LESIONS CAUSED IN SUCKLING MICE BY CERTAIN VIRUSES ISOLATED FROM CASES OF SO CALLED NON-PARALYTIC POLIOMYELITIS AND OF PLEURODYNIA*

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Interest has been aroused by the reports of Dalldorf and Sickles (1), Dalldorf, Sickles, Plager, and Gifford (2), and of Melnick, Shaw, and Curnen (3), on the isolation of virus strains from "poliomyelitis-like" outbreaks in various localities. The strains isolated thus far are pathogenic for suckling mice and hamsters. Melnick and Ledinko (4) have recently reported the production of fever and a carrier state with the Ohio type of Coxsackie virus in *cynomolgus* monkeys after oral administration. They state also that chimpanzees though showing no clinical signs of illness develop neutralizing antibodies after ingesting Coxsackie virus. Some strains (Dalldorf's KH and TT) affect principally the skeletal muscles, leaving the central nervous system and other viscera intact. With other strains, lesions, not described in detail, have been found in the brain and heart muscle. The viruses are immunologically unrelated to various poliomyelitis strains, Theiler's FA and GD VII strain of mouse encephalomyelitis, mumps, herpes, LCM, encephalomyocarditis (EMC), louping ill, Venezuelan equine encephalitis, and Newcastle disease. Dalldorf has suggested the name "viruses of the Coxsackie group" as a provisional designation (5).

The occurrence of cases of mild "atypical poliomyelitis" in Worcester and neighboring localities during the summer of 1949, led to an investigation by the Massachusetts State Department of Health. Fecal samples and sera were obtained from a number of these patients, and the attempt made to isolate similar viruses.

From the stool of one of these patients (Powers), an interesting virus pathogenic for suckling mice, has been recovered. Since this agent induces pathologic changes quite different from those described by Dalldorf and Melnick and their coworkers, and indeed unlike those caused by any other known virus,

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it seems of interest to present our observations in some detail. The history of the patient, the method of isolation, and the biologic characters of the virus, insofar as they have been studied, are presented in a separate paper (6). A second strain (Matulaitis) has been isolated from another patient; this also produces lesions in suckling mice which will be briefly described.

Three other virus strains (DeMole, McCarthy, and Kine) which also induce a variety of lesions, have been isolated by Weller, Enders, Buckingham, and Finn, from throat washings obtained during an epidemic outbreak of pleurodynia in 1947. A report of the biologic characters and immunologic relations of these strains is published separately (7). We are including in this report a study of the pathologic changes in suckling mice infected with these agents. We shall present also our findings in mice infected with the Connecticut 5, Ohio R, and High Point strains, kindly sent to us by Dr. Melnick, and shall refer briefly to lesions observed in suckling mice infected with the WS No. 4 strain, and with the EMC and Col. SK strains.

Methods

For pathologic study, the entire carcass was fixed for several days in Bouin's fluid; the thoracic and abdominal cavities were opened by a median ventral incision, and a dorsal incision made, as well as a transverse cut below the medulla, to ensure penetration of the fixative to the central nervous system. After fixation, five transections through the trunk were embedded in paraffin, including all the major viscera and spinal cord at various levels. The brain was carefully dissected out after fixation, and was cut at three levels. Hematoxylin eosin was used as a routine stain, but special stains (Giemsa, Mallory's anilin blue fuchsin, Mahan's stain for myelin, Baker's stain for phospholipids) were employed on occasion. In a few mice, the brain was embedded in gelatin and frozen sections stained with Sudan IV for fat.

LESIONS PRODUCED BY VARIOUS AGENTS

I. Powers Virus.—

Results of Intraperitoneal Injection.—

Heart.—Many of the mice inoculated during the first 5 days of life have shown gross lesions in the form of greyish patches in the wall of the ventricles, readily detected with the naked eye. In some animals they were very conspicuous,—in others, minute examination with the aid of a hand lens was needed for their detection. The auricles were invariably distended with blood. Microscopic lesions of the myocardium were found in most of the mice inoculated during the first 5 days of life with lethal amounts of the virus.

The initial change was a patchy necrosis of the muscle fibers, which lost their fibrillar structure, became swollen and intensely eosinophilic, and tended to fragment into hyaline clumps. The necrosis affected the muscle of the auricles (Fig. 1) as well as that of the ventricular walls and interventricular septum. There was, in the early stages, an acute inflammatory reaction in which polymorphonuclears, lymphocytes, and large mononuclears participated in

varying proportions. Phagocytosis of fragments of the necrotic muscle fibers by mononuclear cells was occasionally seen. The necrotic material and leucocytes rapidly disappeared, giving way to large areas (Fig. 2) composed of the collapsed stroma and spindle-shaped cells with vesicular and often irregular elongated nuclei. It was difficult to decide whether these were the nuclei of young connective tissue cells or of young regenerating myoblasts. The nuclei were indistinguishable from those of the muscle nuclei at the edge of the lesions. It seems probable that the nuclei of the muscle cells may escape destruction, only the contractile portion of the fiber undergoing necrosis, and that in these young animals, if they survive, regeneration of new cardiac muscle fibers may be possible. Some of these fusiform cells with fibrillar cytoplasm resemble embryonic muscle fibers.

It is not certain whether these areas of necrosis in the myocardium would eventually result in extensive fibrous scarring. In a mouse which had been infected by the cerebral route, and in which large areas of myocardial degeneration with calcification were found after 6 days, no new formation of well formed collagenous fibers could be demonstrated with the Mallory anilin blue fuchsin stain. The degenerated fibers and cell detritus took a pale blue stain, but the blue staining material was not fibrillar. Another mouse which was partially protected by human convalescent serum was killed 27 days after inoculation, having shown no symptoms. In addition to discrete calcified deposits in the interscapular fat, the myocardium showed several compact collagenous scars as remains of a previous non-fatal myocarditis.

In a few mice surviving for 5 days or longer, some of the necrotic fibers had become calcified (Fig. 3).

It should be emphasized that the cardiac lesions are patchy rather than diffuse. In none of our preparations was the entire myocardium affected. Nor is the endocardium or pericardium involved, although when the lesions reach the surface, a few free wandering cells may find their way into the pericardial sac. We have seen no lesions of the blood vessels or thrombus formation.

Lungs.—In very young mice inoculated with a large dose of virus, the changes in the lungs are obvious, and a distinctive part of the pathological picture.

There is usually extreme emphysema, with dilatation of the bronchi and ductuli alveolares (Fig. 4 and 5). This affects a large part of the lung, but in some areas, there is more or less complete atelectasis. The alveolar walls, though thinned out in some places, are rather cellular and rigid in others, and may contain an increased number of histiocytic cells. There is, however, no edema or inflammatory exudate in the alveolar spaces, and the bronchi also, apart from flattening of the epithelium consequent upon their dilatation, are not affected. There is thus no clear evidence of any inflammatory process in the respiratory tissues. There is great distention of the pulmonary veins, probably due to the cardiac insufficiency and auricular dilatation. We have noted in several animals, a broad strip of collapsed lung immediately contiguous to the distended right auricle. Mechanical pressure may be a factor in the pathogenesis of the pulmonary lesions, but it is probably not the only one, and the cause of this striking lesion remains unexplained.

Adipose Tissue.—The most interesting and distinctive lesions in this disease are those of the adipose tissue. Especially affected are the lobules of embryonic fat in the cervical region, in the axillae, the interscapular fat pad, and less often the fat of the mediastinum and mesentery. Although the intensity and distribution of the lesions vary from one animal to another, they are present to some degree in virtually every infected mouse.

The lesions are essentially coagulative necrosis of the individual fat cells, which show pycnosis and fragmentation of their nuclei, and breaking up of the cytoplasm into coarse, eosin-staining clumps. There is in the early stages a violent inflammatory reaction, with polymorphonuclears, histiocytes, and small lymphocytes the nuclei of which undergo chromatorrhexis into coarse fragments. Under the polarizing microscope, numerous anisotropic acicular crystals, presumably fatty acid, can be demonstrated in the necrotic fat lobules. The perilobular areolar tissue is edematous, and infiltrated with various types of wandering cells—polymorphonuclears, lymphocytes, histiocytes, and occasional mast cells. As the lesion progresses, polymorphonuclears diminish in numbers, and fibroblasts appear (Fig. 6). The new connective tissue may take on a myxomatous character.

In mice which had survived for a week or more, the lobules of inflamed and necrotic fat became extensively calcified (Fig. 7). This was indicated macroscopically by the chalky white appearance of the fat lobules. The calcium deposits in the early stages were in the form of dust-like particles. These became larger and confluesced into coarse clumps, more or less of the same size and shape as the original fat cells. In a more advanced phase, all suggestion of cellular structure was lost, and the greater portion of the fat lobule, especially the peripheral portion, was represented by large blue-staining plaques of calcified material. Foreign body giant cells appeared about the calcific masses, and even engulfed the smaller deposits.

Although some relatively unaltered fat cells may survive in the center of the calcified lobules, much of the original adipose tissue that is not calcified becomes replaced by young connective tissue.

Several mice which had been inoculated after the period during which they are susceptible to lethal infection, or which had been protected by immune serum, were found to have small localized deposits of calcium, well encapsulated, in the cervical or interscapular fat pads, in the absence of any other lesions.

It is worth noting that the subcutaneous fat, which is inconspicuous in new born mice, was not affected.

Liver.—In mice dying or sacrificed during the 1st week of life, the liver was the seat of active hematopoiesis. This is a normal feature at this age. However, there were present in many of the liver sinusoids, large, more or less oval cells, staining intensely with eosin. The cytoplasm of these cells was sometimes hyaline, sometimes vacuolated, presumably containing lipoid material. The nucleus was eccentric, and usually pycnotic and degenerating. Sometimes the cells were so numerous as to block the sinusoids. They contained no iron-reacting material. Such cells were present in about half the inoculated mice. A more detailed description of these peculiar elements will be given in discussing the liver lesions caused by the DeMole and Conn. 5 viruses.

Pancreas.—Lesions of the pancreas were present in approximately half of the mice in which this organ was included in the sections.

The changes ranged in severity from an acute interstitial edema with cellular infiltration of polymorphonuclears and histiocytes, to almost complete destruction of all the acinal cells. In the latter type of lesion, which was the one most often found, there was extreme disruption of the acinar structure (Fig. 8). The glandular cells could still be identified in places, but they were detached from each other, their nuclei pycnotic and distorted, and their cytoplasm coarsely eosinophilic. Fragments of the broken down cells were ingested by phagocytes. The stroma was edematous, and there was usually a profuse cellular reaction of polymorphonuclears and histiocytes.

In contrast to this destructive change in the acinal epithelium, the ducts and islands of Langerhans appeared to be refractory, and remained intact in the midst of the devastation. The confines of the islands became somewhat indistinct and the finding of occasional mitoses suggested attempts at proliferation of the island cells.

In analyzing a portion of our material, it was discovered that the mean survival period of mice with pancreatic lesions was 4.2 days (17 animals), whereas those in which the pancreas was unaffected lived for 9.4 days (30 animals). This suggests that the pancreatitis is at least a contributing factor in hastening the fatal outcome. The average age at which the animals were inoculated was virtually the same in both groups, so that this was not the determining factor for the shorter survival period in the group with pancreatic lesions.

In most cases in which the pancreas was severely damaged, the intestines were found to contain large masses of amorphous pink-staining material, the nature of which was not further investigated. One may surmise that it is in some way related to the lack of digestive ferments normally supplied by the pancreas.

Central Nervous System.—The earliest lesions in the brain were found in mice sacrificed 5 days after intraperitoneal injection of infected brain tissue.

The first lesions were seen chiefly in the cortical grey matter and brain stem, as necrobiosis of scattered individual ganglion cells, with a sparse polymorphonuclear reaction. The dead cells were sometimes invaded by leucocytes. Chromatin fragments were scattered through these areas. The necrotic ganglion cells were shrunken and had lost their nuclei. The nuclei of the oligodendroglia (?) also became shrunken, lost their chromatin structure, and became converted into dense pycnotic spheres. There was great congestion of capillaries and venules, but no thrombus formation. Many of the small vessels were surrounded by a thin mantle of lymphoid cells.

At 7 days after intraperitoneal inoculation, the lesions were of about the same intensity. The only additional feature was a slight patchy infiltration of the meninges with lymphocytes.

At 9 days, there was a full blown meningoencephalitis with poorly circumscribed lesions scattered through all parts of the brain. Again, there was necrosis of individual cells, but the most striking feature at this stage was the presence of many microglial cells, with pale deformed rod-shaped nuclei. There were no fat granule cells. Many of the venules were surrounded by broad cuffs of lymphoid and adventitial cells, occasionally in mitosis. The meninges were in places quite densely infiltrated with wandering cells, chiefly mononuclear, but amongst them an occasional polymorphonuclear leucocyte.

At 14 days after intraperitoneal injection, there was again a severe encephalitis, but the lesions, except in a few areas, had become localized (Fig. 9). They were now essentially areas of complete necrosis, and many of them had undergone early calcification, as evidenced by the bluish staining of the necrotic ganglion cells and cell processes. There were many distorted nuclei and nuclear fragments, whose derivation was difficult to decipher. The meninges still showed infiltration in places. The cerebellum was not affected.

In a mouse examined at 18 days, the scattered lesions were well circumscribed, and calcification had proceeded further (Fig. 10). They were present in both hemispheres—not in brain stem or cerebellum, and they were not numerous. Over a short stretch, the pyramidal cells of the hippocampus had become calcified, though retaining their contours. About and between these encrusted ganglion cells were the distorted nuclei of microglial elements.

Spinal Cord.—Lesions were not invariably found in the cord, even when the brain was quite severely hit.

When present, the lesions were essentially similar to those in the brain. There were in some sections, moderate mononuclear infiltrations of the meninges. Focal areas of perivascular cuffing, microglial infiltration with occasional polymorphonuclears, and chromatorrhexis of individual nuclei were the principal features. There was no extensive destruction of ganglion cells.

In one mouse killed 16 days after intraperitoneal injection of 7th passage material, a large necrotic focus with calcification, identical with those seen in the brain, was found in the central grey matter (Fig. 11).

Thymus Gland.—The thymus gland was the seat of "acute involution," pycnosis, and fragmentation of small lymphocytes, sometimes with phagocytosis by reticular cells. These lesions may be regarded as non-specific. The lymphoid tissue in general and the spleen showed no changes.

No significant lesions were discovered in other organs or tissues. In none of the experimental animals, were there any lesions of skeletal muscle—a negative finding of importance since it differentiates this disease from that caused by the strains isolated by Dalldorf and Melnick and their coworkers.

We should also like to emphasize the absence of stainable microorganisms, inclusions, or elementary bodies.

Lesions Following Intracerebral Inoculation.—

The pathologic changes in the heart, lungs, adipose tissue, and pancreas were indistinguishable from those produced by intraperitoneal injection. The cerebral lesions, however, were more intense, and presented certain features not seen in animals infected by the intraperitoneal route.

There was found after 3 days, diffuse necrosis of a large portion of the cortical grey matter of the cerebrum. The alveus hippocampi was also affected, but the nucleus dentatus was spared, as were the mid-brain, cerebellum, pons, and medulla. In the affected areas, ganglion cells had disappeared, and pycnotic nuclear remains could no longer be identified. Many small spherical bodies staining greyish blue with hematoxylin were scattered through these areas, and were thought to be free nuclei which had escaped destruction. The ground substance was rarefied and cribriform. The capillaries were engorged with blood cells, but aside from the intense congestion, there were no vascular lesions. There was little or no inflammatory reaction and the meninges were not infiltrated with wandering cells.

In mice surviving for 6 days or longer, much of the necrotic tissue had undergone liquefaction, so that even in the gross, the cortex had a honeycombed appearance. Microscopically, the cortex over the greater portion of the convexity had been replaced by irregular cavities, ragged or sometimes lined in part with flattened glia cells (Fig. 12). Many large phagocytes containing fat globules, cell detritus, or ingested nuclear fragments were present at the margins of the cavities, or floating free in the cavities. There were also many acicular, anistropic crystals presumably of fatty acid. The capillaries and venules were resistant to digestion, and were found intact within the cavities. The cerebellum and medulla were not affected, and spinal cord changes were minimal.

It is evident that Powers virus brings about a colliquative necrosis of the cortical grey matter to a degree not observed with other encephalitogenic agents—at least in weanling or older animals. Further studies are needed to ascertain whether this is a specific pathogenic effect of this particular virus or whether the brain cortex of infant mice is prone to undergo malacic softening in response to a variety of agents. We have not been able to produce similar liquefying lesions in infant mice with the JHM virus, the Col. SK virus, WEE, or Lansing, nor with suspensions of carcass from normal mice. DeMole and Conn. 5 strains which are immunologically related to each other do, however, produce identical effects.

On the other hand, cystic softening of the cortex has been observed in the brain of suckling *rats* inoculated intracerebrally with the JHM virus (8).

Age Susceptibility to Powers Virus.—

It has been pointed out (6) that the Powers virus is lethal only when injected intraperitoneally into 3 day old, or younger mice. By the intracerebral route, death may ensue in 5 day old mice, but rarely in older animals. Weanlings and older mice are entirely insusceptible.

Although fatal infections can be produced only during the first few days of postnatal life, anatomical lesions may be found also in animals inoculated after the 3rd day, and sacrificed at varying intervals thereafter. It is interesting to compare the age susceptibility of different tissues, and although our material is insufficient for a statistical analysis of the problem, certain inferences seem justified on the basis of our present experience.

1. Pancreatic necrosis was found only in animals infected during the first 5 days of life and not thereafter.

2. Myocarditis did not occur in mice inoculated after the 6th day.

3. Lesions of the adipose tissue could be produced up to the 10th day. Several mice, which had shown no signs of disease, and in which no other visceral lesions were present, were found to have massive fat necrosis with calcification.

4. The susceptibility of the central nervous system to the virus also persists until the 10th day.

Further experience will be necessary to establish the validity of these observations, but the probability that different tissues vary in age susceptibility to the effects of this virus is of more than passing interest.

Protection Experiments.—

The general results of exposing the virus before intraperitoneal inoculation to specific immune sera, and to acute and convalescent sera obtained from patient Powers, and other human cases, have been presented in the previous paper (6). Pathologic examination of representative mice from these experiments has shown an excellent correlation between the presence or absence of lesions,

and the results of the neutralization tests. Thus of 19 mice treated with immune serum protecting against 100 to 700 LD_{50}, 17 had normal organs, and in the remaining 2, the lesions were limited to healed calcific deposits in adipose tissue. On the other hand, the two sera (ER and JM) which contained no neutralizing antibodies against the Powers virus, as determined in the usual way, also failed to protect against the development of lesions.

Specific protection against pathologic changes was also well demonstrated by experiments in which the mothers had been immunized by repeated injections of virus, and the new born young given a challenge dose of virus.

TABLE I

Powers Virus: Protection Experiments on Offspring of Vaccinated Mothers

Mouse No.	Virus	LD_{50}	Route	Survival	Lesions	Protective antibodies
				days		
3756	Powers 10^{-4}	700	I.P.*	8	0	6/6‡
3801	Powers 10^{-4}	700	I.P.	21	0	6/6
3803	Powers 10^{-1}	1,000,000	I.P.	15	0	7/7
3825	Powers 10^{-1}	1,000,000	I.P.	14	0	6/7
3802	Powers 10^{-1}	1,000,000	I.C.§	21	+++ (Porencephaly)	2/6
3743	Conn. 5 10^{-3}	1,000	I.P.	3	+++	0/6
3785	Ohio (40 per cent carcass)	?	I.P.	4	+++	0/6

* Intraperitoneal.

‡ Numerator = survivors, denominator = No. inoculated.

§ Intracerebral.

As is shown in Table I, the offspring of mothers vaccinated with the Powers virus were resistant to the homologous virus, but not to the Conn. 5 or Ohio strains.

None of the unchallenged offspring of vaccinated mothers have become spontaneously ill with the disease.

II. MATULAITIS STRAIN.–

This strain, isolated from the stools of another patient in the Worcester area has been maintained by passage of 40 per cent carcass suspensions inoculated intraperitoneally into day old suckling mice. The patient's serum did not neutralize Powers virus.

Only 21 mice have been examined, of which 7 showed no lesions. Of the remaining 14, 4 presented lesions of varying severity in the myocardium, 8 had calcified foci in adipose tissue, 4 showed slight perivascular infiltration about some of the cerebral arterioles, and 3 more intense cortical lesions; 1 had fairly marked *myositis* limited to one psoas muscle. One mouse was killed

10 days after intraperitoneal inoculation of 40 per cent carcass suspension. It had flaccid paralysis of hind legs, and was tremulous and excitable. The muscles of the hind limbs were the seat of very extensive myositis with active regeneration. There was acute rather widespread encephalomyelitis, chiefly perivascular.

No lesions were found in liver or pancreas in any of the animals.

III. DeMole "Pleurodynia" Virus.—

During the late summer and fall of 1947, an epidemic of acute febrile illness occurred in and about Boston. On the basis of symptomatology, the disease was diagnosed as epidemic pleurodynia. A detailed clinical study of 114 cases admitted to the Boston City Hospital was made by Finn, Weller, and Morgan (9). Attempts at that time to isolate a virus were not successful. However, throat washings and acute and convalescent sera were obtained from a number of the patients, and preserved in the frozen state. The demonstration by Dalldorf and Melnick and their respective coworkers that suckling mice are susceptible to various virus strains obtained from stools and throat washings of cases diagnosed as "non-paralytic poliomyelitis" or "aseptic meningitis," and the report by Melnick, that certain of the cases from which such strains had been recovered presented the clinical picture of pleurodynia, prompted a renewed study of the preserved material.

From two of the patients (DeMole and Kine) viruses have been obtained which are highly pathogenic for suckling mice during the first few days of postnatal life. We are concerned here with the pathologic alterations in the infected mice. As will be shown, they resemble those produced by the Powers virus, but with certain striking differences.

Material Studied.—We have thus far completed the study of 80 mice. Of these 37 were inoculated with original throat washings or with varying dilutions up to 10^{-6} of brain suspensions, 31 by the intracerebral route, 6 intraperitoneally. 43 mice received in addition to the infective brain suspension immune mouse serum, acute or convalescent sera from the original patient, or from other cases, or immune mumps serum as control. The mice were almost all 1 day old when inoculated, but one 4 day and one 7 day old mouse were included. The survival period ranged from 2 to 52 days, the animals being sacrificed when moribund, or in the absence of signs, for pathologic examination.

Lesions.—In an occasional animal, it was noted that the liver was unusually pale and moist, and with a hand lens, minute greyish foci were seen uniformly distributed through its substance. The lungs were definitely emphysematous, and frequently an increase of light yellow fluid in the abdominal cavity was recorded. The heart, unlike that of mice infected with Powers virus, showed no macroscopic lesions. In mice dying between the 3rd and 6th day of life, striking microscopic alterations were found in the *liver.*

The liver cord alignment was entirely lost, owing to detachment of the individual liver cells (Fig. 13). These had undergone a peculiar, but almost universal degenerative change, which was best studied in sections stained overnight in buffered Giemsa, and differentiated in colophonium alcohol. Each liver cell in such preparations was composed of a mass of granular eosinophilic cytoplasm; only the periphery of the cell retained its normal basophilic staining. The nucleus was either shrunken, pycnotic, or sometimes fragmented, or it had undergone karyolysis, with loss of the normal chromatin staining. The nucleolus was often oxyphilic.

Throughout the liver, there was, as is normal at this age, very active hematopoiesis. The sinusoids contained large colonies of normoblasts. The nuclei of many of them had been freed, and took a slate grey stain in hematoxylin and eosin preparations. Such isolated, pale staining nuclei were sometimes seen within the cytoplasm of liver cells. Groups of large basophilic cells with deeply staining vesicular nuclei probably represented myeloblastic elements. Megakaryocytes were quite numerous. In addition there were polymorphonuclears, which were concentrated in the vicinity of the large bile ducts (pericholangitis) or occasionally invaded necrotic liver cells (Fig. 14).

The most interesting feature was the occurrence within the sinusoids of large, irregularly oval, rather refractile cells, staining very deeply with eosin. In some, no nucleus could be distinguished—in others, a small shrunken and flattened mass of nuclear material remained at the periphery of the cells. These cells were almost always free, but occasionally one found swollen Kupffer cells, still attached to the wall, but containing in their cytoplasms, masses of ingested, eosinophilic material. It seems probable, therefore, that the free bodies arise from exfoliated Kupffer cells.

In some areas, these peculiar structures were so large and abundant as virtually to block the sinusoids, and possibly produced degeneration of contiguous liver cells by depriving them of access to blood.

We are puzzled as to the source and nature of the material contained within these cells. Frozen sections of formalin-fixed material stained with Scharlach R show small droplets of neutral fat within the liver cells, but the elements under discussion do not stain, or at most take a faint yellowish tinge. They are very pale blue with Nile blue sulfate. With the polarizing microscope, they disclose no doubly refractile material. They contain no stainable iron.

When paraffin sections of Bouin-fixed material are treated with Sudan black, these oval bodies stand out as completely blackened against the paler parenchyma. But they are equally well demonstrated in preparations stained by Mahan's myelin method, in which again, they are completely blackened (Fig. 15). Indeed the reaction to Sudan black and Mahan's stain is indistinguishable from that of myelin. Since the material has passed through ascending grades of alcohol, chloroform, and xylol, does not stain with Sudan III, and is not anisotropic, it is evidently not composed of neutral fat or cholesterol esters. More probably it contains phospholipids. This is confirmed by the use of the Baker stain (10).[1] The material stains bluish black, and is completely dissolved by pyridine. This reaction is given by egg lecithin, brain lecithin, cephalin, and sphingomyelin, but not by steroids or other lipids. Although the staining reactions are those of myelin, it would be unjustified to identify it chemically without further evidence.

As to the origin of the material, we are completely in the dark. The central nervous system at this age is completely devoid of myelin, nor can it derive from the breakdown products of nerve tissue, since it appears before the brain or cord shows any histologic evidence of damage. The inoculum itself cannot be the source of the material, since it is found in animals after intracerebral injection of 0.02 cc. of clear centrifuged inoculum diluted to 10^{-4}. The material

[1] We are indebted to Dr. Helen Wendler Deane of the Department of Anatomy for preparations stained by this method.

is not composed of ingested R.B.C. Nor does it come from degenerated liver cells, since the cells are found not infrequently in livers in which there is little or no change in the hepatic parenchyma.

Whatever the origin and chemical nature of the material in the cells just described may prove to be, their occurrence in the liver appears to be a feature of the lesions produced by the Powers, DeMole, Kine, and Conn. 5 viruses. It should be noted here that similar cells are not found in spleen, lymph nodes, or bone marrow—a point against the idea that this material indicates a general metabolic disorder.

The lesions produced by the DeMole virus may be summarized as those of a diffuse hepatitis, affecting practically the entire parenchyma. They are present in practically all the mice inoculated, whether by the intracerebral or intraperitoneal route, that succumb within the first 4 days after injection.

Pancreas.—A number of the mice have shown severe lesions of this organ. Many of the glandular acini were more or less completely necrotic, the individual cells detached, and intensely eosinophilic, their nuclei pycnotic, fragmented, or missing. The stroma was edematous, and loosely infiltrated with wandering cells, chiefly large mononuclears. The ducts and islands of Langerhans were spared (Fig. 16). The lesions did not always affect the entire organ.

Fat necrosis with calcification was found occasionally, but less frequently than with the Powers virus (Figs. 17, 18).

Central Nervous System.—Cerebral lesions have been found thus far only in mice infected by the intracerebral route, and not in all of these.

Of 4 mice, inoculated with 3rd passage brain and dying on the 3rd day, only one was found to have lesions of the brain, namely a few cortical hemorrhages which might have been traumatic, and a small area of necrosis in the alveus hippocampi. A mouse, which had received convalescent serum with virus and survived for 8 days, showed diffuse necrosis of the cortex and hippocampus, as well as small encephalitic foci in the pons. Three other mice, given a 10^{-6} dilution of 3rd passage infected brain and killed 8, 8, and 13 days after inoculation, showed well marked cerebral lesions, in the form of a diffuse cortical necrosis, with involvement of the overlying meninges (Fig. 19).

A mouse, which had been given 0.03 cc. of a 5 per cent suspension of 1st passage brain suspension and which was killed 14 days after inoculation, was found to have a very marked hydrocephalus, with large areas of liquefaction in the contiguous brain tissue (Figs. 19 and 20). That this lesion was not due to a congenital malformation was evidenced by the inflammatory reaction in the walls of the porencephalic cavities.

It is interesting that with one exception, none of the mice with well developed cerebral lesions had hepatitis or pancreatitis. It appears that when the disease affects liver or pancreas, death ensues before the lesions of the brain develop.

Spinal Cord.—Lesions of the spinal cord were found in most of the mice with encephalitis. They were confined to the grey matter. Necrosis and loss of ganglion cells, microglial infiltration and interstitial edema, capillary congestion,

and occasionally slight mononuclear infiltration of the meninges were the main features.

Thymus.—This organ, when included in the sections, invariably showed "acute involution," with extreme pycnosis, fragmentation, and altered staining of the small thymocytes. The lymphoid tissues in general were not affected.

No lesions were found in the *skeletal muscles.*[2] This is a negative finding of importance in distinguishing this virus from those reported by Dalldorf, Melnick, and their coworkers. The strains isolated by them were described as producing a generalized myositis. *Myocardial lesions* were also absent or minimal. When present, they consisted of hyaline necrosis of small groups of fibers in left ventricle or septum without inflammatory reaction. The *lungs* were often emphysematous, or partly atelectatic, but showed no inflammatory changes. No significant alterations were found in other viscera. Subcutaneous edema, however, was noted in a few animals. In summary, the most distinctive lesions thus far noted have been diffuse hepatitis, pancreatitis, and encephalomyelitis.

IV. Kine Virus (Pleurodynia).—

This was isolated from throat washings collected during the 1947 pleurodynia outbreak and is immunologically related to the DeMole virus.

We have examined sections of 28 mice, inoculated when 1 day old with original throat washing material, or with brain suspensions from 1st, 3rd, and 4th passage animals, diluted 10^{-2} and 10^{-4}. 27 were injected intracerebrally, 1 by the intraperitoneal route.

Lesions of the liver identical with those caused by the DeMole strain, were found in 7 mice, fat necrosis in 7, encephalitis in 14, and myelitis in 3. The myocardium was affected in 4; the lungs were emphysematous and atelectatic. There were no lesions of the skeletal muscles.

The one mouse which had been inoculated intraperitoneally showed severe necrosis of the pancreas, but this organ was normal after intracerebral injection. Included are 8 mice receiving convalescent serum from 2 patients of the same outbreak. 3 showed lesions in brain and adipose tissue. 1 also had myocarditis.

V. McCarthy Virus (Pleurodynia).—

This strain also was isolated from throat washings of a patient with epidemic pleurodynia, but has not been studied immunologically. 12 mice, inoculated intracerebrally with original throat washing material, or with suspensions of infected mouse brain in dilutions of 10^{-2} to 10^{-6}, have been studied. The mice were killed at periods of from 2 to 14 days after inoculation.

[2] One mouse constitutes an exception to this statement. It had been inoculated at the age of 1 day with 3rd passage material plus Bell convalescent serum 1:10. When sacrificed on the 6th day, it was found to have slight encephalomyelitis, but no visceral lesions. The psoas muscle on each side had degenerated, and there was moderate histiocytic reaction. No other muscles were affected.

Myocardial lesions were found in 3 mice, in one of which there was extensive calcification. In only one, dying on the 5th day, was there severe hepatitis, and in none was the pancreas involved. 3 animals had lesions of fat tissue, 2 of which were calcifying. In 8 of the 12, lesions were present in the brain; they were very extensive in the 4 mice which survived for 14 days, and in one of these there were large areas of porencephaly. The spinal cord was unaffected, and no lesions were found in the skeletal muscles. It would seem on the basis of this limited material, that the heart is more severely and frequently affected than with the DeMole strain, but that the liver lesions are of less frequent occurrence, and that the pancreas escapes entirely. However, the pathogenicity for mice of this agent is low, and attempts to adapt the virus further have not been carried out.

VI. Connecticut Strain of C Virus.—

Dr. Melnick has very kindly sent us three of the strains isolated by his group from cases of "non-paralytic" poliomyelitis. One of these, designated as Conn. 5, and obtained from pooled feces, has been passaged through several litters of suckling mice, 1 to 3 days of age, and 29 of these have been used for study of the pathology. The signs of disease in the living animals were indistinguishable from those caused by the Powers and DeMole viruses.

The lesions have proved to be identical with those caused by the DeMole virus with which it is immunologically closely related.

There have been found myocarditis, emphysema, hepatitis, pancreatitis, fat necrosis with calcification, and encephalomyelitis. In one mouse inoculated with 10^{-3} brain suspension intracerebrally, and sacrificed after 7 days, there was liquefaction necrosis of the cortex such as was found in a number of mice infected with the Powers and DeMole viruses.

We have found no significant lesions of the skeletal muscles. This was unexpected, since myositis has been regarded as a feature of infection with the Conn. 5 strain (11). Because of this discrepancy, and to exclude the possibility that one of our strains had been inadvertently introduced, the experiments were repeated with a second sample (Conn. 5-M17) generously sent by Dr. Melnick. This was inoculated intraperitoneally into nine 1 day old mice in dilutions of 10^{-3} and 10^{-6}. These were sacrificed when moribund at 4 and 5 days. Again careful examination of the limb and trunk muscles disclosed no significant lesions.

The possibility that different strains of mice may react in different fashion to this agent, should be investigated. We have recently examined 3 mice obtained from an outside source. These were inoculated intraperitoneally with 10^{-5} carcass suspension of Conn. 5 material. They became ill and were killed 4 and 6 days later. Two of the 3 had definite and fairly extensive necrosis and fragmentation of the hind limb muscles. This exceptional finding suggests that differences in strain susceptibility may underlie the apparent contradiction between our observations and those of the Connecticut investigators.

Another possibility is that the discrepancy with regard to the occurrence of myositis may be due to differences in the conduct of the experiments. Dr. G. C. Godman has kindly submitted a series of preparations of mice inoculated with the Conn. 5 strain, and we can confirm his observations as to the frequent presence of myositis in his material. From him, we have learned that the mice were infected on the 4th or 5th day of life, and that the average period before signs of disease appeared was about 7 days. In our experiments, the mice were almost always inoculated on the 1st day after birth, and the survival period was considerably shorter. Whether this difference in experimental technique adequately explains the absence of muscle lesions in our material, will have to be determined by further study.

VII. Ohio R Strain of C Virus.—

This strain, for which we are also indebted to Dr. Melnick, was isolated from the stools of one of four patients diagnosed as poliomyelitis. Its serologic relationships have been described by Melnick, Ledinko, Kaplan, and Kraft (12). The virus is lethal for 1 day old mice. 18 such animals have thus far been studied by us, their survival time after intraperitoneal injection ranging from 4 to 12 days. Myocarditis, emphysema, fat necrosis, and encephalomyelitis, and in one animal only, pancreatitis and diffuse hepatitis, were found. In contrast to the mice infected with Powers, DeMole, and Conn. 5 viruses, lesions of the skeletal muscle were encountered in 5 of the 18 animals. Since these conformed in all respects to those recorded by Dalldorf and by Melnick and their coworkers, a detailed description is unnecessary.

VIII. High Point Strain of C Virus.—

This strain was obtained from sewage during the summer of 1948, at High Point, North Carolina, and was sent to us by Dr. Melnick. 14 suckling mice, infected by the intraperitoneal route, have thus far been studied. 13 of these showed striking lesions of the skeletal muscles, both of the trunk and extremities. Involvement of the diaphragm and intercostal muscles probably explains the accompanying pulmonary atelectasis. There were no lesions of the myocardium, liver, pancreas, adipose tissue, or central nervous system.

The pathologic effects thus offer a basis for sharply separating this agent from the other strains studied. Whether this will hold on further passages, remains to be determined.

IX. WS No. 4 Strain of C Virus.—

This virus was obtained from pooled feces secured in Winston-Salem, North Carolina, in 1948.

Two mice, fixed in Bouin's fluid, were received from Dr. Joel Warren. They had been inoculated intraperitoneally with 10^{-3} and 10^{-5} brain passage suspensions, and were sacrificed after 2 and 4 days respectively.

In both mice, the pancreas was almost completely necrotic. There was found also widespread necrosis of the skeletal muscles of the trunk and extremities, with little or no inflammatory reaction. The other tissues and viscera were not abnormal, save that in the 2 day old mouse, the epithelium of the convoluted tubules of the kidney had undergone a peculiar lytic process, with complete loss of nuclear staining. This was apparently not due to postmortem autolysis, since the tissue had been fixed immediately after death. Additional material will be needed to interpret the significance of this unusual lesion.

X. EMC (Encephalomyocarditis) Virus.—

Dr. Joel Warren, of the Department of Virus and Rickettsial Diseases of the Army Medical Department Research and Graduate School, Washington, has also sent us for study, Bouin-fixed material of five suckling old mice, sacri-

ficed 24 and 48 hours after intraperitoneal injection of 10^{-3} and 10^{-5} suspensions of the Florida strain (86B, 86D) of the EMC virus, which was originally isolated by Helwig and Schmidt (13) from cases of spontaneous myocarditis in a gibbon and a chimpanzee.

Although this virus has been shown by Dalldorf to be immunologically unrelated to his strains of Coxsackie virus, and differs also in being pathogenic for older mice, hamsters, and guinea pigs, it nevertheless produces in suckling mice, widespread necrotizing lesions of the skeletal muscles, particularly of the trunk, indistinguishable from those described by Dalldorf and Melnick and their coworkers as characteristic of certain strains of Coxsackie virus.

The muscle fibers are swollen, hyaline, fragmented, separated by edema, and there is a variable amount of polymorphonuclear and histiocytic response (Fig. 21). The limb muscles in the few animals examined, were less affected than those of the abdomen, thorax, and diaphragm, but this may not prove true of animals surviving for a longer period. Myocardium, liver, pancreas, adipose tissue were not affected. In one of the five animals, sacrificed after 24 hours, a single focal area of necrosis, with polymorphonuclear reaction and regional infiltration of the meninges, was found in the cerebral cortex. The spinal cord showed no lesions.

It will be desirable to confirm these observations with more material, yet it seems evident at this time that the production of myositis in suckling mice may be accomplished with other viruses than those of the Coxsackie group. Indeed Dalldorf (2) has found lesions in the spinal muscles in hamsters infected with MM virus.

XI. Columbia SK Virus.—

This virus has been shown by Rustigian and Pappenheimer (14) to produce rapid and extensive necrosis of muscle when injected intramuscularly into weanling mice. The muscle lesions remain limited to the inoculated limb, although the central nervous system soon becomes infected.

We have examined 6 suckling mice inoculated intraperitoneally with 10^{-5} dilution of infected brain suspension. They were killed 2 and 3 days later when moribund. Acute necrotizing lesions were found in the muscles in some mice limited to thoracic and abdominal muscles, in others affecting also the diaphragm and limb muscles. Myocardium, lungs, liver, pancreas, and adipose tissue were not involved, but early necrotizing lesions of the brain were seen in 4 of the 6.

The Col. SK virus, like the EMC with which it is serologically related, is thus capable of exciting myositis in new born mice.

DISCUSSION

The lesions produced by the Powers, DeMole, and Conn. 5 agents in suckling mice are in some respects quite unlike those observed with any other viruses. Thus the massive necrosis of acinal pancreatic tissue, which is found in mice inoculated during the first 5 days of life, is a unique feature. Mumps virus, as is

well known, may cause clinical symptoms of pancreatitis; though little is known of the underlying pathology, it is obvious that the symptoms caused are not due to a destructive lesion such as we have described. One may surmise that the loss of external pancreatic secretion in these new born animals, may have far reaching physiological effects, and may be largely responsible for early death.

The susceptibility of the pancreas to the Powers virus appears to be limited to the first 5 days of postnatal life. At least, we have found no lesions of this organ in mice infected on and after the 6th day. The resistance of the islands of Langerhans and ductal tissue to the destructive effects of this agent, is interesting.

The inflammatory and necrotizing lesions of the embryonic fat lobules, with their tendency to rapid calcification, are also most interesting and distinctive. That they are not secondary to liberation of lipolytic ferments from the degenerating pancreas is certain, since they are frequently found in the absence of any lesions of this organ. The virus can readily be recovered from the ground-up adipose tissue when dissected free of other tissues. This, however, is not conclusive evidence of affinity for fat tissue, since virus has been recovered from heparinized blood.

The underlying chemical reactions involved in the fat destruction remain to be investigated—particularly, the problem as to whether the virus, or a toxic product, may have fat or lipid-splitting activity, or whether it merely activates preexisting lipolytic enzymes. Whatever the mechanism, it appears that the susceptibility of the embryonic adipose tissue to these agents is very great, and that it persists when most of the other organs and tissues have become refractory.

The production of lesions in the adipose tissue by other viral agents has, to the best of our knowledge, not been previously observed. Duran-Reynals (15) has fully described the outbreak of a disease in rabbits, characterized by massive necrosis of intraabdominal fat. Although he strongly suspected a viral etiology, the occurrence of similar lesions in controls injected with testicular extract or Ringer's solution made it impossible for him to bring definitive proof.

The disease of mink known as pan-steatitis, non-supperative panniculitis, or "yellow fat disease" is characterized by focal inflammatory lesions scattered through adipose tissue (16). Through the courtesy of Dr. Davis, and of Brigadier General Raymond Dart of the Armed Forces Institute of Pathology, we have had opportunity to compare the lesions in this disease with those produced in suckling mice by these viral agents. There is little resemblance. The etiology of the mink disease is not known, although a nutritional disturbance has been assumed.

The cause of relapsing febrile nodular non-suppurative panniculitis in man (Weber-Christian disease) is also unknown, although the possibility of a viral

infection has been discussed. So too, nothing is known as to the etiology of the very rare fat necrosis of the newborn (17). The lesions as described in these two diseases bear little resemblance to those in the suckling mice.

It is interesting that Antopol (18) has recently described the occurrence of necrosis and calcification in the hibernating gland of older mice injected with massive doses of cortisone.

The liver lesions which are especially severe in mice infected with the DeMole and Conn. 5 viruses also offer interesting and distinctive features. Most characteristic are the many oval cells in the sinusoids which are filled with material not chemically defined, but giving the staining reaction of myelin or phospholipids. We have not encountered similar cells in other viral infections of mice. Unfortunately, we have been able to arrive at no conclusion as to their nature or significance.

Lesions of the myocardium, often of great severity and extent, have been found in a fair proportion of mice inoculated with the Powers and Conn. 5 viruses but very exceptionally with the DeMole virus. Although they constitute an important part of the general picture, they are by no means pathognomonic for this particular group of viruses. Spontaneous cardiac lesions of the "rheumatic" type have been seen in otherwise normal mice (19, 20). Such spontaneous lesions have not been noted in our stock.

Myocarditis, chiefly interstitial and unaccompanied by massive necrosis of muscle, is a feature of the disease caused by the EMC, Mengo, MM, and Col. SK viruses, but the character of the lesions as well as the different age susceptibility and lack of immunologic relationship distinguishes the myocarditis of suckling mice from that caused by the encephalomyocarditis group of viruses. In a limited number of suckling mice inoculated with EMC and Col. SK, and surviving for only 2 days, the myocardium has not been affected.

The extreme pulmonary emphysema and bronchial dilatation—often combined with areas of complete atelectasis—are difficult to explain, although they are an obvious and striking feature. Inflammatory lesions are conspicuously absent, and there is nothing to suggest partial bronchial obstruction by secretion or exudate. Whatever its pathogenesis, it seems logical to ascribe to this condition of the lungs the cyanosis which often marks the terminal stages of the disease.

The lesions of the central nervous system produced by the Powers, DeMole, and Conn. 5 viruses are essentially identical and exhibit certain unusual characters. The damage caused by these viruses is especially severe in the cortical grey matter. When the necrosis of the tissue is extensive, liquefaction follows. In a number of mice surviving for 10 days or more, the entire cortex over the convexity was converted into a series of intercommunicating ragged cysts, bridged by vascular strands. In some of our preparations there has been complete destruction of the tissue between the meninges and the ependymal lining

of the ventricles. Leakage or rupture of the fluid-filled cavities into the ventricles may explain the frequently associated hydrocephalus.

Extensive colliquative necrosis of cortical tissue is certainly an unusual, if not unique feature of viral encephalitis. However, we have observed similar, if less extreme liquefying lesions in infant white rats, inoculated with the JHM virus.

Some of the surviving mice have shown focal necroses with calcification in various parts of the brain, and very occasionally, in the grey matter of the spinal cord. Identical lesions of the focal type have occurred with all three virus strains.

The pathologic alterations noted in other organs are few and relatively unimportant. The thymus was the seat of very active destruction of small thymocytes—often with chromatorrhexis and chromatolysis. This "acute involution" is probably not a specific change. Submucous hemorrhages and edema of the urinary bladder, also are probably not significant. There have been no lesions of the gastrointestinal tract, although we have noted in a number of animals that the intestinal contents consisted of masses of an intensely eosin-staining material the nature of which we have not determined. It does not appear to be altered blood. There have been no lesions of the kidneys, lymphatic glands or spleen, bone marrow, endocrine organs (thyroid and adrenals), gonads, or bones, all of which have been routinely examined.

The comparative distribution of lesions produced by the various strains is shown in Table II.

The number of plus signs indicates roughly the frequency and relative intensity of the lesions. A numerical comparison of incidence is of little value, because of varying dosages and routes of administration.

It is evident from these studies that pathogenicity for suckling mice is common to a number of virus strains, isolated from different source materials and geographic locations. Some have derived from acute febrile illnesses, without paralysis, but usually with spinal fluid pleocytosis, and occurring in sporadic outbreaks (Powers, Conn. 5, Ohio R, Matulaitis); some have been associated with symptoms designated as those of epidemic pleurodynia (DeMole, Kine, McCarthy); one strain has been obtained from sewage (High Point); another from cases of spontaneous myocarditis in a gibbon and chimpanzee (EMC); still another (Col. SK) is of questionable human origin, but immunologically related to the Mengo, MM, and EMC strains. It is obvious that the production of lesions in new born mice is not of itself sufficient to characterize a homogeneous group of agents.

On the basis of pathology, the different strains which we have examined may be tentatively separated into four fairly well defined classes:—

I. Strains having a predilection for skeletal muscle, and producing no noteworthy changes in other organs or tissues (High Point). Dalldorf's group A

probably falls within this class, as do the strains described by Armstrong *et al.* (21).

II. Strains without effect upon skeletal muscles, but producing profound changes in myocardium, liver, pancreas, adipose tissue, and central nervous system (Powers, DeMole, Kine, McCarthy, Conn. 5).

III. Strains producing lesions in both skeletal muscle and viscera (Ohio R, WS No. 4, Matulaitis).

IV. Strains producing lesions in muscle and central nervous system (EMC, Col. SK), but no visceral lesions.

This grouping is admittedly provisional, and further experience with other strains of mice differing in susceptibility from those of our laboratory stock may

TABLE II

Comparative Pathology

Virus	Powers	Matulaitis	De Mole	Kine	McCarthy	Conn. 5	Ohio R	High Point	WS No. 4	EMC	Col. SK
No. of mice	86	21	80	16	12	39	18	14	2	5	6
Heart	+++	+	±	0	+++	++	++	0	0	0	0
Lungs	+++	+++	+++	++	+	+	±	++	0	0	0
Liver	+	0	+++	+++	+	+++	+++	0	0	0	0
Pancreas	+++	0	+++	+++	0	+++	+	0	+++	0	0
Adipose tissue	+++	+	++	+	++	±	+++	0	0	0	0
Skeletal muscle	0	+	0	0	0	0	++	+++	+++	+++	+++
Brain	+	+	+	++	+++	+++	+++	0	0	±	+++
Cord	+	±	+	+	0	+	++	0	0	0	+

The number of plus signs indicates roughly the frequency and relative intensity of the lesions. A numerical comparison of incidence is of little value because of variations in dosage and route of administration.

lead to a revision. Reference has been made in the text to the occurrence of muscle lesions when the Conn. 5 virus is inoculated into mice from another source, although it has never been observed in our own laboratory stock. Moreover, we do not wish to imply that strains within each group are identical. Indeed there is already good evidence that such is not the case. Thus, in group II, the Powers virus is not immunologically identical with the DeMole and Conn. 5 strains.

Even within the same group, different strains have shown diversity with regard to the frequency with which a particular organ is affected, and with regard to the severity of the lesions. Comparison is rendered difficult, however, because of individual and litter variation in susceptibility, and because of differences in dosage and route of inoculation. It will require a large number of observations under strictly standardized conditions to establish the validity of these pathological differences on a sound statistical basis.

SUMMARY

A study has been made of the lesions produced in suckling mice by the following viruses: Powers, Matulaitis, DeMole, Kine, McCarthy, Conn. 5, Ohio R, High Point, WS No. 4, EMC, and Col. SK.

Pathologic alterations have been found in myocardium, lungs, liver, pancreas, thymus, brain and spinal cord, adipose tissue, and skeletal muscles.

A comparison of the lesions produced by the individual strains has disclosed certain differential features which are discussed in detail. Within the group of so called Coxsackie viruses, myositis has not proved to be a constant finding, and it may occur in suckling mice infected with other types of virus.

Addendum.—Since this manuscript was submitted for publication, Dalldorf (*Bull. New York Acad. Med.*, 1950, **26,** 329) has described the pathological changes produced in infant mice by two groups of Coxsackie viruses. The lesions found by him in group A infections showed only a generalized destruction of striated muscles. The lesions observed with group B infections included focal areas of myositis, and changes in the central nervous system and adipose tissue comparable to those herein described.

We are greatly indebted to Dr. J. L. Melnick for putting at our disposal strains of Conn. 5, Ohio R, and High Point virus; and to Dr. Joel Warren for mice infected with EMC and WS No. 4 strains. We wish also to thank Miss Sheila Richardson and Miss Thelma LeBlanc for technical assistance.

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EXPLANATION OF PLATES

PLATE 5

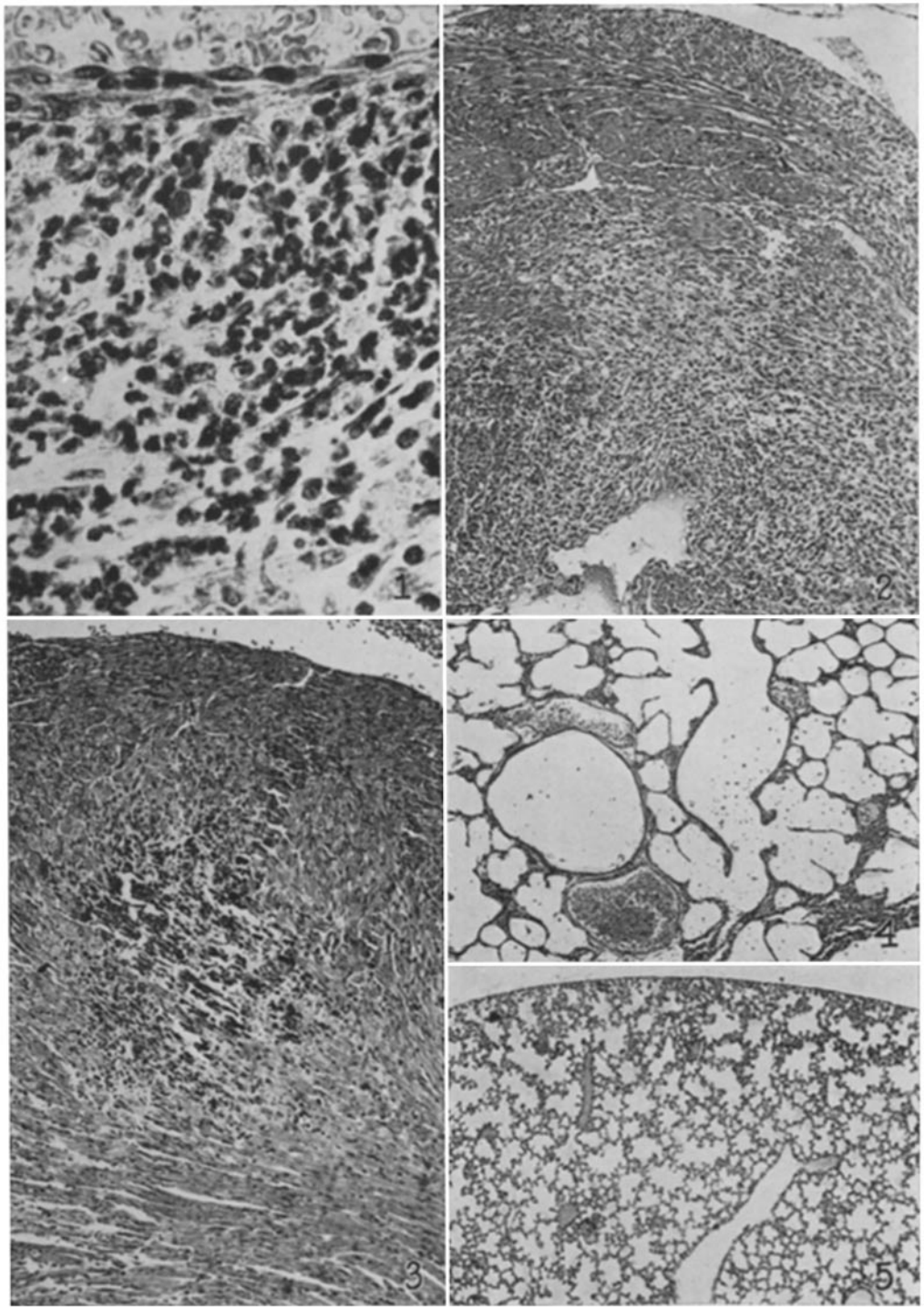
FIG. 1. Mouse 3301. Inoculated intraperitoneally at age of 1 day with 3rd passage brain material of Powers virus. Killed on 5th day. Auricular wall showing acute myocarditis. Complete degeneration of muscle fibers, profuse infiltration of leucocytes. Hematoxylin and eosin. × approximately 750.

FIG. 2. Mouse 3259. Inoculated intraperitoneally at age of 2 days with feces suspension (Powers). Moribund at 5 days. Heart: large area in which the muscle fibers are replaced by spindle-shaped cells, with scattered leucocytes. Hematoxylin and eosin. × approximately 95.

FIG. 3. Mouse 3570. Inoculated intracerebrally at age of 4 days with 11th passage of Powers virus (ground carcass). Killed on 6th day. Focal areas of myocardial necrosis with early calcification. Hematoxylin and eosin. × approximately 95.

FIG. 4. Mouse 3273. Inoculated intraperitoneally at age of 1 day, with 1st passage brain material of Powers virus. Killed on 7th day. Acute emphysema. Extreme dilatation of bronchi and alveoli. No inflammatory changes. Hematoxylin and eosin. × approximately 40.

FIG. 5. Mouse 3295. 10 days old. Normal lung for comparison with Fig. 4. Hematoxylin and eosin. × approximately 40.



(Pappenheimer *et al.*· Lesions in suckling mice)

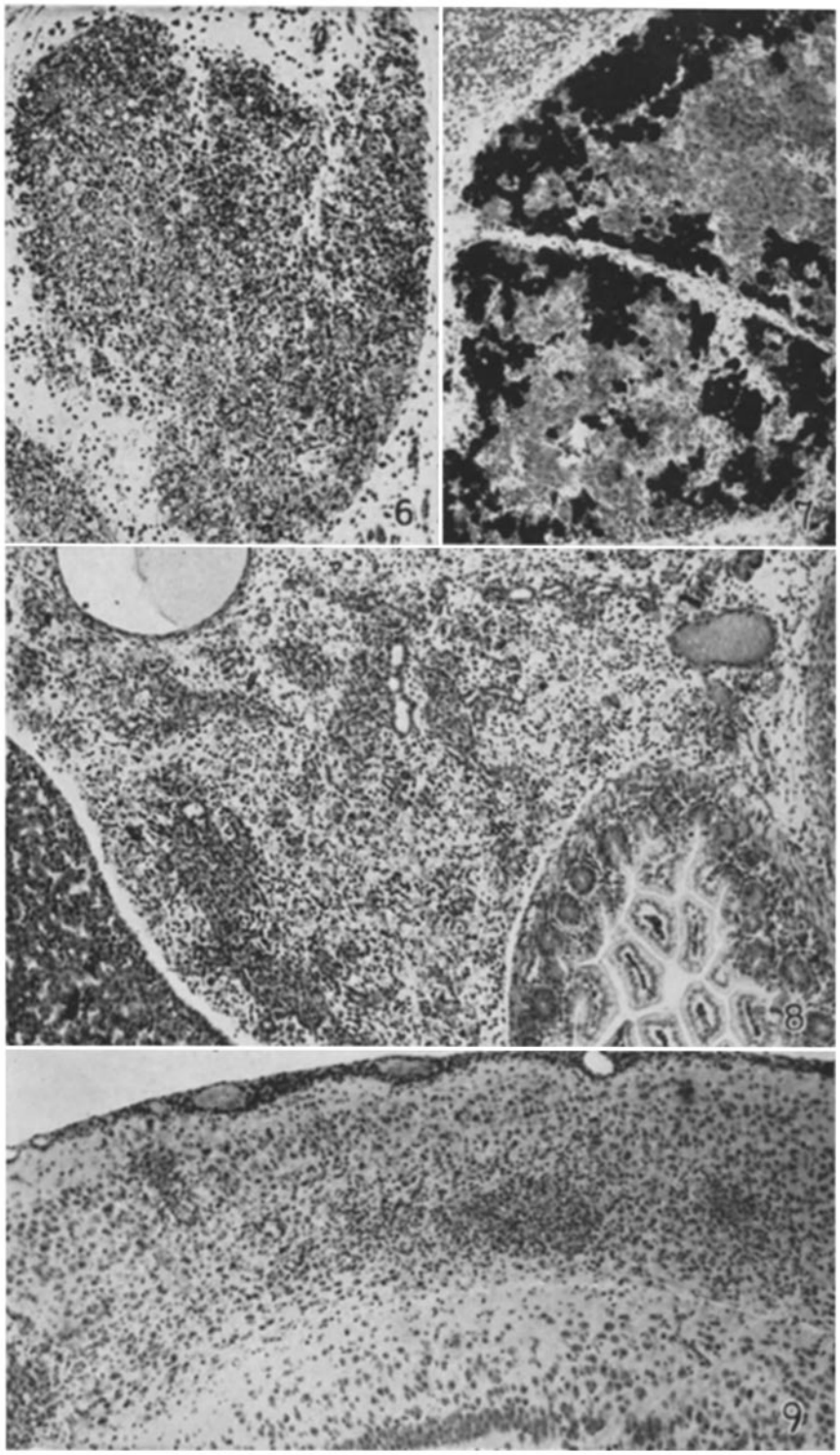
PLATE 6

FIG. 6. Mouse 3262. Inoculated intraperitoneally at age of 2 days with fecal suspension containing Powers virus. Killed on 7th day. Embryonic fat lobules in cervical region showing necrosis of fat cells and acute inflammatory reaction. Hematoxylin and eosin. × approximately 105.

FIG. 7. Mouse 3570. Inoculated intracerebrally at age of 4 days with 11th brain passage material of Powers virus. Killed on 6th day. Fat lobules in cervical region showing extensive necrosis with calcification. Frozen section—Hematoxylin and eosin —Sudan IV—von Kossa × approximately 95.

FIG. 8. Mouse 3491. Inoculated intraperitoneally at age of 2 days with 10th passage brain material (Powers virus). Killed when moribund 2 days later. Pancreas—almost complete necrosis of acini with acute inflammatory reaction. Note persistence of ducts and islands of Langerhans. Hematoxylin and eosin. × approximately 100.

FIG. 9. Mouse 3318. Inoculated intraperitoneally at age of 3 days with 2nd passage brain material (Powers virus). Ataxia and tremors first noted on 11th day. Killed after 14 days. Brain—diffuse cortical encephalitis with inflammatory reaction concentrated in several areas. Lymphocytic infiltration of meninges. Hematoxylin and eosin. × approximately 95.



(Pappenheimer *et al.*: Lesions in suckling mice)

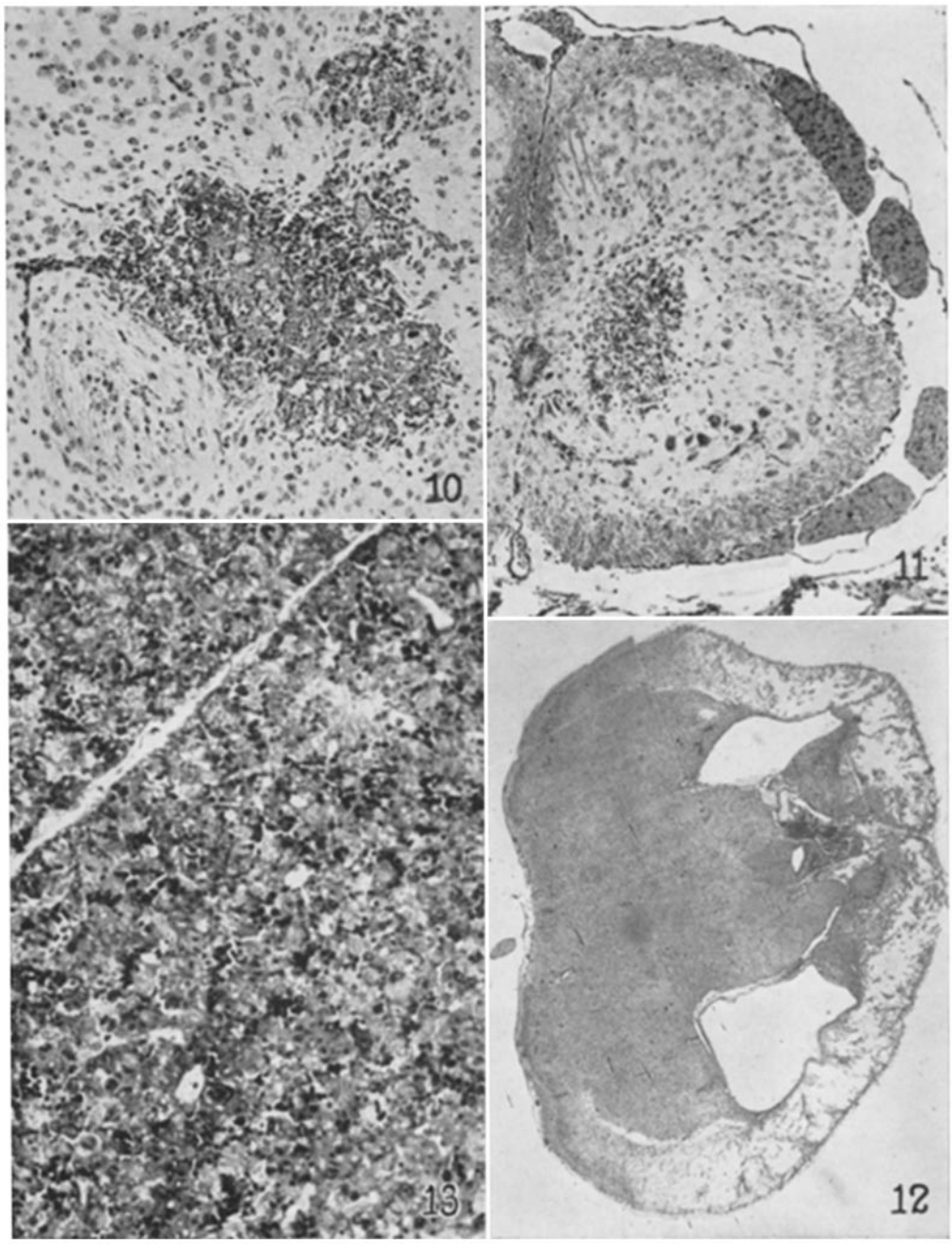
PLATE 7

FIG. 10. Mouse 3340. Inoculated intraperitoneally at age of 3 days with 2nd passage brain (Powers 10^{-1}). Diffuse incoordination noted on 11th day. Sacrificed 18 days after inoculation. Brain—areas of focal necrosis with early calcification in midbrain. Hematoxylin and eosin. × approximately 120.

FIG. 11. Mouse 3464. Inoculated intraperitoneally at age of 3 days with 7th passage brain (Powers 10^{-1}). Partial paralysis. Killed 16 days after inoculation. Spinal cord—large area of focal necrosis with beginning calcification in grey matter of lumbar region. Hematoxylin and eosin. × approximately 110.

FIG. 12. Mouse 3570. Inoculated intracerebrally on 4th day with 11th passage ground carcass material of Powers virus. Stunted growth—tremors. Killed on 6th day. Brain—liquefaction necrosis of entire cortex over convexity. Moderate dilatation of lateral ventricles. Encephalitic foci in midbrain. Hematoxylin and eosin. × approximately 20.

FIG. 13. Mouse 3589. Inoculated intracerebrally when 1 day old with 0.02 cc. of 3rd passage infected mouse brain suspension 10^{-2} (DeMole virus). Killed on 3rd day. Diffuse hepatitis. Giemsa stain × approximately 215.



(Pappenheimer *et al.*: Lesions in suckling mice)

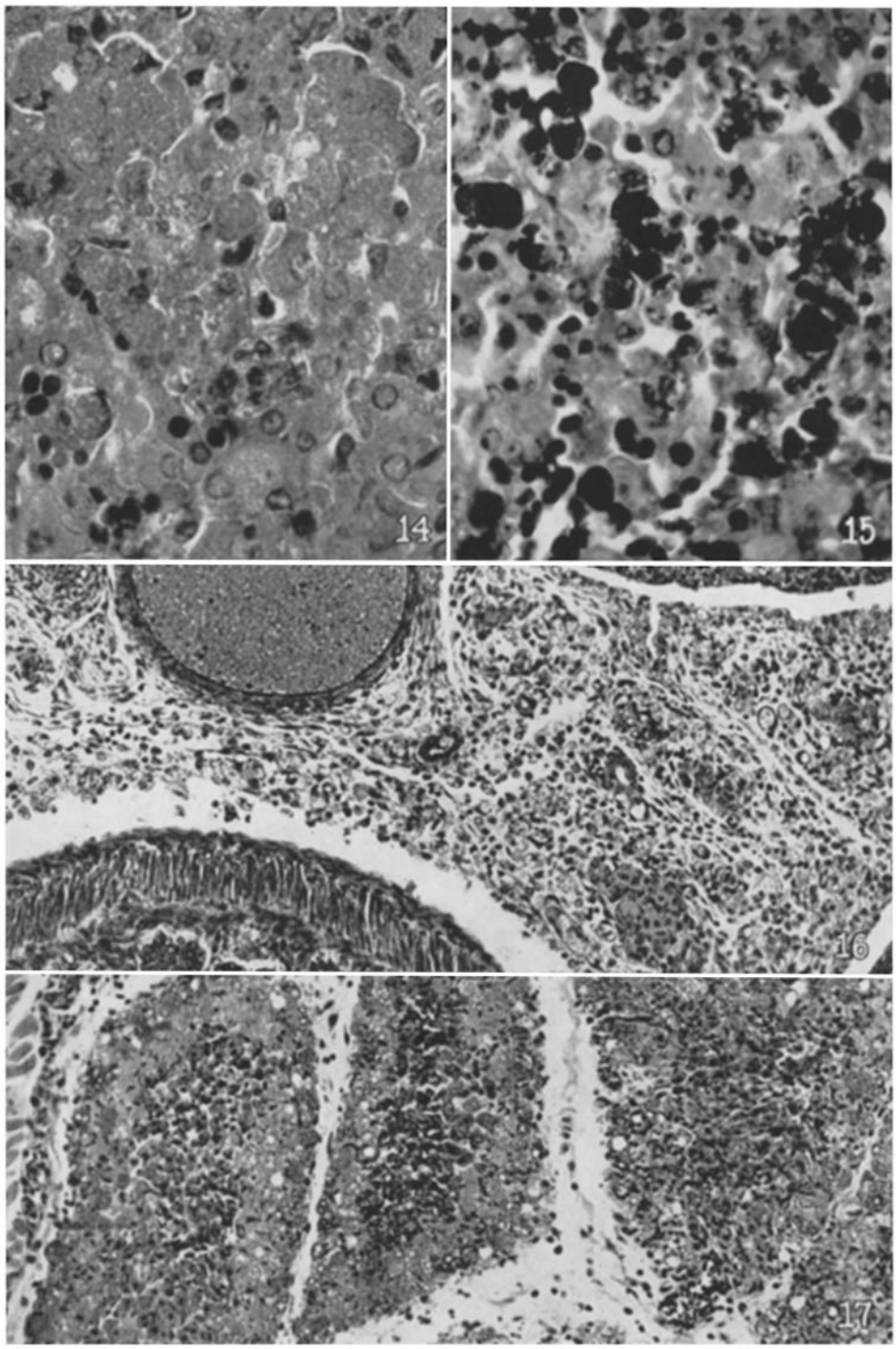
PLATE 8

FIG. 14. Mouse 3589. Inoculated intracerebrally when 1 day old with 0.02 cc. of 3rd passage infected mouse brain, 10^{-2} DeMole virus. Diffuse hepatitis. Note degenerative changes in liver cells, oval phagocytic cells in sinusoids, active hematopoiesis. Giemsa stain × approximately 620.

FIG. 15. Mouse 3686. Inoculated intracerebrally when 1 day old with 3rd passage brain suspension, 10^{-4} DeMole virus. Liver—diffuse hepatitis. Note black-staining phagocytes in sinusoids. Mahan's myelin stain × approximately 580.

FIG. 16. Mouse 3665. Inoculated intraperitoneally when 1 day old with 3rd passage brain suspension, 10^{-4} DeMole virus. Killed on 3rd day. Diffuse pancreatitis. Almost complete necrosis of acini, inflammatory infiltration. Note persistence of ducts and islands of Langerhans. Hematoxylin and eosin. × approximately 185.

FIG. 17. Mouse 3687. Inoculated intracerebrally when 1 day old with 3rd passage brain suspension of DeMole virus plus acute phase serum K. Killed on 7th day. Interscapular fat showing peripheral necrosis of cells with beginning calcification and inflammatory infiltration. Hematoxylin and eosin. × approximately 100.



(Pappenheimer *et al.*: Lesions in suckling mice)

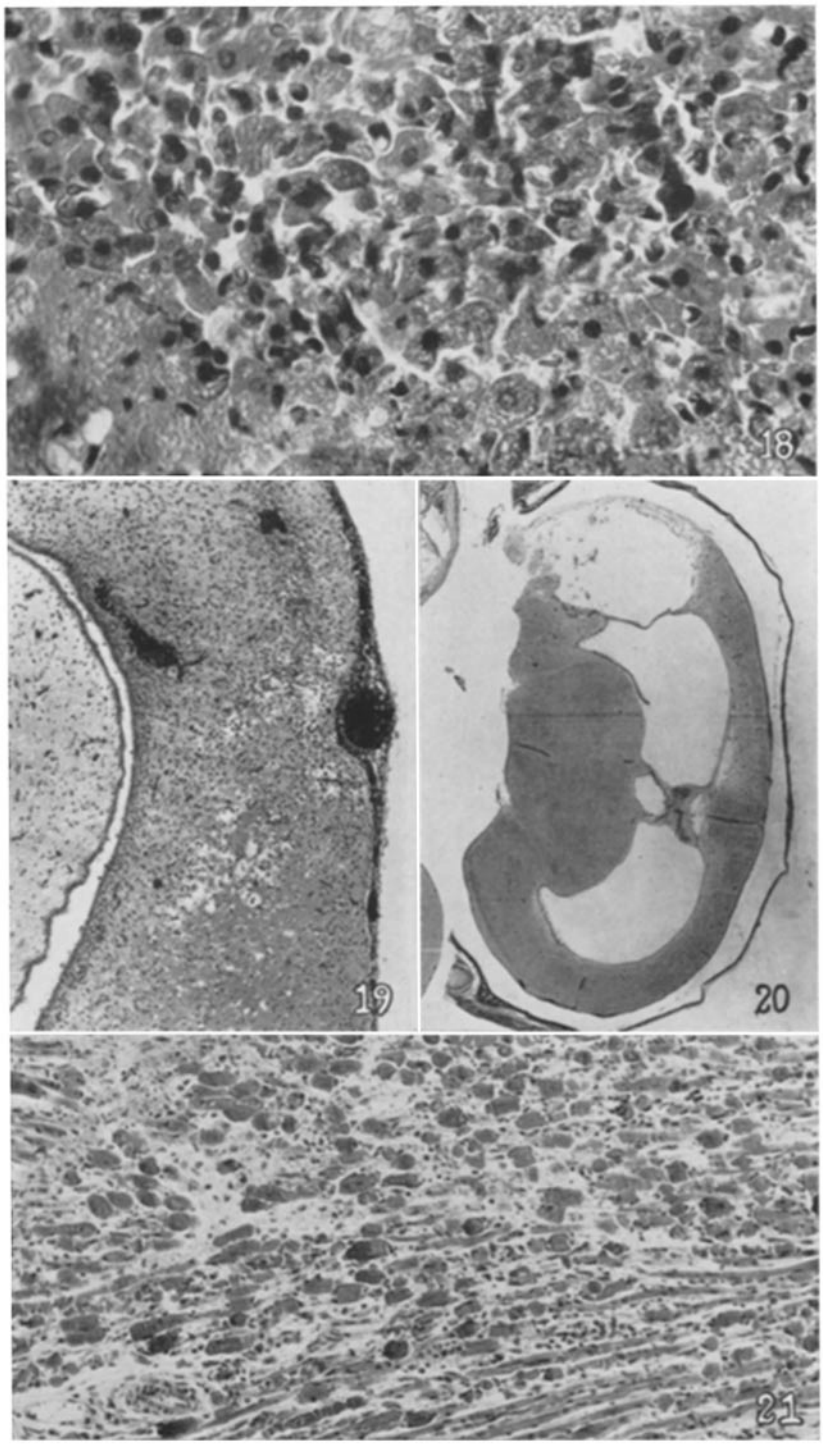
PLATE 9

FIG. 18. Same as Fig. 17. Hematoxylin and eosin. × approximately 620.

FIG. 19. Mouse 3668. Inoculated intracerebrally when 1 day old with 3rd passage infective brain suspension, 10^{-6} DeMole virus. Killed on 13th day. Brain showing diffuse cortical necrosis with beginning liquefaction. Hematoxylin and eosin. × approximately 100.

FIG. 20. Mouse 3579. Inoculated intracerebrally when 1 day old with 1st passage infective brain suspension, 1:20 DeMole virus. Killed on 14th day. Brain showing marked hydrocephaly and porencephaly. Hematoxylin and eosin. × approximately 18.

FIG. 21. Mouse 3925. 1 day old mouse inoculated intraperitoneally with EMC virus (86D) 10^{-5}. Sacrificed 2 days later. Leg muscle showing necrosis and fragmentation of fibers, interstitial edema, and slight inflammatory reaction. Hematoxylin and eosin. × approximately 195.



(Pappenheimer *et al.*: Lesions in suckling mice)