MURINE INFECTION WITH REOVIRUS: I. PATHOLOGY OF THE ACUTE PHASE*

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LITTLE is known about the natural history of reovirus infection or the association of infection in man with any well-defined clinical syndrome. The three antigenic types so far described appear to be sufficiently ubiquitous to make any attempt at association with clinical illness difficult (Stanley, 1961*b*). Although reoviruses have been isolated from the faeces of healthy children, evidence is accumulating to suggest an association with infections of the respiratory and enteric tracts (Sabin, 1959; Rosen, Hovis, Mastrota, Bell and Huebner, 1960; Ferris, personal communication). In addition, reoviruses have been isolated from children with steatorrhoea (Sabin, 1959; Stanley, 1961*b*) and more recently from patients with hepatitis and encephalitis, including one fatal case (Joske, Keall, Leak, Stanley and Walters, unpublished). It is of great interest that nearly all the clinical features of infection described by Stanley, Dorman and Ponsford (1953, 1954) in mice have now been observed in humans, namely—pneumonia, steatorrhoea, alopecia, hepatitis and encephalitis. For this reason and because little is known of the nature of the lesions in mice or man except for the early work of Van Tongeren (1957), it was decided to carry out a detailed study of the infective process in mice.

Van Tongeren (1957) made a limited histopathological investigation of infected mice in his study of a familial infection with hepato-encephalomyelitis virus (reovirus type 3). He described hepatocytic necrosis and degeneration, with associated periductal and perivascular inflammation. Cerebral, cerebellar, pontine and quadrigeminal lesions of neuronal necrosis, glial-cell proliferation and slight perivascular cuffing were also found after subcutaneous inoculation of a faecal suspension of the virus. No meningitis or neuronophagia was observed.

After three alternate mouse-egg passages of the virus myocarditis and myositis appeared in some animals. A further passage increased the strike rate to 90 per cent and discrete foci of necrobiosis in the brown fat of the shoulder girdle also appeared.

No pancreatic lesions were observed.

He also observed increased numbers of giant cells in bone marrow and the mononuclear infiltration of renal glomeruli, which were not a feature of our material.

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The prototype strain of type 3 originally referred to as hepato-encephalomyelitis virus (HEV) when the strain was first isolated and described (Stanley *et al.*, 1953), was selected as being the most suitable for this study because—type 3 strains consistently produce a characteristic clinical picture after infection of mice; the mice may be infected by the oral route with low concentrations of virus; and the quantitative distribution of this strain (HEV) in the organs of mice at different times after infection has already been defined (Stanley *et al.*, 1953).

MATERIALS AND METHODS

Virus strain.—Type 3 prototype strain HEV : the 50 per cent infective dose (ID_{50}) by the oral route in infant mice was 0·01 ml. of a $10^{-5.5}$ dilution of the stock suspension. The ID_{50} by the intraperitoneal route was 0·05 ml. of a 10^{-7} dilution.

Mice.—Prince Henry strain (Stanley *et al.*, 1954). Seventeen litters (136 infant mice) were inoculated orally with virus and two litters (16 mice) were inoculated with Hanks' salt solution (HSS) alone as controls. All infant mice were inoculated at approximately 24 hr. after birth. The litters were pooled, mixed and evenly redistributed to the mothers prior to inoculation.

In daily observations the following signs were looked for : peritoneal exudate, retardation of growth, emaciation, uncoordination, tremor, paralysis, jaundice, oily hair effect (OHE) and alopecia. After inoculation mice were observed daily and touched only with sterile forceps. Mice that were removed were weighed, killed by ether and examined post-mortem. Mice dying during the course of the experiment were discarded. Some animals were fixed *in toto* in Heidenhain's Susa and some in 10 per cent formalin (pH7).

Paraffin sections of Susa-fixed material were cut at 5 μ and stained by the following methods : Harris' haematoxylin and eosin, Heidenhain's iron haematoxylin, Gordon and Sweet (reticulin), Periodic acid-Schiff (PAS), Lendrum's phloxine tartrazine, Aniline blue-chromotrope, and von Kossa. Frozen sections of formalin-fixed tissue were stained for fat with Oil Red O.

Sections of the following tissues were examined : brain, thoraco-cervical cord, sacrospinalis muscle, thymus, lung, trachea, heart, oesophagus, stomach, small intestine, colon, liver, pancreas, spleen, vertebral bone and marrow, adrenals, kidneys, bladder and dorsal skin. The salivary glands were examined only in the oral inoculation experiment.

EXPERIMENTAL

Experiment I. Oral Inoculation. Series 1a

In this series, together with series 1b (*vide infra*), the mice were observed for 27 days, during which 43 test and 10 control animals were removed for post-mortem and histopathological examination. The inoculum consisted of a 10^{-2} dilution of stock virus in HSS.

The outstanding clinical feature was retardation of growth (Fig. 1) which was apparent 10 days after inoculation. Thereafter animals showed progressive emaciation. The most frequent phenomenon, occurring consistently after 7 days, was OHE (Fig. 2), accompanied by and probably due to production of faeces with a high fat content since the mothers also showed OHE. This was followed by tremor and uncoordination associated with the development of encephalitis. All signs were maximal between the 8th and 15th days, during which time 5 animals died. Alopecia was not observed in this experiment.

Macroscopic appearances at autopsy

The animals killed after 7 days were wasted and showed OHE. The brain was swollen and congested after the 10th day. In the abdomen a peritoneal exudate, either bile-stained or haemorrhagic, was frequently present. Affected livers were enlarged, dark in colour with small circular yellow lesions measuring up to 3 mm. in diameter on all surfaces (Fig. 3). After the first week the gut appeared reddened and distended and in 2 animals was grey in colour. In some animals the heart showed small circular grey epicardial foci. Haemorrhagic areas were occasionally found in the lungs. The other viscera were normal macroscopically.

Histopathology

Liver.—The liver appeared normal during the first 3 days after inoculation of the virus. On the 4th day lesions appeared beneath the capsule and near the centrilobular veins. They consisted of occasional spherical aggregations of proliferating mononuclear cells and a few segmented leucocytes (Fig. 4). The aggregates caused pressure atrophy of hepatocytes, some of which showed eosinophilic degeneration of their cytoplasm. The mononuclear cells were small and possessed an oval to round vesicular nucleus, occasionally with a prominent nucleolus. Their cytoplasm was scanty and difficult to distinguish from the pale pink ground substance.

Midzonal and centrilobular eosinophilic necroses with polymorphonuclear leucocytic and histiocytic infiltration at the periphery occurred on the 5th day (Fig. 5). In some foci cystic change developed due to lysis of the eosinophilic coagulum. The cellular aggregates were increased in size and number and were associated with hepatocytic necrosis. A few small, dense, globular, eosinophilic bodies, often containing nuclear remnants, were observed either

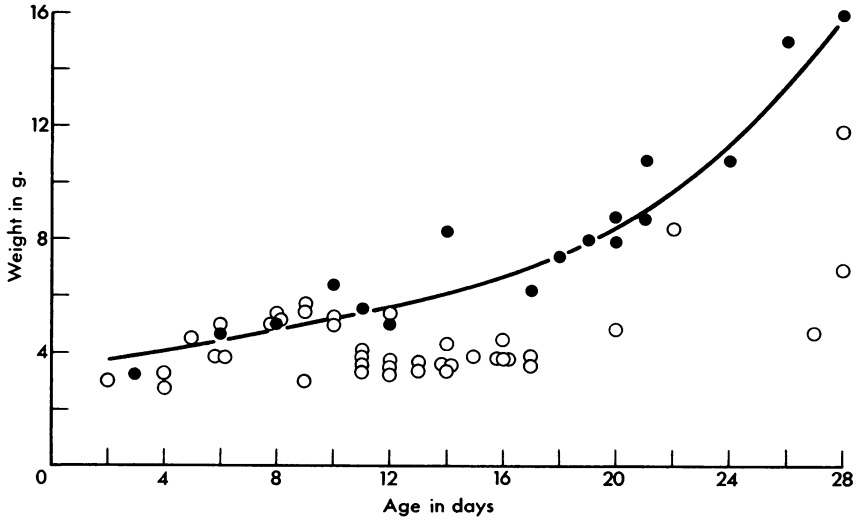


FIG. 1.—Graph showing retardation of growth in infected mice. ● = uninoculated mice ○ = inoculated mice.

lying free or within macrophages (Fig. 6). These were similar in appearance to the Councilman bodies of infectious hepatitis and other viral infections of the liver.

Other parenchymal cells were bi- and multi-nucleated but mitoses were infrequent. Many sinusoids were distended by protein-rich fluid, while others were occluded by small collections of leucocytes.

By the 6th day foci of proliferating mesenchymal cells were present in all lobular zones. These varied in size from those associated with recent necrosis of one or more hepatocytes to larger areas involving considerable cellular necrosis with coalescence of adjacent foci in neighbouring lobules. Mitoses and other cellular regenerative phenomena were still present.

Augmentation of these lesions was apparent on the next (7th) day. The areas of mesenchymal proliferation appeared as roughly-circular, oedematous granulomata composed of a few segmented leucocytes, many macrophages and lymphocytes admixed with oval cells with large, vesicular, pale nuclei and the debris of hepatocytic necrosis. Councilman bodies were prevalent, appearing small, round, hyaline and densely eosinophilic, but sometimes containing basophilic detritus.

Cellular degeneration and necrosis was widespread. The hepatocytes swelled to approximately 3 to 4 times their usual size. The nucleus remained normal in its dimensions but was less basophilic. The cytoplasm showed granular eosinophilic degeneration, being broken up

into small pink, circular globules or bubbles (Fig. 7). These condensed or else enlarged and the remaining cytoplasm became hydropic. The eventual appearance was that of a ballooned and transparent cell, divided by fine eosinophilic septa, and possessing a pyknotic nucleus with a few globules and flecks of eosinophilic material in its cytoplasm.

Other cells showed coagulative necrosis. Such necroses were small or of the zonal type with faint outlines of the cell and nuclear membranes still present (" ghost lobules ") (Fig. 8). Hyperplasia of sinusoidal histiocytes was pronounced and a few central veins had thickened walls which were infiltrated by leucocytes.

The changes on the 8th day were similar, although more widespread necrosis had led to parenchymal disarray.

On the 9th day every lobule showed at least one lesion, varying from large areas of eosinophilic coagulative necrosis (Fig. 9) to isolated ballooned or necrotic cells. Acidophilic bodies were plentiful and when very small mimicked the appearance of inclusion bodies even as far as staining purple-red with the phloxine-tartrazine reagent. Hyperplasia of Kupffer cells and sinusoidal collections of macrophages, lymphocytes, polymorphs and debris were pronounced. Portal tracts and central veins were inflamed.

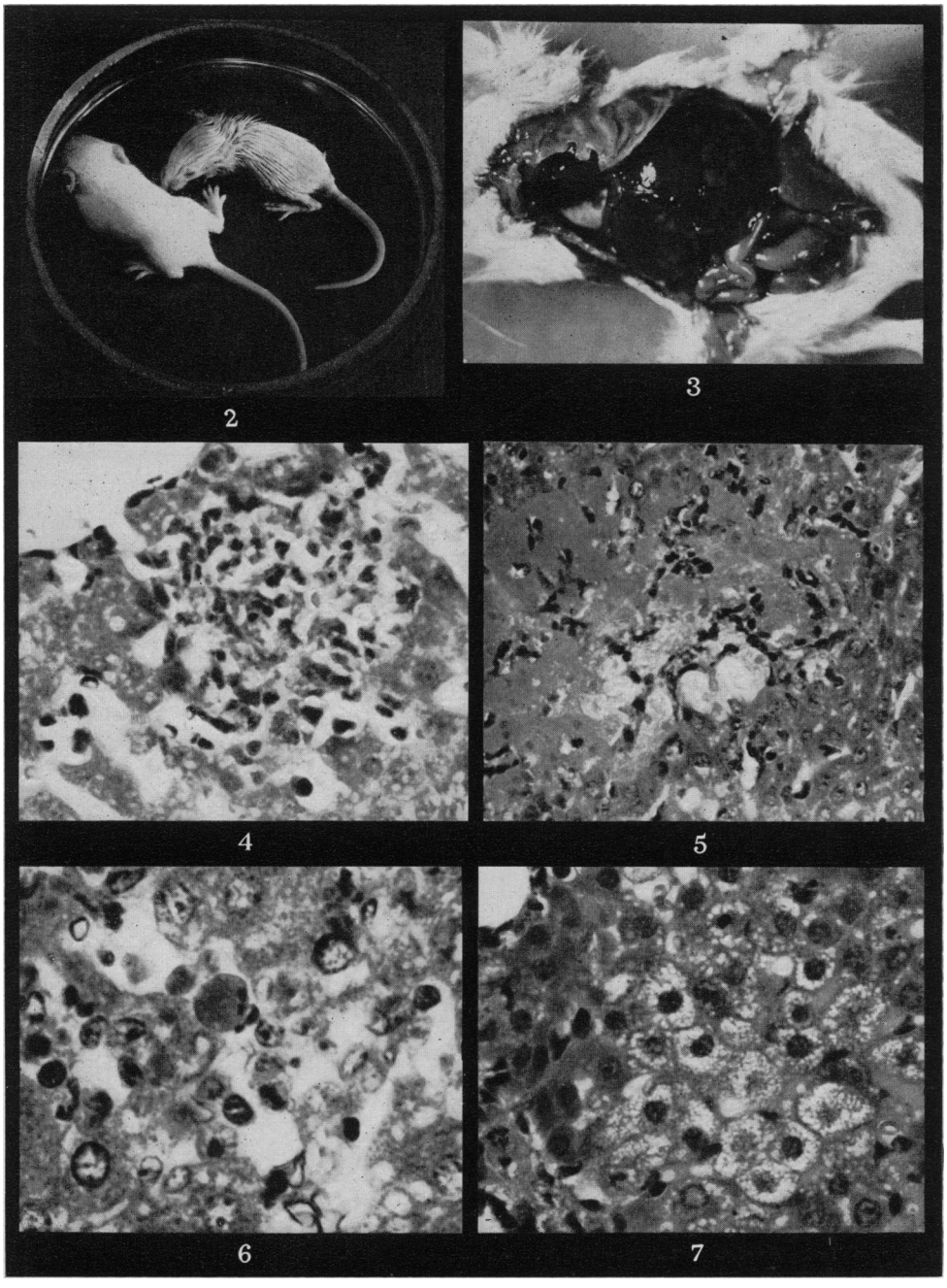
By the 14th day lysis of many necrotic cells in the larger areas of zonal necrosis had occurred, the spaces being filled with fibrin, erythrocytes and eosinophilic remnants. Peripheral organization of these lesions had begun, but fresh necroses still occurred and large bands of oval mononuclear cells traversed the lobules. Capsular depressions overlay areas of necrosis. Regenerative activity was still marked.

No evidence of retention of bile was seen throughout the entire experiment.

Pancreas.—By the 2nd day after inoculation occasional acinar cells showed cytoplasmic basophilia despite loss of zymogenic granules and nuclear lysis. Slight dilatation of most centroacinar ducts and small ductules was present. These changes became more pronounced on the next day and both cytoplasmic vacuolation and granular eosinophilic degeneration

EXPLANATION OF PLATES

FIG. 2.—Litter mates. One mouse shows oily-hair effect.
FIG. 3.—Macroscopic appearance of liver in mouse killed 11 days after oral inoculation.
FIG. 4.—Liver day 4. Earliest lesion near central vein. Haematoxylin and Eosin. ×400.
FIG. 5.—Liver day 5. Coagulative necrosis. H. and E. ×250.
FIG. 6.—Liver day 6. Eosinophilic body containing nuclear remnants lying in a hepatic sinusoid. H. and E. ×250.
FIG. 7.—Liver day 6. Hepatocytic swelling and eosinophilic bubbling of cytoplasm. H. and E. ×400.
FIG. 8.—Liver day 8. Zonal necrosis (" ghost lobule "). H. and E. ×250.
FIG. 9.—Liver day 9. Eosinophilic zonal necrosis. H. and E. ×100.
FIG. 10.—Pancreas day 10. Eosinophilic lobular acinar necrosis. H. and E. ×75.
FIG. 11.—Heart day 8. Early focal myocardial necrosis. H. and E. ×184.
FIG. 12.—Heart day 12. Basophilic clumping of necrotic myocardial material. Von Kossa. ×184.
FIG. 13.—Skeletal muscle day 7. Early basophilic fascicular necrosis. H. and E. ×210.
FIG. 14.—Sketetal muscle day 10. Myositis. H. and E. ×250.
FIG. 15.—Skeletal muscle day 14. Basophilic clumping of necrotic muscular material. H. and E. ×130.
FIG. 16.—Liver day 15. Cystic degeneration in an area of zonal necrosis. H. and E. ×250.
FIG. 17.—Liver day 18. Cystic area of necrosis containing numerous acidophilic bodies. H. and E. ×250.
FIG. 18.—Liver day 24. Hepatocytic regeneration. H. and E. ×130.
FIG. 19.—Liver day 24. Hepatic regeneration. Mitoses. H and E. ×100.
FIG. 20.—Liver day 24. Proliferation of oval cells. H. and E. ×400.
FIG. 21.—Liver day 25. Hepatocytic regeneration showing proliferation of oval cells in relation to an area of zonal necrosis. H. and E. ×240.
FIG. 22.—Pancreas day 11. Pancreatitis. H. and E. ×100.
FIG. 23.—Cerebrum day 12. Neuronal hydropic degeneration and satellitosis. H. and E. ×220.
FIG. 24.—Cerebrum day 6. Eosinophilic bubbling of neuronal cytoplasm mimicking inclusion bodies. H. and E. ×400.



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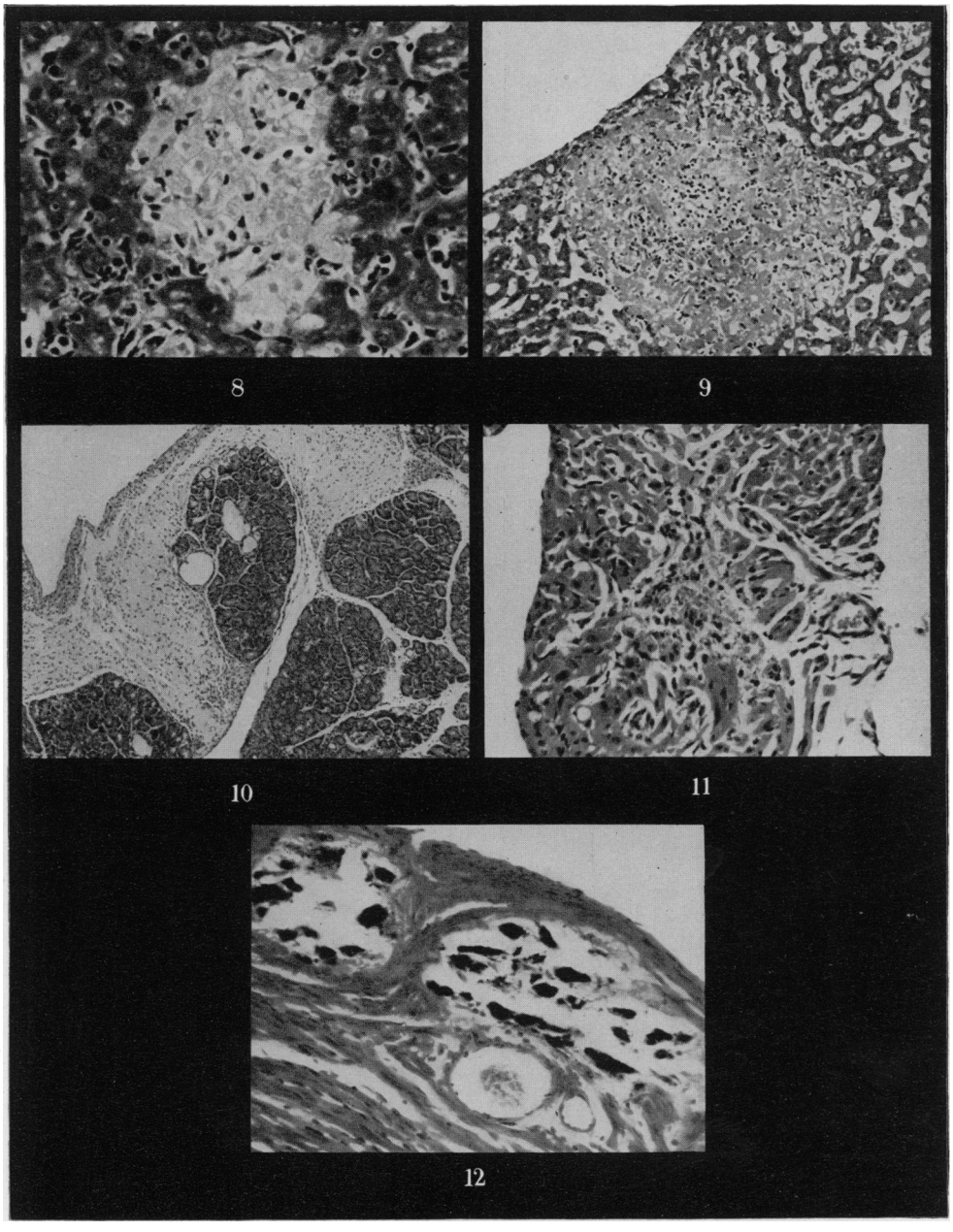

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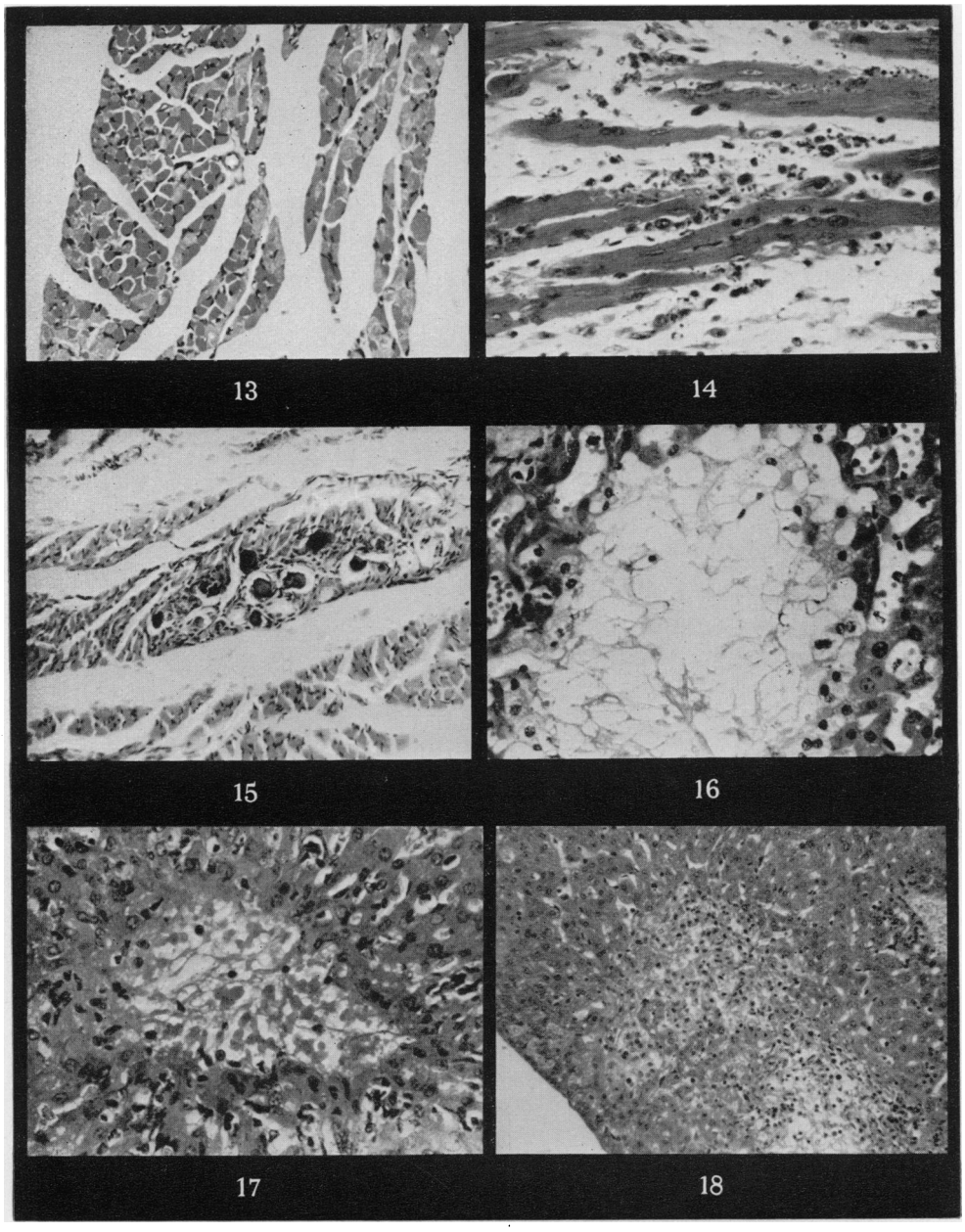

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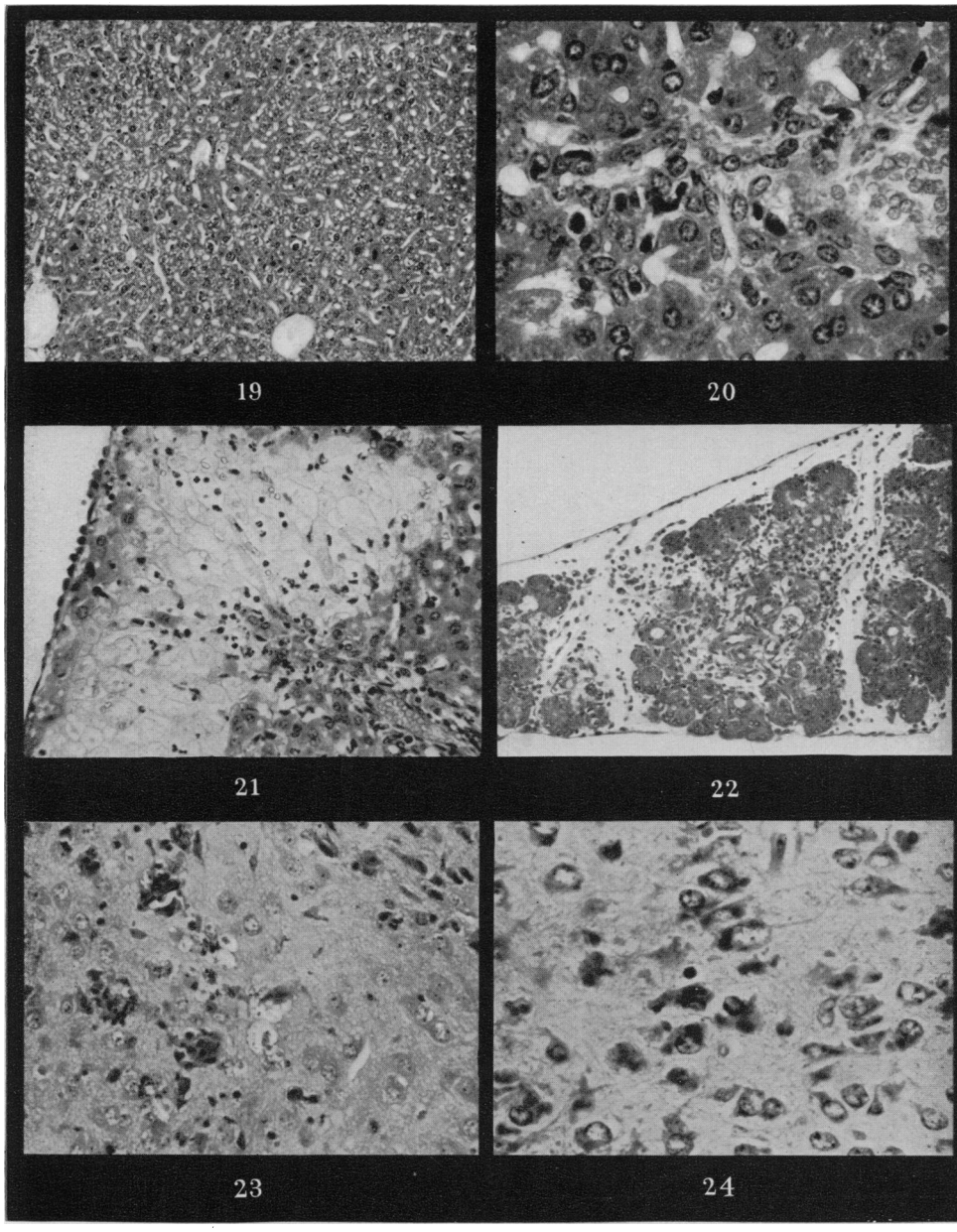

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occurred. Some acinar cells were necrotic and appeared as dense, eosinophilic globules which still retained nuclear residues. Ductular cells were prominent.

These changes persisted to the 6th day. Only a meagre leucocytic response to the now more-widespread glandular degeneration and necrosis was evident and the viscus continued to increase in size.

By the 7th day every lobule possessed at least one necrotic acinus. The centro-acinar ducts were now moderately dilated with atrophy of the acinar cells. Ductules were greatly dilated and lined by flattened cells. The tissue was oedematous and a moderate infiltration with polymorphs, lymphocytes and macrophages found.

On the following day necrotic acini were replaced by amorphous eosinophilic debris and a little areolar tissue. Necrosis of the cells lining larger pancreatic ducts was seen, the desquamated cells appearing as spherical hyaline bodies, similar in appearance to Councilman bodies in the liver.

Cellular vacuolation, nuclear swelling and intense basophilia of the basal perinuclear portion of the acinar cells occurred on the 9th day.

On the 10th day whole lobules showed eosinophilic necrosis (Fig. 10), while in those remaining the acinar cells were basophilic apart from a small eosinophilic tip. Crystalline material condensed out in sheaves in these basophilic areas.

Some necrotic acini had undergone lysis and here only an interstitial exudate composed of polymorphs, lymphocytes and spindle-shaped cells was seen. In the larger ducts necrotic columnar cells were replaced by oval ductular-like cells. Hyperplasia of ductular cells was associated with small foci of eosinophilic acinar necrosis.

Two weeks after inoculation there was total basophilic necrosis of the pancreas with shrinkage in bulk of this tissue.

The islets remained normal throughout the entire period.

Salivary glands.—The changes were comparable to those observed in the pancreas. Epithelial degeneration and necrosis, ductular hyperplasia and mild chronic inflammation occurred in small foci in most lobules.

Heart.—No changes occurred in the first week. Lesions consisting of small necrotic foci in the papillary muscles of the left ventricle were seen in one animal of 4 slaughtered on the 8th day. They were composed of structureless and pale eosinophilic granular fragments of necrotic myocardial cells associated with oedema, nuclear and cytoplasmic debris, a little fibrin and a scant infiltration of lymphocytes and macrophages (Fig. 11).

On the next day all animals showed more foci, which were larger and found not only beneath the endocardium but also deep within the myocardium and on the epicardium of both ventricles. A fine basophilic stippling of the cytoplasm of many muscle cells was apparent and numerous eosinophilic hyaline bodies were found. These combined with the phloxine-tartrazine dye.

By the 10th day the necrotic foci were still extending but the granular necrosis was now coarse and markedly basophilic. Fresh foci appeared in which the myocardial fibres swelled, lost their striations and became fragmented. The nuclei underwent lysis, karyorrhexis or pyknosis. Sometimes the fragmented cytoplasm was diminished to form small hyaline bodies, but more frequently appeared as tiny circular basophilic granules. The cellular response was meagre and lymphocytic and histiocytic in nature.

Over the superficial foci swelling of pericardial serosal cells was seen on the 10th day. The centres of many foci appeared less dense and small capillaries together with a few fibroblasts were found.

Clumping of necrotic debris into small roughly-circular, intensely-basophilic bodies was seen on the 12th day. These bodies were stained black by the von Kossa technique (Fig. 12) and were mauve-red with the PAS reagent.

By the 13th day the heart appeared normal.

Skeletal muscle.—The muscle of the sacrospinalis group remained normal until the 6th and 7th days, when many fasciculi, either singly or in isolated groups, showed a coarse eosinophilic granulation. In other areas the muscle bundles became swollen, fragmented and lost their striations. Their staining was at first pale pink but later became slightly basophilic (Fig. 13). These changes were unaccompanied by an inflammatory cellular exudate.

Over the next week the necrotic muscle became deeply basophilic and developed at first fine and then coarse granules which were black with the von Kossa reagent. Segmented leucocytes, lymphocytes, macrophages, fibroblasts and pyknotic sarcolemmal cells were not infrequently seen amongst the necrotic muscle at this stage (Fig. 14).

At 14 days the basophilic granules had clumped into roughly-circular intensely-basophilic bodies identical in appearance to those seen at this time in the myocardium (*vide supra*) (Fig. 15). Shrinkage of some bundles was evident.

Central nervous system.—No histopathological changes were observed in either the brain or thoraco-cervical cord throughout the first 8 days after oral inoculation. On the 9th day the brain became congested and neurones both singly or in small scattered groups showed degenerative and necrotic changes. The neurones were swollen with their cytoplasm staining deeply eosinophilic. This change sometimes involved the whole cytoplasm but in other cells the coagulum was aggregated into small globular clumps giving an appearance of eosinophilic bubbles (see Fig. 24). Occasional segmented leucocytes were seen associated with nuclear or cytoplasmic debris in some of the larger necrotic foci. These changes were most prominent in the brainstem and the cerebral hemispheres. Neither cord nor cerebellum were involved at this stage.

By the 10th day the changes were more florid and perivascular cuffing of capillaries by polymorphonuclear leucocytes appeared together with neuronal satellitosis. The leptomeninges were infiltrated with round cells and leucocytes. By the 14th day the encephalitis was more diffuse and severe, and several small haemorrhages were found in areas of necrosis. Inclusion bodies taking up the phloxine tartrazine reagent were present in many affected neurones (see Fig. 24).

In the thoraco-cervical cord only an occasional neurone showed eosinophilic cytoplasmic bubbling.

Lungs.—In both experiments approximately one half of the animals showed patchy areas of alveolar haemorrhage and pulmonary oedema without any leucocytic reaction. This was found from the second day onwards, but its occurrence was unpredictable. The lesion was not observed in control animals.

Alimentary canal.—The changes were similar in all groups and were thought to be autolytic. They consisted of dilatation of the central lymphatics of intestinal villi together with distension of submucosal lymph channels.

Spleen.—Some animals showed follicular hyperplasia.

Skin.—One animal on the 26th day showed reduction in hair follicles, oedema and subepidermal infiltration with polymorphs, lymphocytes and macrophages.

The remaining organs examined, including the thymus, were normal histologically.

Experiment I. Oral Inoculation. Series Ib

The inoculum consisted of a 10^{-3} or 10^{-4} dilution of stock virus in HSS. The animals receiving these dilutions of virus were considered in one group as the lesions were comparable, despite the difference in dose.

Histopathology

Liver.—The first animals were examined 11 days after oral inoculation and when compared with the lesions of animals in experiment I*a* the whole process was less florid.

By the 15th day the granulomata appeared more organized but still very cellular. Cystic change in the necrotic areas became the dominant feature by the 18th day (Fig. 16). The cysts appeared empty or contained fibrin, cellular debris, or hyaline bodies simulating inclusions (Fig. 17). Oval cells (" bile ductular cells ") were found proliferating in other cystic foci.

On the 20th day large areas of eosinophilic necrosis were obvious and appeared either as " ghost lobules " or contained an amorphous acidophilic coagulum devoid of cellular detail. In these areas there was often loss of the reticulin framework. Parenchymal collapse, shrinkage and early organization had led to capsular depressions. Regenerative phenomena involved the parenchymal cells and rows of new hepatocytes began to invade the necrotic areas (Fig. 18). This regenerative activity was profound on the 24th day and binucleate hepatocytes, cells with giant nuclei and showers of mitotic figures were seen in every lobule (Fig. 19). Necrosis was still present and ballooned cells and cells showing eosinophilic cytoplasmic bubbling were numerous. Proliferation of oval cells was pronounced. These cells occurred in clusters, often with tiny lumina, and appeared to insinuate between the hepatic cords or to replace them. They arose from the regions of the portal tracts and showed

marked mitotic activity. Other oval cells arose from the centrilobular zones and appeared unrelated to pre-existing ductules (Fig. 20).

The changes on the next day were similar with increasing repair of the cystic areas by columns of oval cells and small hepatocytes migrating into these foci (Fig. 21). The reticulin framework in the cystic areas became condensed and appeared related to the presence of the oval cells. In a few areas pericellular collagen was seen.

Although on the 26th day isolated necrosis of cells was still in evidence, regeneration was the dominant feature, and all cystic foci and ghost lobules showed either multiplication of hepatocytes or oval cells from the periphery. Cellular bands composed of round and oval cells traversed the lobules and joined these healing areas. Virtually no collagen was deposited in association with this change, yet the parenchymal architecture was distorted by segments of hepatic tissue being cut off by these cellular bands. Capsular depressions were also frequent.

Pancreas.—By the 11th day the pancreatic exocrine tissue showed centro-acinar and ductular dilatation with concomitant degeneration and necrosis of glandular cells. This occurred in single cells, in groups of cells and acini, or as a lobular eosinophilic necrosis appearing as pale pink amorphous masses similar to the lobular eosinophilic necrosis described in affected livers (see Fig. 10). A pronounced inflammatory exudate composed of segmented leucocytes, lymphocytes, plasma cells and macrophages was seen related to these areas of necrosis in the interstitium and in the tissues surrounding the pancreas (Fig. 22). Amorphous faintly blue-staining areas of fat necrosis were present in other areas.

Similar morphological changes were seen throughout the next week.

Heart.—Minimal necrotic changes occurred in animals receiving the smaller doses.

Skeletal muscle.—The changes were similar to, but less marked than, those in series I*a*.

Central nervous system.—On the 11th day the brain was normal in appearance, but by the next day several small and scattered foci of neuronal degeneration and necrosis had developed in the cerebral hemispheres. Pronounced ballooning of some of the affected neurones was also present (Fig. 23). Nearby capillaries were engorged and the perivascular spaces filled with segmented leucocytes and lymphocytes.

Four days later small petechial haemorrhages, a meagre cellular exudate comprising polymorphs and round cells, and neuronal satellitosis had been added to the basic lesion of neuronal degeneration. The foci appeared larger and more numerous and were seen not only in the hemispheres, but also in cerebellar nuclei, hypothalamus and pons.

The reaction appeared less florid from this stage until the 25th day although animals slaughtered at this time showed some variation.

No change was found in the thoraco-cervical cord in this group.

Experiment II. Intraperitoneal or Intracerebral Inoculation

The inoculum consisted of a 10^{-2} dilution of stock virus in HSS. Two litters were inoculated by the intraperitoneal route and two by the intracerebral route. Mice were removed at daily intervals for post-mortem examination. All mice again showed retardation of growth and emaciation (see Fig. 1). Signs of encephalitis appeared on the 5th day after inoculation and many mice died soon after. The OHE was not observed. Macroscopic appearances did not differ significantly from those observed after oral inoculation.

Histopathology

Liver.—The first changes after intraperitoneal inoculation were seen on the 4th day and were the same as those found after oral inoculation. After one week the stigmata of the type of viral hepatitis already described in experiment I were present.

After intracerebral inoculation changes were again first seen on the 4th day and by the 6th day the hepatic lesions were comparable with those in the other groups.

Pancreas.—Pancreatitis appeared after 2 or 3 days in this experiment and the lesions were morphologically comparable to those seen after oral inoculation.

Heart.—Necrosis was observed in only one animal 10 days after intraperitoneal inoculation.

Skeletal muscle.—Necrosis was observed in only one animal 10 days after intraperitoneal inoculation.

Central nervous system.—After intraperitoneal inoculation no changes were seen in the brain until the 6th day when there was early congestion and mild pia-arachnoiditis.

On the 3rd day after intracerebral inoculation the lesions first became manifest and progressed in severity until the 6th day. Widespread severe diffuse meningo-encephalitis was

present. The brain and brainstem were markedly congested and oedematous and many neurones showed clumping of their cytoplasm as well as karyolysis, karyorrhexis and pyknosis. The cytoplasmic and nuclear remnants of these changes simulated inclusion bodies (Fig. 24) and the phloxine tartrazine reaction was positive. There was " falling out " of the ground substance, together with small haemorrhages, satellitosis and infiltration with polymorphs, lymphocytes and macrophages. Perivascular cuffing was marked.

The meningeal vessels were also congested, and fibrin, polymorphs and round cells were present in the subarachnoid space.

Changes in other organs were similar to those described in expt. I.

DISCUSSION

These pathological changes may be related to the concentrations of virus in different organs at differing times. Stanley *et al.*, (1953) using the present virus and the same strain of mice found the titre of virus in the liver reached its peak on the 4th day after intraperitoneal inoculation, thereafter declining progressively. In the present studies hepatic lesions followed this sequence, initial changes being apparent on day 4, while new necrotic foci appeared on all subsequent days. In the brain the virus reaches its maximum concentration slightly later, at day 8 or 9, and pathological changes appeared correspondingly at day 9, reaching their greatest severity at day 14. Detailed virus titres in other organs have not so far been reported. The more severe lesions produced with greater inocula of virus and the varying severity of changes in liver and brain after intraperitoneal and intracerebral inoculation also suggest the changes produced are due to direct viral invasion of cells.

This cellular invasion produced two major morphological sequelae—eosinophilic coagulative necrosis and hydropic degeneration (" ballooning "), of which the first is more frequent and manifests as a variety of subtypes.

These modifications of eosinophilic coagulative necrosis are well seen in the liver in the present experiments. When the process is widespread and involves either the whole or the majority of cells in a lobule, the so-called " ghost-lobules " are produced. These in turn may undergo further degenerative changes, lysis or cystic degeneration, organization (although this is not a feature of the acute stage of the disease), or else regeneration.

When single isolated cells or small groups are affected, a further range of lesions emerges. Sometimes these cells shrivel into dense and deeply eosinophilic hyaline masses often containing nuclear detritus (Councilman bodies) which are found in sinusoids, or necrotic and cystic foci, and sometimes engulfed by macrophages.

The eosinophilic change in affected cells may be either finely or coarsely granular. When fine, the granules are at first eosinophilic but soon become basophilic, and in striated muscular tissue condense and aggregate into large dark-blue spherical globules that are stained black by the von Kossa technique. These spherules are also faintly PAS positive, and in the heart the lesion mimics the basophilic myocarditis described by Grosberg and Gerstl (1961) who related this particular lesion to shock. The coarse granular type (" eosinophilic bubbling ") usually occurs alone, but is sometimes found associated with hydropic degeneration.

The results of stains for inclusion bodies were difficult to interpret, although the Councilman bodies and cells showing coarse granular degeneration took up the stains. It is improbable, however, that these inclusions represent viral

aggregates, being rather cytoplasmic accumulations of degenerative products in the dying cell (Kakulas and Gibb, 1963).

After hepatic necrosis numerous sequelae are possible. Continuing massive necrosis may lead to death of the organism, while at the less extreme end of the spectrum isolated necrotic cells are quickly lysed and replaced by new cells by means of regenerative processes in neighbouring hepatocytes. Fibrosis is another outcome of hepatic necrosis, but in the acute experiments described it did not feature prominently.

Another lesion seen after hepatic necrosis is proliferation of oval or ductular cells, and in our experiments is an important component of the morphological picture. This proliferation is unrelated to biliary obstruction which was entirely lacking in this acute phase of the disease.

Whenever a sufficiently large area of the hepatic lobule becomes necrotic, oval (ductular cell) proliferation accompanied by prolific mitotic activity appears. These cells stream into the degenerated foci, often accompanied by small young hepatocytes, and finally the damaged area becomes filled with these two types of cells as well as leucocytes. In most instances the final result is return of the necrotic area to normal. This will be considered fully in further studies to be published.

Two possible conclusions emerge from this apparent metamorphosis of ductular cells into hepatocytes. The first is that oval cell proliferation is a passive accompaniment of hepatic regeneration, offering no basis for the manufacture of new hepatocytes. When these are formed by the division of surrounding parenchymal cells, the oval cells disappear like the inflammatory cells in some imperfectly determined manner.

The second conclusion is that the proliferation of oval cells plays a major and important role in hepatic regeneration and that these cells make a profound contribution to the numbers of new hepatocytes which eventually fill the breaches on the reticulin framework. This latter view seems to be the correct one and we would therefore join those who believe the so-called " bile-duct " cell capable of generating hepatocytes.

All type 3 reovirus strains we have studied produce the same clinical syndrome in infant mice of the Prince Henry stock. Presumably the microscopic picture of the lesions would be the same. Reovirus types 1 and 2 rarely produce OHE, liver lesions, or steatorrhoea. This marked difference which separates reovirus type 3 from the other two types is augmented by other differences in behaviour. For example, type 3 strains are more often isolated from mice than types 1 and 2 (Joske *et al.*, unpublished). The haemagglutinin of type 3 viruses is distinct from that of types 1 and 2 and from that of any other virus yet described (Gomatos and Tamm, 1962). Although we have confirmed some of the observations of Gomatos and Tamm (1962) with regard to the haemagglutination of ox erythrocytes, we have not correlated the affinity of virus for such cell receptors with the pathological picture.

Nearly all strains of reovirus isolated in Australia have been recovered from infants and children (Joske *et al.*, unpublished). Although many of the children had mild respiratory or intestinal tract infections some had steatorrhoea and others hepato-encephalitis. The one fatal human case of hepatitis and encephalitis which we have studied showed lesions remarkably similar to those described for mice in this paper. (This case will be reported in full, elsewhere.)

In any discussion of hepatitis in man, therefore, it is necessary to consider reovirus type 3 as a possible causal agent as well as hepatitis *A* and *B* viruses, the agent(s) of infectious mononucleosis, and other viruses which occur less frequently in temperate zones. In certain tropical areas diseases such as yellow fever must also be included. In neonatal hepatitis, herpes simplex and cytomegalic inclusion disease viruses must be grouped with reovirus type 3 as agents known to cause hepatitis in man.

The wide range of organs showing pathological changes in murine infection with reovirus type 3 makes this agent comparable with the virus causing human hepatitis. Hepatitis *A* and *B* infection may result in encephalitis and pancreatitis as well as hepatitis (Joske, 1955). In mumps, encephalitis and hepatitis may occur as well as pancreatitis while all three may be associated with the infectious mononucleosis syndrome. This pantropism linking reovirus type 3 with agents causing human hepatitis further differentiates it from other viruses causing hepatitis in mice such as murine hepatitis virus (Gledhill and Andrewes, 1951), and herpes simplex virus. However, the MHV3 agent of Dick, Niven and Gledhill (1956) has an organ specificity similar to that of reovirus type 3.

One interesting and consistent feature of mice infected with reovirus type 3 is marked retardation of growth. This, together with coagulative hepatic necrosis, OHE, and alopecia is, of course, characteristic of the runting syndrome (graft-host reaction). The implications of this similarity will be discussed in detail in a further communication.

SUMMARY

The histopathological changes occurring in infant mice following oral, intraperitoneal or intracerebral inoculation with reovirus type 3 have been studied during the first 28 days after infection. Changes were observed from the fourth day onwards, affecting chiefly the liver, pancreas and nervous system, and to a lesser extent salivary glands, heart and skeletal muscle. The pathological picture was of focal parenchymal degeneration and necrosis associated with mild inflammation, with clinical manifestations of retardation of growth, ataxia, steatorrhoea and oily hair effect.

The photomicrographs were prepared by Mr. H. Upenieks, University of Western Australia.

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