MURINE INFECTION WITH REOVIRUS.
III. PATHOLOGY OF INFECTION WITH TYPES 1 AND 2*

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ACUTE and chronic disease of mice following neonatal infection with type 3 reovirus has been described in detail in previous papers (Walters, Joske, Leak and Stanley, 1963 ; Stanley, Leak, Walters and Joske, 1964). There are, however, considerable differences between types 1 and 2 reovirus and type 3. Strains of types 1 and 2 do not as a rule produce the syndrome in mice which characterizes all type 3 strains we have tested (Stanley, 1961). Type 3 strains, but not types 1 and 2, agglutinate bovine erythrocytes (Eggers, Gomatos and Tamm, 1962). Gomatos (1963, personal communication) has shown that the nature of the interaction of reovirus type 3 with receptor substance is unique. Finally, observations in this laboratory suggest that the epidemiology of type 3 infection in animals does not always parallel that of types 1 and 2.

For these reasons, and because it was considered that a comparison of the infections produced by different types of reovirus might throw light upon the pathogenesis of reovirus infection in mice, it was decided to make a detailed study of the changes produced in infant mice by reovirus types 1 and 2. The results of this study comprise the present paper.

MATERIALS AND METHODS

Virus strains.—Reovirus type 1—" Lang " strain as 19th (Expt. 1) or 23rd (Expt. 2) monkey kidney tissue culture passage. Reovirus type 2—" D-5 " strain as 9th (Expt. 1) or 12th (Expt. 2) monkey kidney tissue culture passage.

Mice.—Prince Henry strain (PH) (Stanley, Dorman and Ponsford, 1954) were inoculated within 24 hr. of birth. General examination of mice and histopathological methods were described previously (Walters *et al.*, 1963).

Virus isolations.—Brain, liver and gut from the experimental mice from Expt. 2 were tested for infective virus using infant mice (intraperitoneal inoculation) and mouse-fibroblast L cell tissue cultures. As it happened, all virus isolations were made in tissue culture ; they were typed by haemagglutination-inhibition (HI) tests using reovirus type-specific antisera, and all proved to be the same type of reovirus as was inoculated. Tests for infective virus were considered negative when mice remained symptomless for 14 days and no cytopathic effect or haemagglutinin for human group O cells was observed after two passages of the specimen in L cells.

Serum from the experimental mice was tested for HI antibodies to all 3 types of reovirus, using methods described previously (Stanley and Leak, 1963*a*). Since only small quantities of serum were obtained from individual mice, the initial dilution of treated serum tested was 1/20.

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EXPERIMENTAL

Experiment I.—The Acute Phase of the Disease with Reovirus Types 1 *and* 2

This includes observations made up to and including the 18th day after inoculation.

Ia. Oral infection with reovirus type 1

Eight litters of infant mice were inoculated orally with approximately 0·01 ml. of undiluted virus suspension having a TCD_{50} in L cell tissue culture of 10^6/ml.

The majority of mice showed no clinical evidence of infection. A few appeared to be emaciated, retarded in growth and incoordinated. Eight of the 65 mice died between the 11th and 16th day. At post-mortem examination, some mice removed after the 8th day showed lesions in the liver or heart macroscopically similar to those observed in mice infected with reovirus type 3.

Histopathology of reovirus type 1 *acute infection*

Two mice were examined daily from the 4th to 18th day after inoculation.

Liver.—Small foci of necrotic hepatocytes were seen on the fourth day. On the next day larger lesions exhibited eosinophilic debris, nuclear remnants, leucocytic infiltrations and hyaline bodies appeared. Fatty change in hepatocytes, associated with lysis and thinning of the liver cords followed, the sinusoids being filled with the debris of the cellular necrosis. After one week there were numerous small necrotic foci in all parts of the lobule and fatty metamorphosis was more pronounced. Larger foci containing aggregates of macrophages were also seen. Coagulative eosinophilic zonal necroses, usually found beneath the capsule, were present early in the second week of the infection (Fig. 1). In the portal tracts there was mild ductular proliferation and infiltration with segmented leucocytes. Ballooned hepatocytes were seen not infrequently. The liver became increasingly congested on subsequent days and this, coupled with the atrophy and thinning of the hepatocytic plates, mimicked the appearance of " subacute red atrophy " (Fig. 2). Two weeks after inoculation subcapsular areas of haemorrhage, atrophy and eosinophilic zonal necrosis were present together with a more diffuse fatty infiltration of hepatocytes. During the third week the intensity of the necrotic lesions diminished although the fatty change was unaltered.

Pancreas.—The acinar cells showed degranulation and swelling with early lysis and replacement by oval ductular cells ; on the fourth day acini showed eosinophilic coagulative necrosis, and cytoplasmic bubbling was present while other cells were shrivelled and resembled hyaline bodies. Interstitial oedema separating the lobules also appeared at this time.

At one week after inoculation areas of active pancreatitis were present. Ballooned and necrotic cells, nuclear and cytoplasmic debris, clumps of condensed cells with meagre amounts of cytoplasm and subacute interstitial inflammatory exudate were present. In a few islets clumps of nesidiocytes were also necrotic. The changes were similar on the 11th day of the infection but were augmented by regenerative phenomena involving not only ductular and acinar cells but also nesidiocytes. Many of the acinar cells showed basophilic necrosis and they were often detached from their acini, forming groups or lying as individual cells in the

oedematous interstitium. After 2 weeks the serosal cells proliferated and sheets of macrophages appeared between the acini. Fatty replacement of some of the necrotic tissue occurred and the process continued until the third week of the infection.

Salivary glands.—By the 5th day a few isolated acini showed necrosis and lysis without any accompanying inflammatory reaction. After one week, many acini were necrotic, basophilic, shrunken and fragmented, appearing as individual cells. During the second week fatty replacement of the atrophic tissue occurred while the necrotic process was still in evidence. Ductular cell proliferation was pronounced. Many macrophages and small round cells had infiltrated the interstitial tissues (Fig. 3). A similar appearance persisted throughout the third week of the period of study.

Heart.—The first lesions appeared on the 5th day after inoculation when small subendocardial foci of muscle necrosis appeared in the left ventricle. These areas were oedematous and the affected muscle at first underwent eosinophilic coagulative necrosis, and then the cytoplasm fragmented. New foci appeared in the walls of both chambers and in the septa until the 7th day when the condition became more severe and widespread. Larger foci appeared and were infiltrated with a variety of leucocytes, mainly mononuclear ; myocytes were also plentiful. The foci were basophilic because of the marked cellular exudate. In a few cases almost the whole of the ventricular wall was involved in the necrosis. The changes were similar after two weeks although the basophilia was more intense. Thereafter in most cases the areas shrank in size and the debris disappeared until finally little evidence of the lesion remained. In a few cases, however, newer necrotic lesions appeared. Basophilic spherules as seen in type 3 myocarditis occurred in only one case.

Skeletal muscle.—No muscular lesions appeared until 11 days after inoculation when mild focal subacute interstitial myositis was found in the muscles of the thigh and cervical region. Similar lesions appeared in the tongue on the following day. The lingual glands also underwent necrosis and provoked an inflammatory reaction. The musculature of the scalp showed necrosis and lysis of fasciculi with sarcolemmal cell proliferation. Foci of subacute panniculitis were observed in the surrounding fat. This became more diffuse 2 weeks after inoculation of the virus, but apart from periarterial collections of round cells in the involved areas and the presence of empty sarcolemmal tubes, no new lesions developed.

Central nervous system.—The first lesions were seen 14 days after inoculation and comprised microscopic foci of neuronal degeneration in the midbrain associated with small collections of round cells. The lesions were confined to this area of the brain throughout the experiment. No lesions were found in the cervical cord.

Lung.—On the 4th day oedema, congestion and small alveolar haemorrhages were obvious. The interstitial tissues were infiltrated with lymphocytes, macrophages and occasional polymorphs. On subsequent days purulent material was sometimes found in bronchioles ; the alveoli became lined by cuboidal cells that were occasionally found to be necrotic and produced areas mimicking hyaline membranes. Eosinophilic hyaline bodies were also occasionally observed. By the 11th day of the disease aggregates of mononuclear cells formed, these contained giant cells in a few cases.

No significant lesions were found in the adrenal, kidney, alimentary canal, skin, spleen, bonemarrow, lymph nodes or thymus.

Ib. Oral infection with reovirus type 2

Six litters of mice were inoculated by the oral route with approximately 0·01 ml. of undiluted virus suspension having a TCD_{50} in L cell tissue culture of 10^6/ml. Two mice were removed daily from the 4th to the 14th day after inoculation.

During the period of observation no clinical evidence of infection was apparent. At post-mortem examination only one mouse, removed on the 10th day, showed macroscopic lesions in the liver.

Histopathology of reovirus type 2 *acute infection*

Animals examined on the 4th day after oral inoculation of virus showed no abnormalities ; the earliest lesions appeared on the 5th day.

Liver.—Small foci of hepatocytic necrosis were seen on the 5th day of the disease. As the necrotic areas enlarged, macrophages and lymphocytes together with one or two polymorphonuclear leucocytes appeared.

By the next day hyaline bodies were present in the necrotic foci and the polymorph content of the inflammatory exudate was increased both in the liver and portal tracts. One week after inoculation fatty change became more marked, and thinning, atrophy and lysis of the hepatic cords was seen. These changes progressed over the following 3 days and severe subcapsular sinusoidal congestion also developed. The appearances again mimicked those of " subacute red atrophy " of the liver.

Occasional small areas of hepatocytic necrosis were seen 12–14 days after infection, as well as isolated ballooned cells, fatty infiltration, sinusoidal congestion and atrophy of liver cell plates.

Pancreas.—In one of the animals basophilic coagulative necrosis of exocrine epithelium was seen on the 5th day. Many of the acinar cells were swollen and there was proliferation of the ductular cells. On the following day many of the acini underwent lysis, while other glandular cells condensed into basophilic rims of cytoplasm surrounding prominent nuclei. These were present either as individual cells or in small clumps. Mitotic figures were commonly observed in both ductular and glandular epithelium. Most of the acini lost their zymogenic granules one week after infection. Interstitial oedema was present on the following day and ductular cell proliferation became more pronounced. In some areas the atrophic acini were lysed and replaced by adipose tissue in which mast cells were frequently seen. Similar changes were seen after two weeks of infection.

Salivary glands.—Early vacuolar degeneration of the acinar cells appeared on the 5th day. Others were necrotic and became replaced by small oval cells ; lysis of the necrotic tissue followed. One week after infection many glandular cells were shrunken ; there was mild interstitial oedema and subacute inflammation. Many of the atrophic acinar cells appeared as a small basophilic rim of cytoplasm surrounding the nucleus. On the 10th day fat replacement of the atrophied tissue commenced. Mitotic activity in ductular and acinar cells was pronounced. The changes were the same 2 weeks after inoculation.

Heart.—The first lesion appeared 7 days after inoculation and comprised oedema and vacuolar degeneration of muscle fibres. On the endocardial surface of the left ventricle, small foci of ambiphilic coagulative necrosis admixed with nuclear debris and an infiltration of lymphocytes, monocytes and polymorphs developed. These foci increased in number and size over the next 3–4 days, but

thereafter were small. Resolution through lysis of the necrotic material quickly followed.

Skeletal muscle.—The first lesions of striated muscle were seen in the muscles of the thigh 6 days after infection. A small area of ambiphilic granular necrosis was present associated with lymphocytic infiltration. On the following day similar lesions were seen in the tongue. The lingual glands became necrotic and fragmented, providing subacute inflammatory reaction.

The cervical muscle showed lesions on the 10th day and hyperplastic sarcolemmal cells became prominent. Mild panniculitis was also occasionally observed in this site. These changes of necrosis, subacute inflammation and proliferation of sarcolemmal cells persisted until the 2 weeks of the study had elapsed.

Central nervous system.—Foci of necrosis and ballooning degeneration of neurones in the thalamic nuclei, together with infiltration by small round cells and occasional large mononuclear cells, were present on the 10th day. Similar changes were found in the cerebellar peduncles and there was marked congestion, perivascular cuffing and neuronal satellitosis. The lesions were found in the same regions during the next 4 days.

No lesions were found in the cervical cord.

Lungs.—These remained normal until 8 days after inoculation, when an interstitial inflammation developed. The lungs were congested and aggregates of lymphoid and reticuloendothelial cells formed as small granulomata. These were usually found near blood vessels and bronchioles. Occasionally small alveolar haemorrhages were also seen. These lesions persisted throughout the test period.

No significant lesions were found in the adrenal, kidney, alimentary canal, skin, spleen, bonemarrow, lymph nodes or thymus.

Experiment II.—The Chronic Phase of the Disease with Reovirus Types 1 *and* 2

A common feature of murine infection with reovirus type 3 was the development of a clinical syndrome similar to runting (Stanley and Leak, 1963*b* ; Stanley *et al.*, 1964). This chronic disease persisted for months. To determine whether a similar chronic process followed infection with reovirus types 1 and 2, the following experiments were performed.

1. *Infection by the oral route (Tables IA and IB)*

Four litters of infant mice were inoculated by the oral route with approximately 0·01 ml. of virus suspension having a TCD_{50} in monkey kidney tissue culture of

TABLE IA.—*Oral Inoculation of Infant Mice with Reovirus Type* 1 (10^5 TCD_{50})

Mouse No.	Age (in days) at p.m.	Prodn. of spec. ab (reciprocal titre)	Isolation of virus at p.m.	Evidence of reovirus infection		
				Clinical	At p.m.	Histopath.
1	35	40	—	+	—	+
2	35	80	—	—	—	+
3	42	>160	—	—	—	+
4	49	>160	—	+	—	+
5	49	>160	—	—	—	+
6	56	40	—	—	—	+

TABLE IB.—*Oral Inoculation of Infant Mice with Reovirus Type* 1 (10^5 TCD_{50})

(Distribution of microscopic lesions)

Mouse No.	Liver	Pancreas	Lung	Salivary glands	Other organs
1	+	+	+	—	—
2	+	+	+	+	—
3	+	+	+	—	—
4	+	+	—	—	—
5	+	+	—	—	—
6	+	—?	+	—	—
Total :	6	5	4	1	—

+ = lesions
— = no lesions

10^7/ml. The mice were examined daily until the 56th day. Of the 27 mice inoculated, approximately half died between the 13th and 15th day ; only 2 of these showed any symptoms and then it was only emaciation. Of the survivors, 3 showed clinical evidence of infection, emaciation and incoordination, during the acute phase. One died at 20 days and was not tested further ; by the 30th day, the other 2 appeared to have recovered but were undersized and remained so. These last 2 mice (1 and 4 in tables IA and IB) were among the 6 removed for testing on the 35th, 42nd, 49th and 56th days.

There was no macroscopic evidence at post-mortem examination of reovirus infection in any of the mice. All animals developed HI antibody to reovirus type 1, but antibodies were not detected to the other 2 types of reovirus. Virus was not isolated from any animal.

Histopathology

Microscopic examination of the tissues revealed lesions suggestive of reovirus infection in all animals.

Liver.—After 5 weeks of the infection the predominant changes were seen in the hepatocytic nuclei which were frequently enlarged and irregular. Bi- and trinucleated cells were common. Similar changes were present one week later. Small foci of hepatocytic necrosis (Fig. 4) with occasional hyaline bodies were found up to 2 months after inoculation, while areas of fatty degeneration, haemorrhage and necrosis were also sometimes observed.

Pancreas.—Many lobules were necrotic, exhibiting basophilic degeneration or acinocytic degranulation after 5 weeks. Early fatty replacement was also present at this time. By the 8th week the pancreas appeared normal apart from areas of adipose atrophy of the exocrine tissue.

Salivary glands.—Vacuolar degeneration of the cytoplasm of glandular cells was observed at the 5th week. Thereafter, the glands appeared to be unaffected.

Lung.—At 35 days after inoculation granulomata were still present in the pulmonary parenchyma (Fig. 5). In some areas there was proliferation of alveolar cells, many of which exhibited eosinophilic necrosis of their cytoplasm with the formation of granular eosinophilic debris and formations resembling hyaline membranes (Figs. 6–8).

These granulomata waned over the next 2 weeks.

Other organs, including heart, skeletal muscle and central nervous system were unaffected.

2. *Infection by the intraperitoneal route (Tables IIA and IIB)*

Four litters of infant mice were inoculated by the intraperitoneal route with approximately 0·05 ml. of virus suspension having a TCD_{50} of 10^7/ml. in monkey kidney tissue culture. The mice were examined and treated as for those inoculated

TABLE IIA.—*I.P. Inoculation of Infant Mice with Reovirus Type* 1 (5×10^5 *TCD_{50}*)

Mouse No.	Age (in days) at p.m.	Prodn. of spec. ab (reciprocal titre)	Isolation of virus at p.m.	Evidence of reovirus infection		
				Clinical	At p.m.	Histopath.
1	35	>160	−	+	+	+
2	35	>160	+ (gut)	?	−	+
3	42	<20	+ (gut)	+	+	+
4	42	>160	+ (brain)	?	−	+
5	49	40	−	+	−	+
6	49	>160	−	?	+	+
7	56	>160	−	+	+	+
8	56	>160	−	?	+	+

TABLE IIB.—*I.P. Inoculation of Infant Mice with Reovirus Type* 1 (5×10^5 *TCD_{50}*)

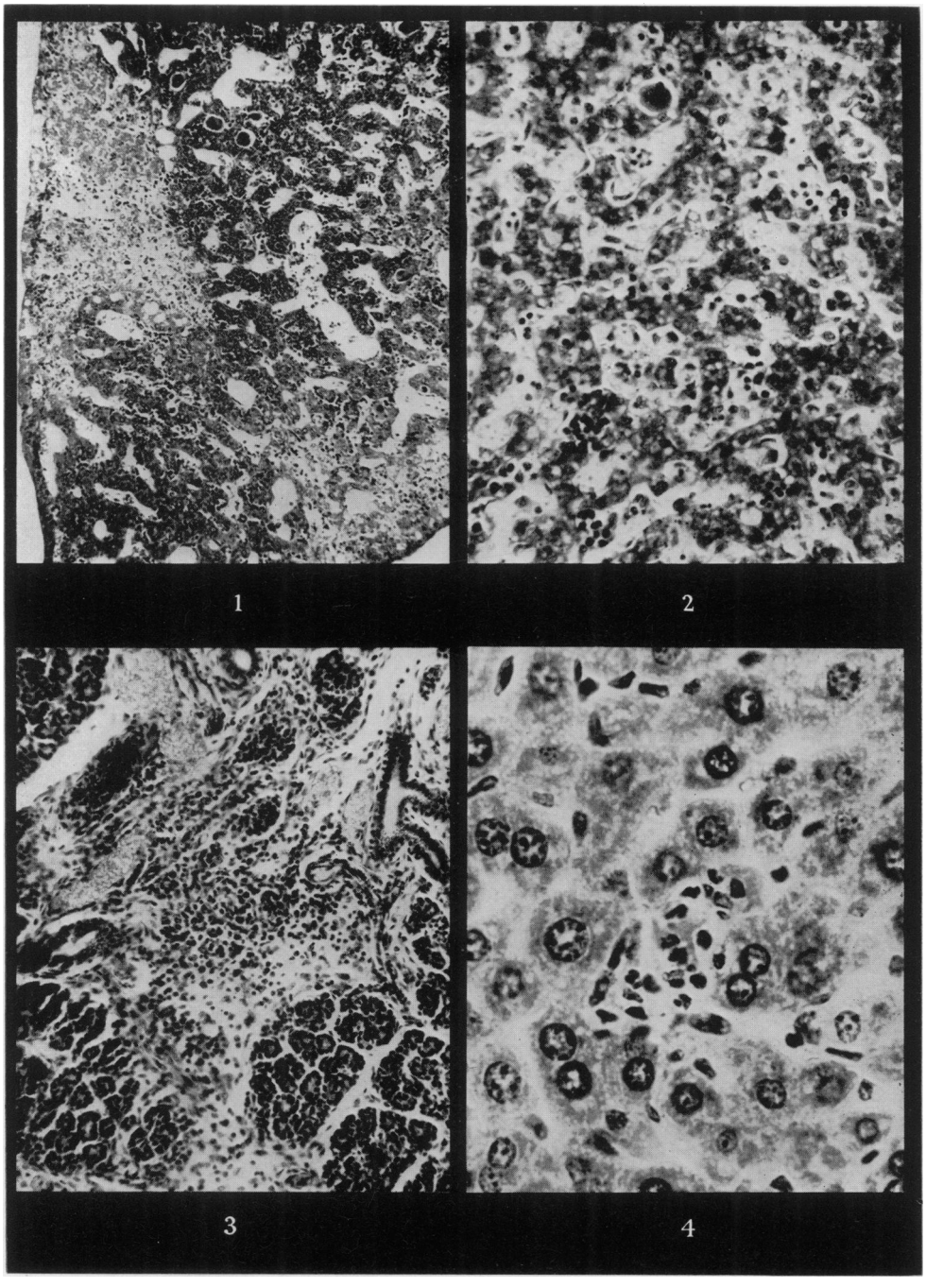
(Distribution of microscopic lesions)

Mouse No.	Liver	Pancreas	Lung	Heart	Salivary glands	Fat	Spleen	Muscle	Brain	Other organs
1	+	+	−	+	+	+	+	−	−	−
2	+	+	+	−			−	−	−	−
3	+	+	−	+	+	+	−	+	−	−
4	+	+	−	−			−	−	+	−
5	+	+	+				−	−	−	−
6	+	+	+	−	+		−	−	−	−
7	−	+		+		+	−	−	−	−
8	+	−		−			−	−	−	−
Total:	7	7	3	3	3	3	1	1		

\+ = lesions
− = no lesions

EXPLANATION OF PLATES

FIG. 1.—Liver (type I oral) 9 days. Subcapsular coagulative necrosis. H. and E. ×100.
FIG. 2.—Liver (type I oral) 15 days. Sinusoidal congestion and atrophy of hepatocytes (" subacute red atrophy "). H. and E. ×250.
FIG. 3.—Salivary gland (type I oral) 11 days. Interstitial sialadenitis with acinar atrophy. H. and E. ×200.
FIG. 4.—Liver (type I oral) 49 days. Small focus of hepatocytic necrosis. H. and E. ×630.
FIG. 5.—Lung (type I oral) 35 days. Pulmonary granulomata H. and E. ×66.
FIG. 6.—Lung (type I oral) 35 days. Proliferation of alveolar cells. H. and E. ×430.
FIG. 7.—Lung (type I oral) 35 days. Early necrosis of alveolar cells. H. and E. ×265.
FIG. 8.—Lung (type I oral) 35 days. Necrosis of alveolar cells and formation of structures resembling hyaline membranes. H. and E. ×430.
FIG. 9.—Heart (type I oral) 35 days. Myocardial necrosis showing amorphous debris. H. and E. ×100.
FIG. 10.—Skeletal muscle (type I oral) 42 days. Myositis. H. and E. ×165.
FIG. 11.—Cervical fat (type I oral) 42 days. Fat necrosis with calcification and giant-cell reaction. H. and E. ×165.
FIG. 12.—Thalamus (type II intraperitoneal) 35 days. Large area of necrosis. H. and E. ×100.



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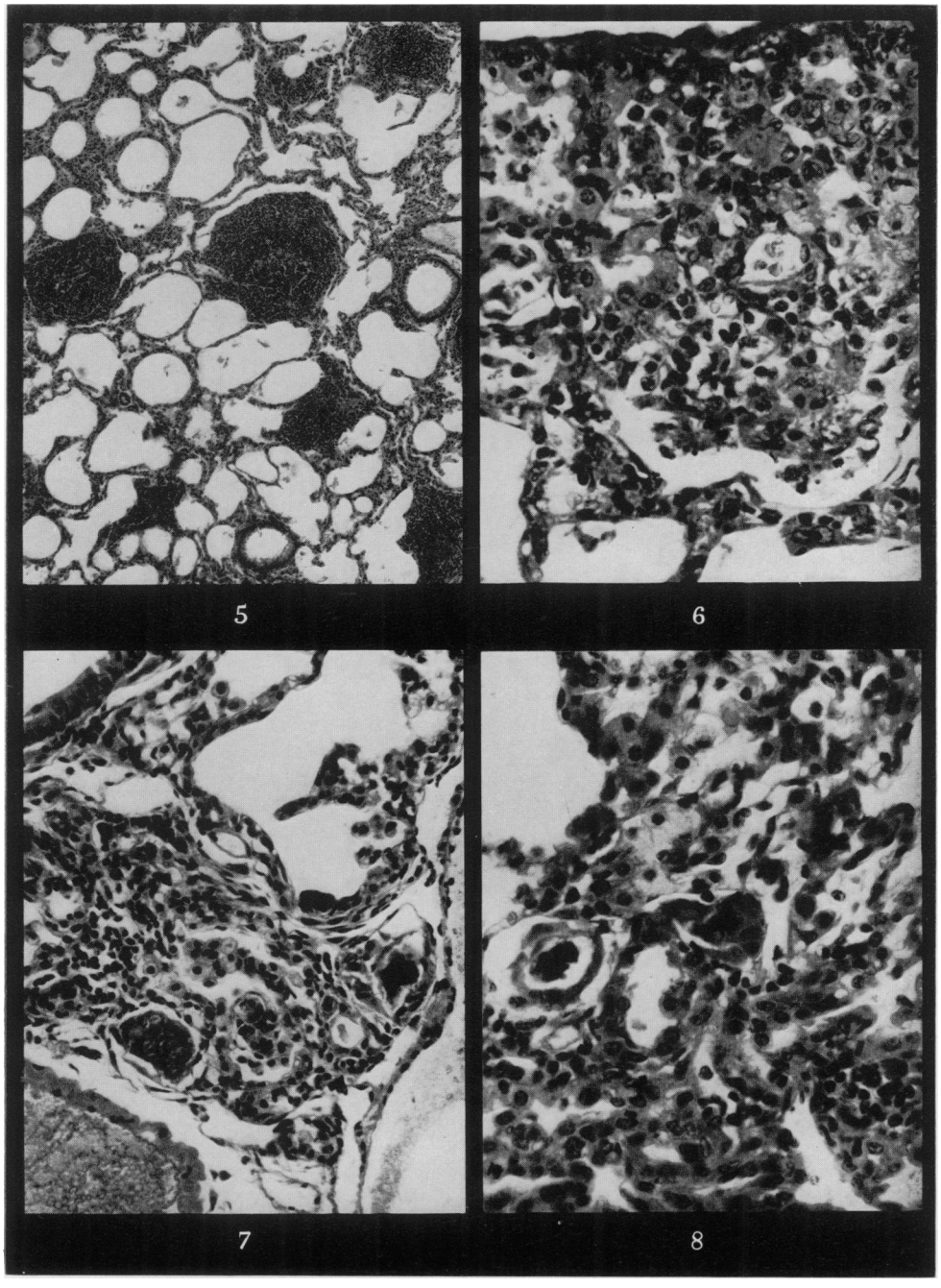

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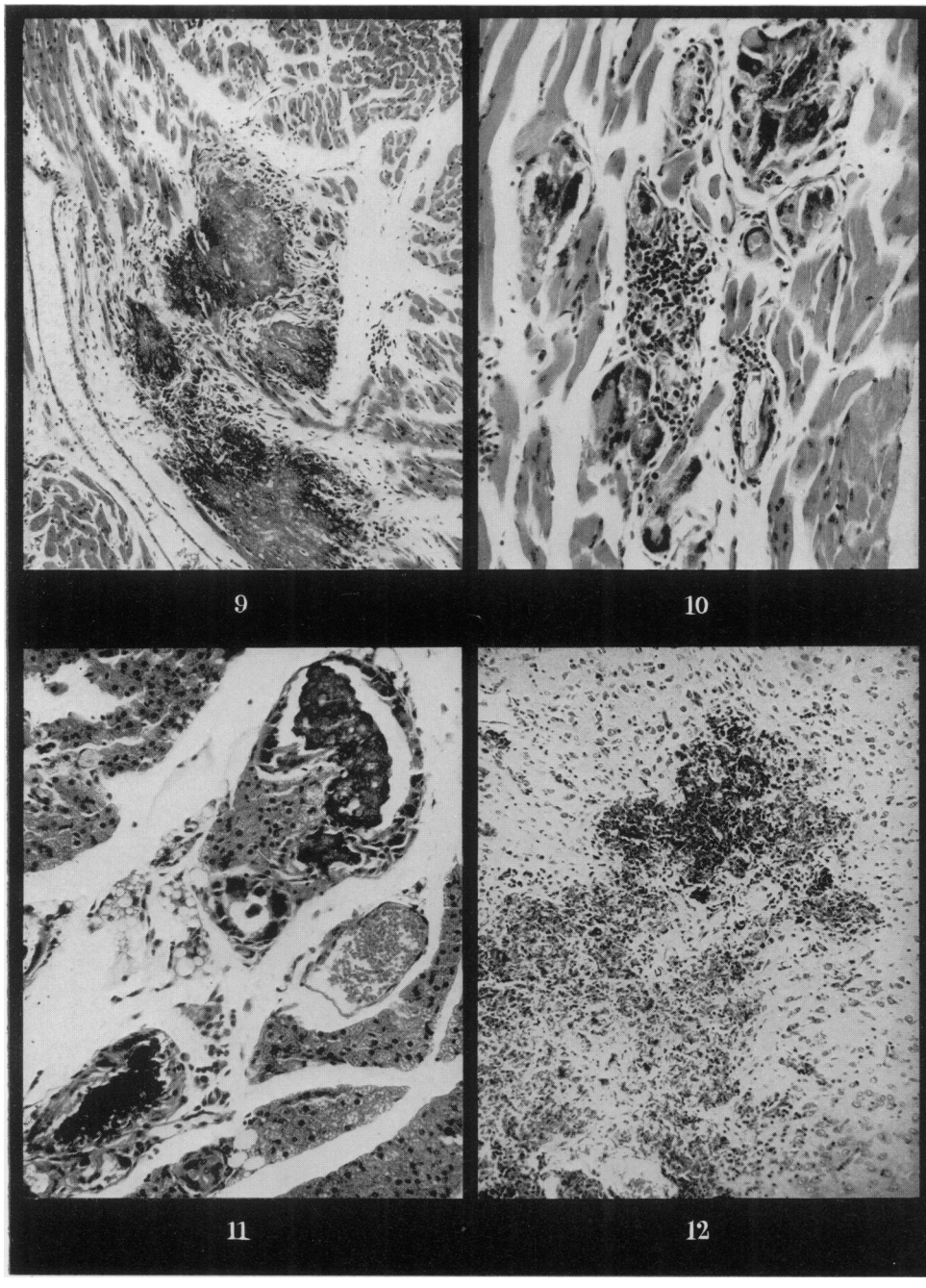

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orally. Approximately half the mice inoculated developed a blood-stained peritoneal exudate during the acute phase ; most of these animals died.

The 8 mice selected for study included some which had shown peritoneal exudate during the acute phase. Four of them also had developed slight retardation of growth, sparseness of hair and roughness of coat but had recovered by the 22nd day. At post-mortem examination, 5 of the mice showed lesions : three in the heart alone, one in the liver alone, and one in both liver and heart. HI antibodies to reovirus type 1 were detected in 7 of the 8 mice ; one of these also had antibody to type 2 reovirus. None of the sera had antibody to type 3 reovirus.

Virus was isolated from brain or gut on 3 occasions (Table IIA).

Histopathology

Microscopic lesions indicative of reovirus infection were present in all animals.

Liver.—At 35 days after inoculation bi- and trinucleated hepatocytes and giant hepatocytes were frequently observed. Mitoses were also prevalent. One case showed an area of necroses undergoing resolution and in another there was subcapsular haemorrhage and necrosis.

These nuclear changes and small foci of hepatocytic necrosis persisted up to 8 weeks after inoculation.

Pancreas.—Areas of basophilic necrosis of acini with shrinkage and acinocytic mitoses were found throughout the test period.

Salivary glands.—The changes were similar to those observed in the pancreas. Areas of acute acinar necrosis with the formation of hyaline bodies were seen during the 6th week of the infection. Fatty atrophy was also found at this time.

Heart.—Myocardial necrosis was present in animals examined on the 35th day of the disease. Within the necrotic areas pale blue amorphous material was present (Fig. 9), which reacted with Schiff's periodic acid reagent, and after six weeks these areas commenced to calcify. Similar necrotic foci were still appearing 8 weeks after inoculation.

Skeletal muscle.—Areas of chronic inflammation were present in only one animal 6 weeks after inoculation. Amorphous basophilic debris and infiltration with mononuclear cells was seen (Fig. 10).

Cervical fat.—Areas of necrosis of the brown fat with calcification and the formation of foreign body giant cells was seen throughout the test period (Fig. 11).

Central nervous system.—In one animal only, 6 weeks after inoculation, a small focus of necrosis was present in the cerebral white matter.

Lung.—This was congested 5 weeks after inoculation, and 2 weeks later a few granulomata appeared, as well as interstitial haemorrhagic pneumonia.

The remaining organs showed no lesions.

3. *Infection by the intraperitoneal route with " partially adapted " virus*

After several passages in infant mice by the intraperitoneal route, both the " Lang " strain of reovirus type 1 and the " D5 " strain of reovirus type 2 regularly produced a clinically recognisable syndrome characterised by blood-stained peritoneal exudate at 4–5 days, followed by death at 8–11 days (Leak and Stanley, unpublished). No histological examination of the mice was undertaken during the mouse adaptation of the strains except for 2 mice in the second Lang mouse

passage. These exhibited unusual symptoms in that they developed a massive peritoneal exudate at the 4th week ; presumably they were infected with partially adapted virus. One mouse was killed on the 29th day. In the peritoneum there was 7·0 ml. of milky fluid, which contained 8·8 g. of fat and 3·4 g. of protein per 100 ml. ; the amylase content was 2340 Somogyi units. The other animal was killed on the 33rd day ; in its peritoneum there was 4·0 ml. of similar fluid, containing 3·7 per cent fat, 3·0 per cent protein, and 1930 units of amylase. In neither instance could virus be isolated from the fluid.

Histopathology

The mesentery contained numerous polymorphonuclear leucocytes, swollen and foamy macrophages, and proliferating reticuloendothelial cells. Mitoses were frequent. There were occasional patches of fat necrosis and great dilation of lymphatics. Occasional giant cells were seen.

Pancreas.—Acute pancreatitis was present, with oedema and peripheral fatty replacement. The acinar cells showed necrosis and lysis as well as numerous mitoses. Many acini were shrunken. In the capsule there was proliferation of mesothelial cells and macrophages and many swollen lipophages.

Liver.—There was an extensive active hepatitis similar to that described above.

Changes in other organs were slight.

TABLE IIIA.—*Oral Inoculation of Infant Mice with Reovirus Type* 2 (10^6 TCD_{50})

Mouse No.	Age (in days) at p.m.	Prodn. of spec. ab (reciprocal titre)	Isolation of virus at p.m.	Evidence of reovirus infection		
				Clinical	At p.m.	Histopath.
1	35	>160	—	—	—	+
2	35	40	+ (brain)	—	—	+
3	42	>160	—	—	—	+
4	42	>160	—	—	—	?
5	49	80	—	—	—	+
6	49	>160	—	—	—	+
7	56	>160	—	—	—	?
8	56	40	—	—	—	+

TABLE IIIB.—*Oral Inoculation of Infant Mice with Reovirus Type* 2 (10^6 TCD_{50})

Mouse No.	(Distribution of microscopic lesions)					
	Liver	Pancreas	Brain	Lung	Skin	Salivary glands	Other organs
1	+	+	—	+	—	—	—
2	+	+	+	+	—	—	—
3	+	+	—	—	+	+	—
4	+	—	—	—	—	—	—
5	+	+	—	—	+	+	—
6	+	+	—	+	—	—	—
7	—	—	—	+	—	—	—
8	+	—	—	—	—	—	—
Total :	7	5	1	4	2	2	

+ = lesions
— = no lesions

IIb. Chronic Murine Infection with Reovirus Type 2

1. *Infection by the oral route (Tables IIIA and IIIB)*

Four litters of infant mice were inoculated by the oral route with approximately 0·01 ml. of virus suspension having a TCD_{50} in monkey kidney tissue culture of 10^8/ml. The mice were examined daily until the 56th day. Two mice were removed on the 35th, 42nd, 49th and 56th days, and were treated as for those inoculated with reovirus type 1.

The mice remained normal throughout the period of observation and no macroscopic lesions were observed at post-mortem examination. However, it would seem that the animals suffered an inapparent reovirus type 2 infection since they all developed HI antibodies to reovirus type 2 but not to types 1 and 3 except for one mouse which developed type 1 antibodies. Virus was recovered, from the brain alone, of one animal killed at the 35th day.

Histopathology

Microscopic examination of the tissues revealed lesions in the liver and pancreas of most animals.

Liver.—Five weeks after infection several hepatocytes were ballooned or lytic. Binucleate liver cells were prevalent. The changes were similar during the 6th week, although the nuclear changes were more pronounced, with giant cells and trinucleate hepatocytes in evidence. One week later numerous foci of necrosis appeared, and were associated with polymorphonuclear leucocytic infiltrations and hyaline globules. No change was apparent two months after infection.

Pancreas.—Basophilic atrophy, fatty replacement of the exocrine tissue and occasional foci of necrosis were seen from the 5th to the 8th week of the test period. Spherical hyaline bodies were often present in relation to necrotic acini. Animals examined during the 8th week, however, showed no microscopic abnormalities.

Salivary glands.—During the 6th and 7th weeks necrosis and lysis of acini were seen, together with fatty replacement of the glands.

Central nervous system.—At the 5th week one animal showed a focus of necrosis in the thalamus with perivascular cuffing.

Lung.—Areas of haemorrhagic pneumonia and granulomata were found during the 5th, 7th and 8th weeks after inoculation.

Skin.—At 6 weeks the epidermis was thinned and showed loss of hair shafts. One week later foci of mild chronic dermal inflammation were present.

The other organs, including heart and skeletal muscle, were unaffected.

2. *Infection by the intraperitoneal route (Tables IVA and IVB)*

Four litters of infant mice were inoculated by the intraperitoneal route with approximately 0·05 ml. of virus suspension having a TCD_{50} of 10^8/ml. in monkey kidney tissue culture. The mice were examined and treated as for those inoculated orally. Four of the mice showed a blood-stained peritoneal exudate during the acute phase of the infection. They had recovered from this by the 10th day. There was no other clinical evidence of infection. At post-mortem examination, 3 of the 4 mice who earlier showed a peritoneal exudate possessed thickened one-lobed livers; otherwise they were normal. Of the 8 mice examined, 5 had HI antibodies to reovirus type 2; antibodies to the other two types of reovirus were not detected in any of them.

TABLE IVA.—*I.P. Inoculation of Infant Mice with Reovirus Type* 2 (5×10^6 TCD_{50})

Mouse No.	Age (in days) at p.m.	Prodn. of spec. ab (reciprocal titre)	Isolation of virus at p.m.	Evidence of reovirus infection		
				Clinical	At p.m.	Histopath.
1	35	40	+ (brain)	—	—	+
2	35	<20	—	+	+	+
3	42	40	—	+	+	+
4	42	40	—	—	—	+
5	49	>160	—	+	—	+
6	49	<20	—	—	—	+
7	56	20	—	+	+	+
8	56	<20	—	—	—	+

TABLE IVB.—*I.P. Inoculation of Infant Mice with Reovirus Type* 2 (5×10^6 TCD_{50})

Mouse No.	Liver	Pancreas	Brain	Lung	Heart	Skin	Salivary glands	Other organs
1	—	+	+	+	+	—	—	—
2	?	+	+	—	—	—	—	—
3	+	+	—	+	—	+	—	—
4	+	+	—	+	—	—	—	—
5	+	+	+	—	—	—	—	—
6	+	+	—	—	—	—	+	—
7	+	+	—	+	—	—	—	—
8	+	+	—	—	—	—	—	—
Total:	6	8	3	4	1	1	1	

+ = lesions
— = no lesions

Reovirus was recovered, on only one occasion, from the brain of a mouse killed on the 35th day.

Histopathology

Microscopic examination of the tissues revealed lesions suggestive of reovirus infection in all animals.

Liver.—Nuclear changes described above were present throughout the test period. During the 6th week hepatocytic mitoses, often bizarre, were frequently seen. One week later tiny foci of necrosis were found together with ductular cell proliferation. These foci persisted into the 8th week of the infection.

Pancreas.—Occasional necrotic acini undergoing lysis were present in an oedematous and mildly inflamed interstitium. Hyperplasia of ductile cells accompanied the necrosis. On succeeding weeks the lobules degenerated, hyaline bodies were formed and adipose tissue replaced the exocrine parenchyma.

Salivary glands.—During the 7th week vacuolation of acinar cells was present.

Heart.—Necrosis of the left ventricle with early calcification was seen during the 5th week after inoculation.

Central nervous system.—A large area of thalamic necrosis was observed during the 5th week of the disease (Fig. 12). Several necrotic foci and perivascular cuffing were also seen during the 7th week.

Lungs.—Congestion, granulomata and occasional haemorrhages were observed throughout the test period.

Other organs, including skeletal muscle, were unaffected.

DISCUSSION

Although the present changes resemble in general those seen after reovirus type 3 infection in neonatal mice (Walters *et al.*, 1963 ; Stanley *et al.*, 1964), there are several important differences, notably affecting the appearances in the lungs and heart.

Pulmonary lesions were frequent in infection with reovirus types 1 and 2, showing a characteristic histopathological appearance. Proliferation of bronchiolar (or alveolar) cells following reovirus infection and their subsequent necrosis resulted in eosinophilic detritus similar to the appearances in hyaline membranes (Fig. 6–8). Barter (1959, 1962) has commented on the pathogenesis of these membranes in human material, and postulated an origin from proliferation and subsequent necrosis of alveolar epithelial cells. The present observations agree well with this concept. It should also be noted that pulmonary lesions were frequent despite a non-respiratory route of infection, suggesting a blood-borne route of infection.

Myocardial necrosis was also frequent and severe in the present experiments, and it seems probable that a proportion of the deaths in the acute stage were the result of heart failure. This heart failure may well have contributed to the profound centrilobular congestion present in the liver (Fig. 2). As with type 3 infection, the myocardial lesions resemble those described by Grosberg and Gerstl (1961), and in both cases stain intensely by the periodic acid-Schiff method.

Necrosis of brown fat in the cervical region is a further point of difference between type 3 reovirus infection, and the chronic phase of type 1 infection. van Tongeren (1957) has also mentioned this finding, which links reovirus infection with the changes seen in animals following poliomyelitis and experimental enterovirus infections (Bodian, 1955).

Conversely, lesions in the central nervous system and skeletal muscle were sparse in the present studies compared with those seen in murine infection with reovirus type 3.

These results again demonstrate the relation between virus strain and the organ specificity of the morphological changes produced.

Of more general importance is the demonstration of continued microscopic lesions in animals which, having survived the acute phase of infection, were clinically and macroscopically normal, and whose weight gain was indistinguishable from that of control mice. This subclinical disease has analogies in other situations. It suggests an experimental model of the situation seen in chronic hepatitis in man, where a viral aetiology has also been postulated but where a history of acute hepatitis is seen in only a proportion of cases (Saint, King, Joske and Finckh, 1953). The situation differs from that seen in scrapie in sheep in the absence of clinical manifestations of an active disease (Palmer, 1957).

As with type 3 infection, there was an apparent recrudescence of the disease at about the 4th week in experiments with " partially adapted " virus.

With all strains, lesions of histologically acute type continued to appear even in the chronic stage of the disease. The frequent mitoses and nuclear abnormalities seen in the liver in the chronic disease (after 35 days) might relate to the similarity between messenger RNA and reovirus (Langridge and Gomatos, 1963) and to the association of reovirus with the spindle apparatus (Dales, 1963 ; Spendlove, Lennette and John, 1963). However, to date none of our mice with reovirus infection have developed neoplastic change.

The virus was isolated in the chronic stage from 5 of 30 animals (see Tables), providing evidence that the chronic disease is at least in part the result of continued virus infection. Most mice in the chronic stage had high titres of antibody to the specific infecting strain but not to other strains of reovirus. The virus may persist in some site (? intracellular) where it is not fully accessible to antibody. It may be noted that all virus isolations were from the central nervous system or gut, and not from the liver. It is, however, still problematical whether a chronic virus infection is alone sufficient to account for the observed changes, or whether some other process, possibly autoimmune, must be considered.

Finally, there was a striking resemblance between the liver lesions in chronic type 1 infection in mice (Fig. 4), and those seen in an infant girl dying of hepato-encephalomyelitis associated with reovirus type 1 (Joske, Keall, Leak, Stanley and Walters, 1964, Fig. 3), although the time relationships are different.

SUMMARY

Murine infection with reovirus types 1 and 2 produces a disease generally similar to that found with reovirus type 3, although cardiac and pulmonary lesions are more prominent and nervous system changes less prominent with types 1 and 2 infection. The histopathological appearances are generally similar with all types, consisting of a recurring granulomatous disease affecting principally liver and pancreas. Types 1 and 2 infection produce a chronic disease in mice which are clinically and macroscopically normal, but with extensive microscopic changes as late as the 56th day after inoculation. Virus was isolated from 5 of 30 mice with chronic disease, despite high titres of specific antibody. In such instances, virus was found in low concentration only in brain or gut and not in liver up to the 42nd day after infection. Infective virus was not demonstrated in any tissue after the 42nd day.

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