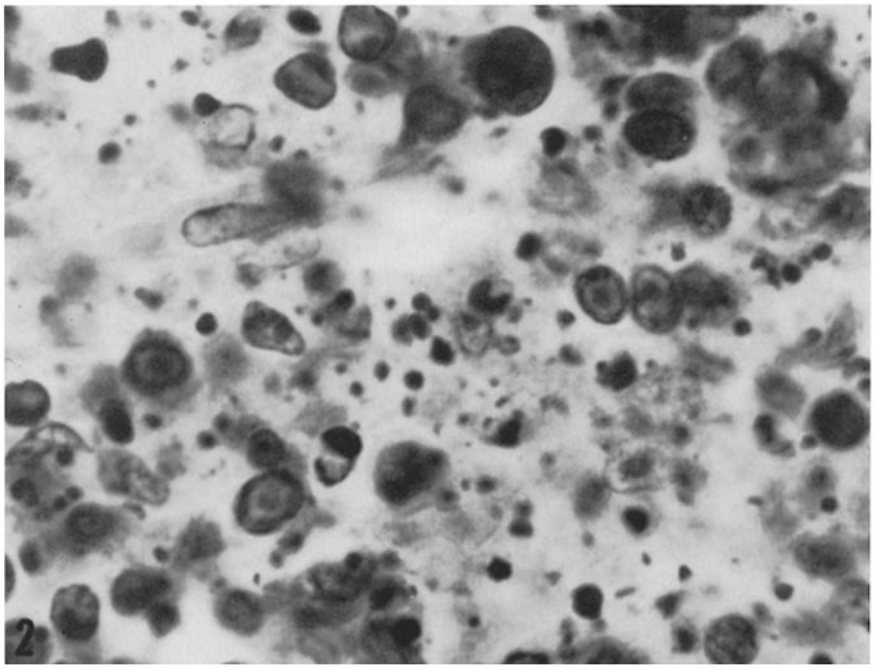
EXPLANATION OF PLATES

PLATE 80

FIG. 1. Early stage of thymic necrosis, showing necrotic material in medulla, cell depopulation and necrosis in cortex, and intranuclear inclusion bodies in thymocytes. XB-4 strain, second mouse passage, 7th day after inoculation. Hematoxylin-eosin. × 295.

FIG. 2. Same section as Fig. 1 at higher magnification to show inclusion bodies. × 1400.



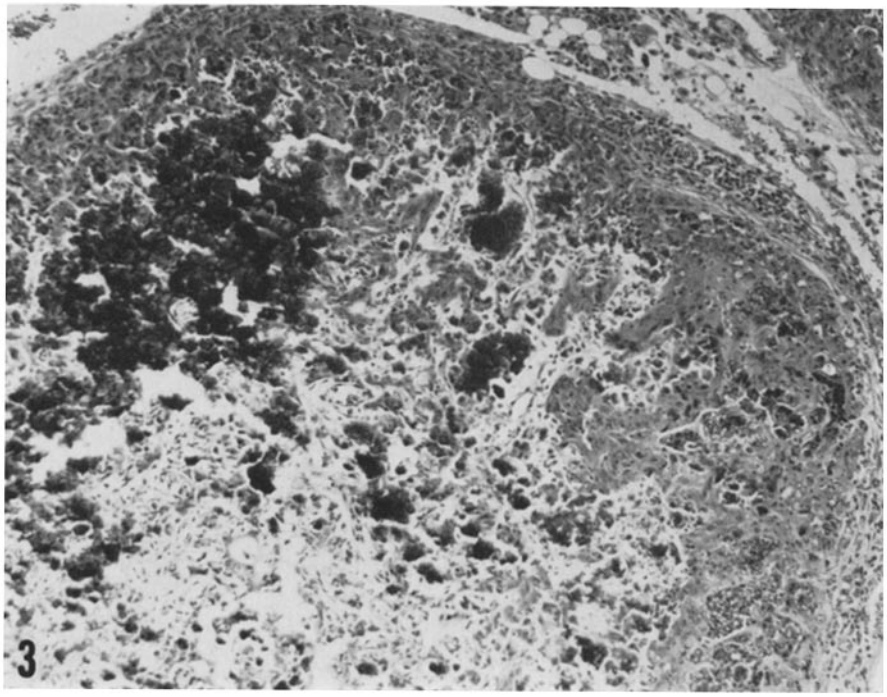


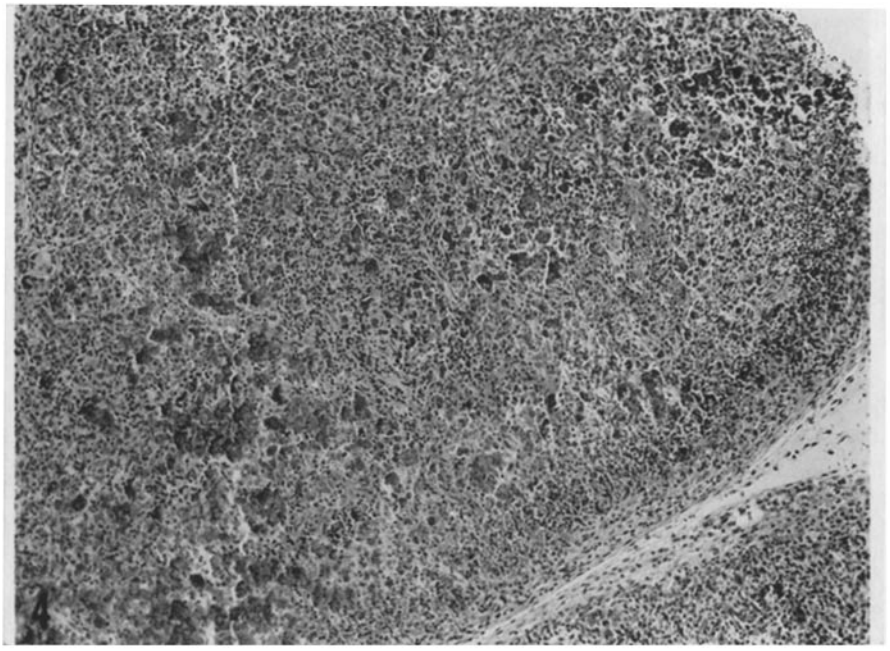
(Rowe and Capps: Mouse virus causing necrosis of thymus)

PLATE 81

FIG. 3. Thymus at height of necrosis. B strain, 14 days after inoculation. Hematoxylin-eosin. × 90.

FIG. 4. Thymus in granulomatous stage. XB-14 strain, second mouse passage, 14 days after inoculation. Hematoxylin-eosin. × 90.



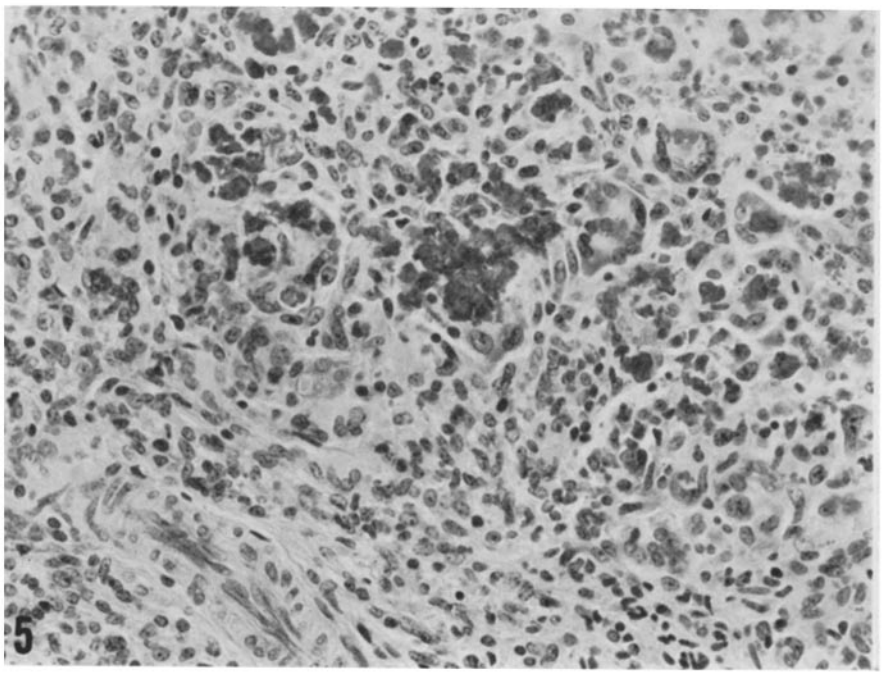


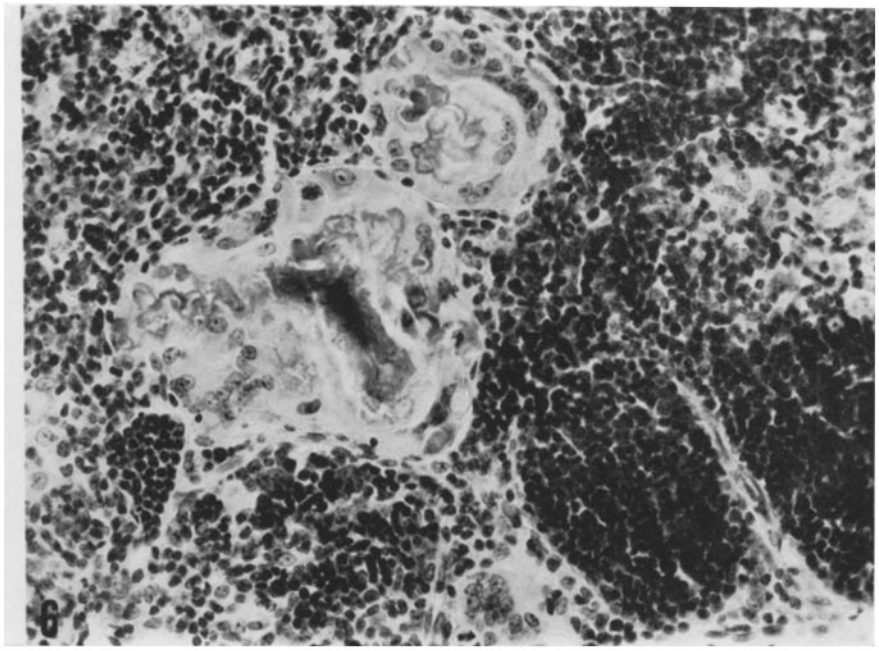
(Rowe and Capps: Mouse virus causing necrosis of thymus)

PLATE 82

FIG. 5. Same section as Fig. 4, at higher magnification to show cellular response with epithelioid and giant cells, and masses of necrotic material. Inclusion bodies are not present. × 295.

FIG. 6. Thymus in late repair stage, showing repopulation of cortex by thymocytes, and calcified amorphous material encapsulated by epithelioid and giant cells. B strain, 61 days after inoculation. Hematoxylin-eosin. × 295.





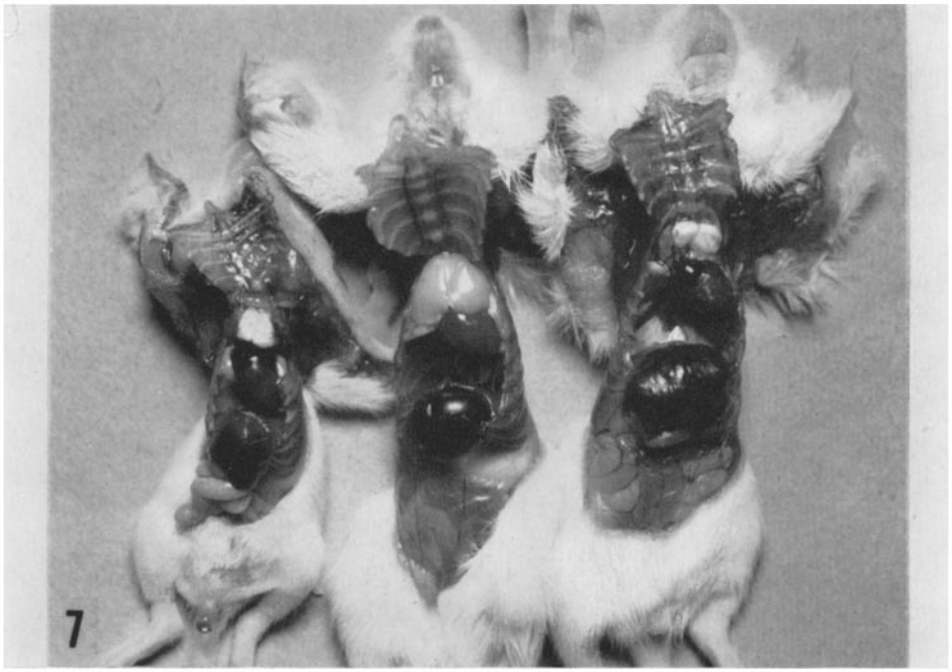
(Rowe and Capps: Mouse virus causing necrosis of thymus)

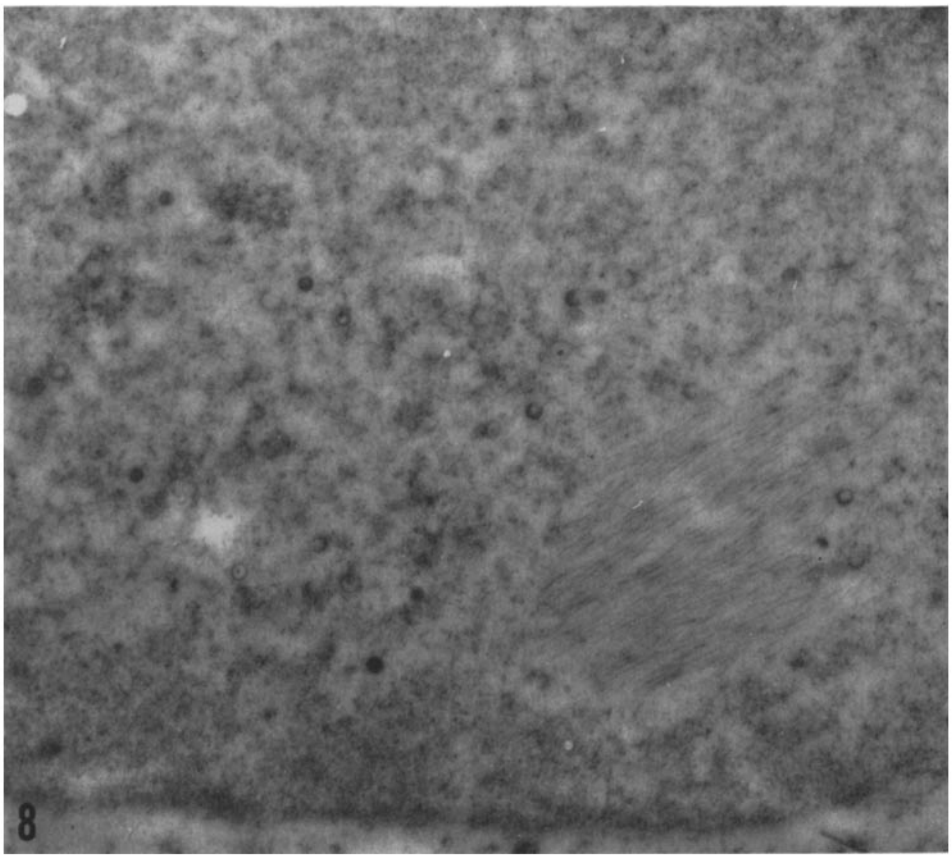
PLATE 83

FIG. 7. Characteristic gross appearance of normal thymus (center) and necrotic thymuses (right and left). Thymus of the mouse on the right has been turned to show the characteristic block-like contours. B strain, 13 days after inoculation. Approximately × 2.

FIGS. 8 to 11. Electron micrographs of the thymus of a suckling mouse 6 days after inoculation with B strain of thymic agent.

FIG. 8. Portion of nucleus with nuclear membrane at bottom. Intranuclear fibrils (lower right) and particles. Here the particles are within an altered area of nucleoplasm. × 23,000.





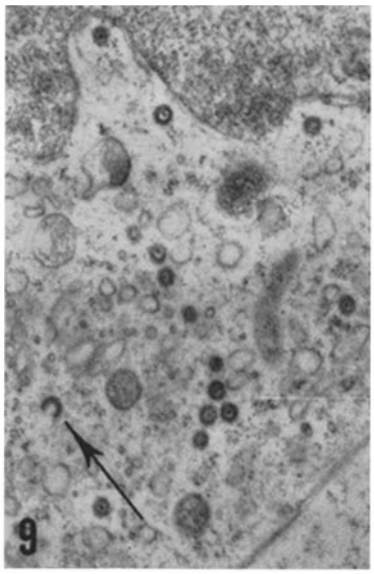
(Rowe and Capps: Mouse virus causing necrosis of thymus)

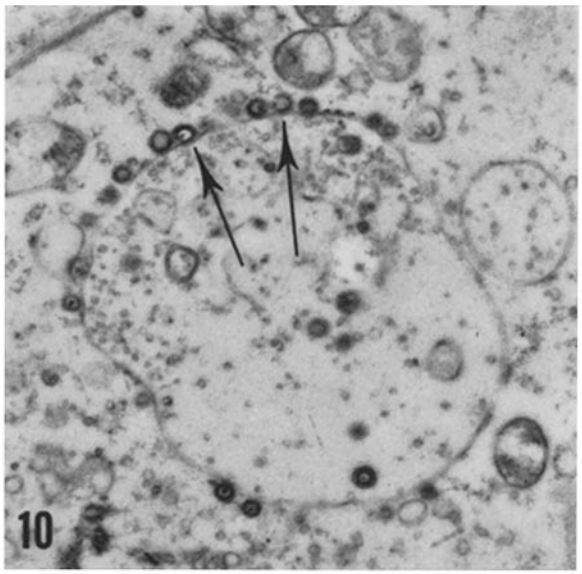
PLATE 84

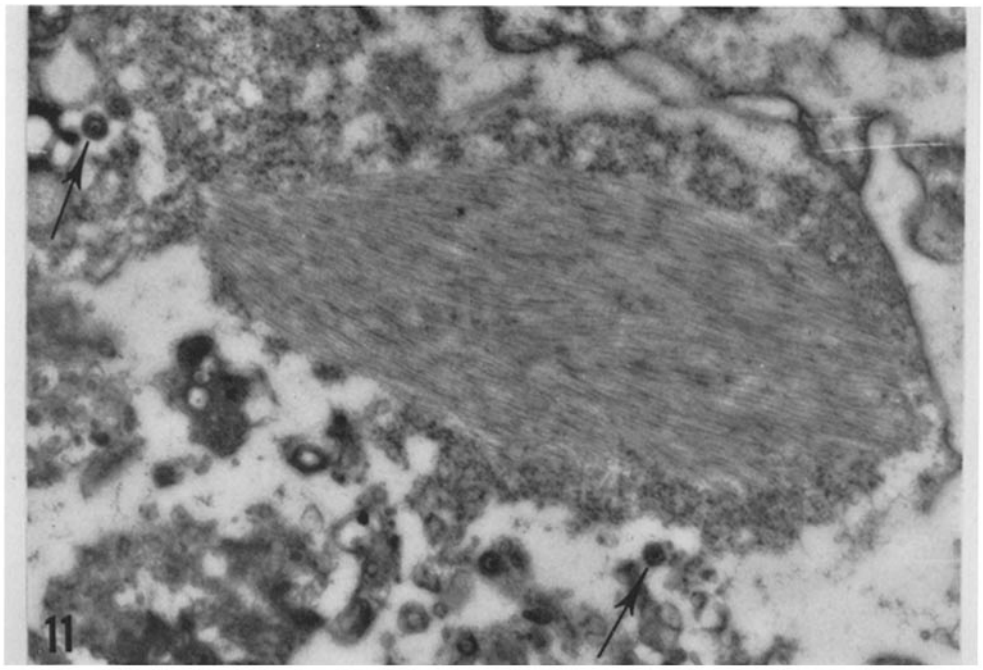
FIG. 9. Portion of cell with segment of plasma membrane at lower right and edge of nucleus at top. The cytoplasm contains thick-walled particles one of which is either broken open or the wall partially formed (arrow). × 18,500.

FIG. 10. Portion of cytoplasm with large vacuole. One particle within the vacuole has an additional membrane. Note the interrupted wall and associated nucleoid of one particle and the thin area of wall opposite the nucleoid in another (arrows). × 18,500.

FIG. 11. Portion of degenerating cell. The granular material about the prominent fibrils is part of the disintegrated nucleus. Note the membrane about the cytoplasmic particles (arrows). × 22,000.







(Rowe and Capps: Mouse virus causing necrosis of thymus)