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Scrapie in Mice: Ultrastructural Observations in the Cerebral Cortex (32680)

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Scrapie is a chronic, progressive, degenerative disease of the central nervous system occurring naturally in sheep and experimentally in goats, rats, hamsters, and mice. The remarkable stability of the scrapie agent in the presence of formalin (1) and heat (1) suggests that the scrapie agent may not be a conventional virus (2). Its resistance to inactivation by ultraviolet light of wavelength specifically absorbed by nucleic acids and its susceptibility to inactivation by electron irradiations are properties that suggest that the scrapie agent may not contain nucleic acid and that its molecular weight is likely to be no greater than 2.0×10^5 which means that its size is probably less than that of any known virus (3).

The work reported in this paper was undertaken to obtain by electron microscopy information of use in elucidating the brain pathology of experimentally induced scrapie in the mouse; in its course a number of previously undescribed structures were observed in the cerebral cortex of scrapie infected mice. It is the purpose of this report to describe these structures.

Materials and Methods. Scrapie agent. The strain of scrapie used was that described by Pattison and Chandler who have reported in detail its isolation from sheep (1) and its adaptation to growth in goats (2) and mice (1).

Source of study materials. At the National

Institutes of Health the scrapie agent was readily transmitted to and maintained in Swiss-NIH mice by brain to brain passage, following procedures already described (4). In the present work saline suspensions were prepared from the infected brains of Swiss-NIH mice at the third and eight passage level and these were inoculated intracerebrally into weanling, specific-pathogen-free, Balb-C mice of a breed developed by L. T. Fitzwater, Animal Production Unit, NIH. Six months post-inoculation when the test mice were in the advanced stages of clinical scrapie their brains were harvested for study. Control material consisted of the brains of specific-pathogen-free mice that were inoculated 6 months previously with a suspension of brain tissue from normal Swiss-NIH mice.

Microscopic techniques. One hemisphere of each brain harvested from scrapie and control mice was frozen at -60°C for future use; the other hemisphere from each mouse was fixed in 3% glutaraldehyde. Small cubes of cerebral cortex were cut from the fixed hemisphere, washed in Millonig's phosphate buffer at pH 7.3 (5), postfixed in 2% osmic acid and embedded in Epon. Thick sections were stained with toluidine blue for examination with the light microscope. Thin sections were mounted on carbon coated grids, stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 1A.

Tests for contaminating viruses. A search

for extraneous agents in scrapie infected and control mouse brains was carried out in 2-day-old suckling mice and in tube cultures of mouse embryo cells employing the same general methods which have proved satisfactory in attempts to recover polyoma and Kilham's "K" (6) viruses from infected mouse tissues. Briefly, the brain hemispheres which were stored frozen at -60°C for 6–10 months were individually triturated in a TenBroeck grinder with sufficient culture medium 199 to give 10% suspensions and these were clarified by spinning at 1000 rpm for 15 min in a horizontal centrifuge. The resulting supernatant fluid was inoculated into 2 litters of 2-day-old mice and into 3 tube cultures of mouse embryo cells. At 14 days postinoculation, the brains of 2 mice in each of the inoculated litters were used to prepare 10% tissue suspensions and these were inoculated into 2 new litters of 2-day-old mice. The remaining mice in the originally inoculated litters and all of the mice in the passage litters were observed daily for 21 days for signs of illness or death. The inoculated cell cultures were observed for 10–14 days when a blind passage was made to new tube cultures of mouse embryo cells. This was done by harvesting the cells and fluids, centrifuging the suspension at 1000 rpm for 15 min to remove debris, and inoculating the supernatant into 3 new tube cultures of mouse embryo cells. The inoculated cultures were examined for 10–14 days for presence of cytopathic change that was not found in the control cell cultures.

Results. Light microscope examination of 29 brains at the low (four) and 4 brains at the high (ninth) passage level showed characteristic (1, 4) extracellular and intraneuronal vacuolation. Large multilobular vacuoles (soap-bubble effect) were in abundance. Light microscope examination of 10 brains obtained from control mice showed only occasional vacuoles which when present were distributed singly and were much smaller than the vacuoles that were seen in the brains of the scrapie infected mice.

Electron microscope study of the cortex of 33 brains obtained from the scrapie infected mice revealed the presence of a number of previously undescribed structures which were not found in the brains of any of 10 control mice. These consisted of (a) enlarged cell processes containing large amounts of particles and rods (b) enlarged cell processes containing a close-meshed network of varicose tubules, and (c) round or oval bodies formed by parallel bands and fibrils.

Cell processes which were strikingly enlarged (5–10 times normal size), were found in the brain cortex of 14 of the 33 scrapie mice examined. In 10 of the low passage and in all 4 of the high passage scrapie brains these processes were partially or almost completely filled with particles and rods ranging in diameter from 320 to 360 Å with electron lucent centers and with walls approximately 95 Å in thickness; the walls were denser in their innermost portion (Figs. 1 and 2). Sometimes it was possible to recognize that the rods were covered with spikes which were perpendicularly arranged at regular intervals (Fig. 2).

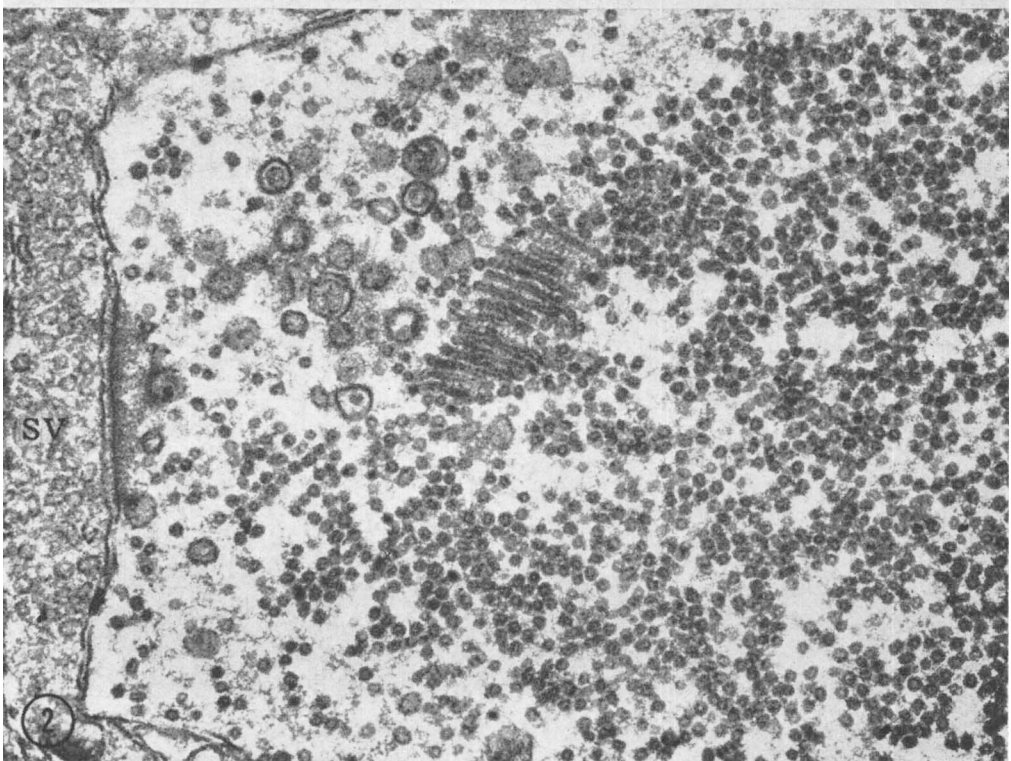
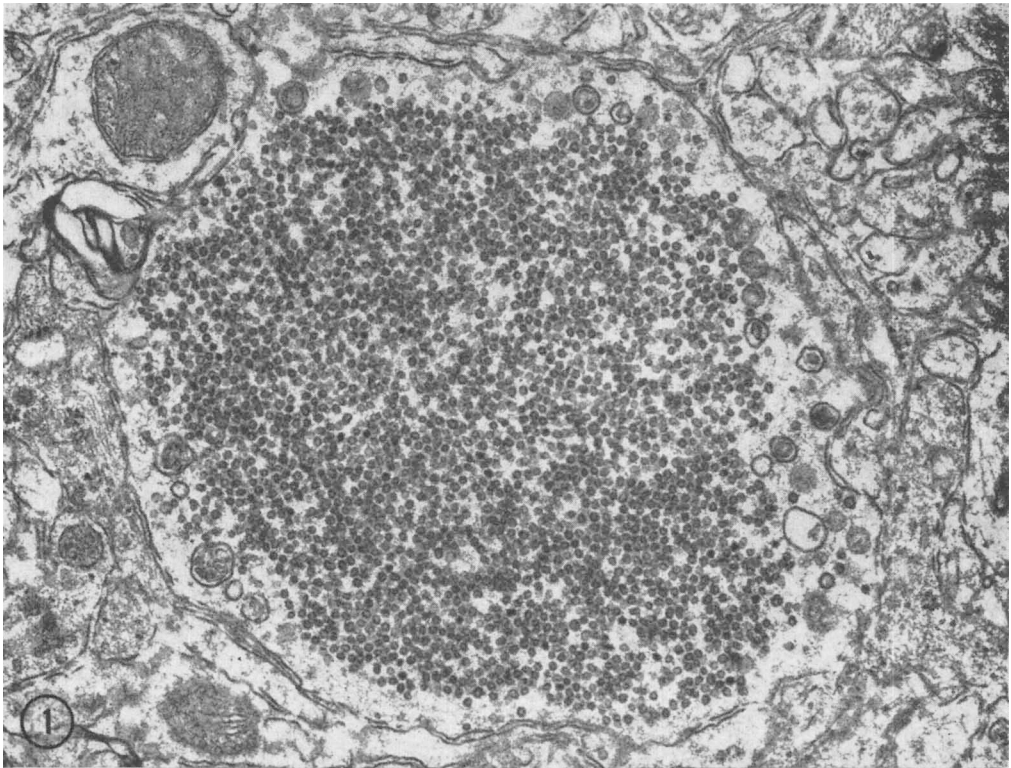
In addition to particles these processes frequently contained some uni- and multi-vesiculated bodies ranging in size from 500 to 2500 Å with walls approximately 100 Å in width (Figs. 1 and 2). The size of the vesiculated bodies varied with the number of contained vesicles. The space within the cell processes which was not occupied by particles, rods, and vesiculated bodies appeared to be empty or filled with filamentous material (Figs. 1 and 2).

The enlarged cell processes were limited by a membrane in which were recognized the three layers characteristic of cell membranes and these made one or more contacts of the synaptic type (Gray type 1) with surrounding cell structures (Fig. 2). The contacts were always established in such way that the enlarged processes containing particles were postsynaptic.

In the brain section of a single mouse,

FIG. 1. An electron micrograph of brain cortex from mice inoculated with scrapie illustrating an enlarged cell process filled with particles. 35,000 $\times$.

FIG. 2. Electron micrograph illustrating part of a process filled with dense particles and rod-like structures. Along the surfaces of the rods, spikes are present. To the left is a synapsis showing synaptic vesicles (SV) on the presynaptic side. 52,500 $\times$.



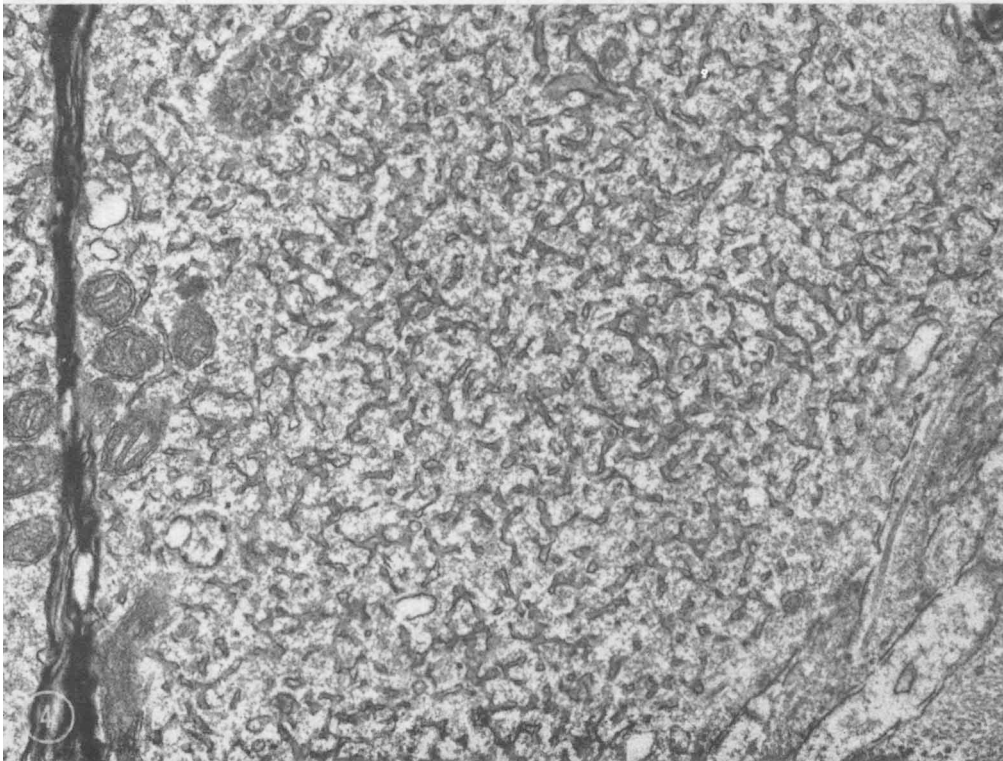
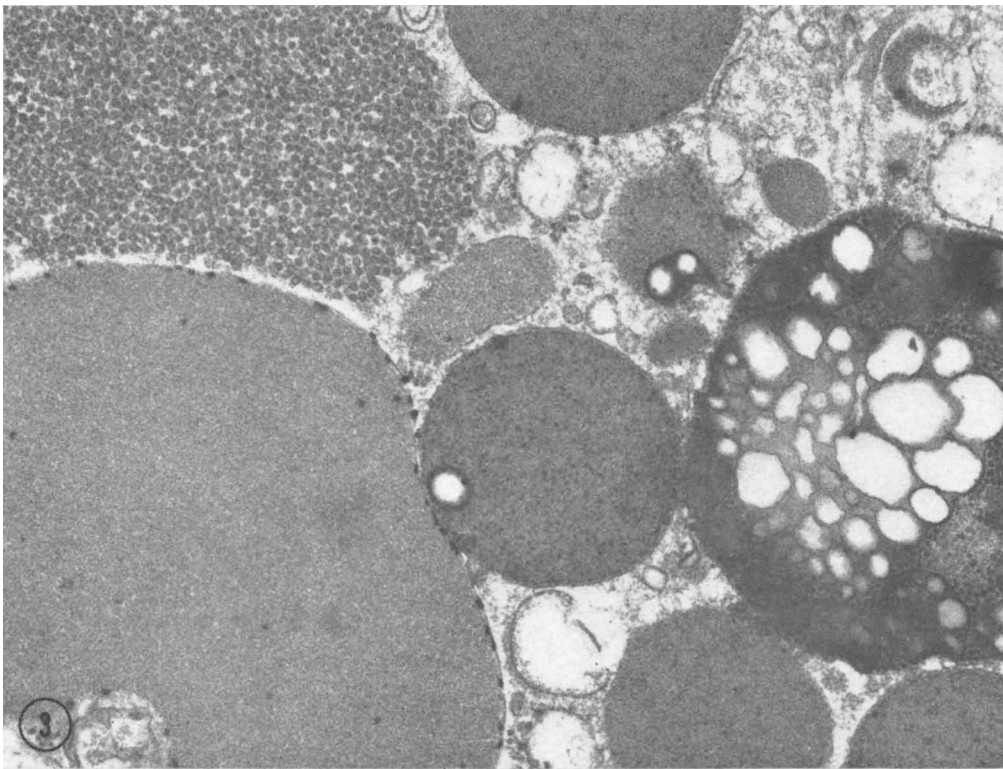


FIG. 3. An electron micrograph showing in the cytoplasm of a cell (glial cell?) a big aggregate of particles together with dense bodies interpreted as phagosomes. 36,000 $\times$.

FIG. 4. Aspects of an enlarged cell process filled with a close-meshed network of varicose tubules containing a dense material. At left a structure formed by myelin-like lamellae. 35,000 $\times$.

particles indistinguishable from those found in these processes were found in the cytoplasm of a cell body (Fig. 3).

In 9 of the 33 scrapie mice examined, strikingly enlarged cell processes (10–30 times normal size) were found which contained a close-meshed network of varicose-tubules 200–500 Å in diameter which were filled with a moderately dense substance (Fig. 4). These enlarged processes contained in addition to the varicosetubules small mitochondria, vesicles, sparse neurotubules and were frequently transversed by an eccentrically situated formation (2 μ long) which was formed by aggregates of myelinlike lamellae (Fig. 4). When synapsis with the surrounding processes were observed they always indicated that the enlarged processes containing varicose tubules were situated in a presynaptic position.

In 9 of 33 scrapie brains examined round or oval bodies consisting of parallel bands 150 Å in width which were separated by empty spaces 50–60 Å wide were found within cell processes (Figs. 5 and 6). They were frequently arranged in concentric rings (Fig. 6). In these instances when synaptic junctions could be recognized the parallel bands were found in postsynaptic processes. In some areas of these bodies another pattern was observed and was constituted by fibrils 50–80 Å wide to which were attached electron dense particles 80 Å in diameter (Fig. 5).

In a few cases, groups of parallel fibrils which were strikingly similar if not identical to those already described were observed inside unidentified cells (Fig. 7).

Failure to detect contaminating viruses in scrapie mouse brain suspension. Suspensions prepared from 33 scrapie infected mouse brains at the fourth (29 brains) and ninth (4 brains) mouse passage level, respectively, were examined in tests conducted in suckling mice and in mouse embryo cell cultures. The tests failed to reveal the presence of polyoma, "K" or any other virus detectable by the procedures employed.

Discussion. The principal aim of this study was to collect information which might add to an understanding of the brain pathology induced by scrapie in the experimentally infected mouse. For this purpose, the cerebral cortex was chosen for study because this brain region of scrapie infected animals has been singularly neglected in previous electron microscope studies of scrapie infected tissues (7–9) although Field *et al.* (7) included the cortex in their electron microscope examination of scrapie infected rat brain. In the present work certain structures were encountered in scrapie infected mouse cortex that were not seen in an extensive examination of appropriate control preparations. While there is no certainty as to how these structures are related directly to scrapie it is worthwhile to bring these findings to the attention of others in order to hasten an evaluation of their significance in the brain pathology in the experimental mouse disease.

The principal changes observed in the cortex of scrapie infected mice were associated in one way or another with synaptic terminals. The strikingly enlarged pre- and postsynaptic cell processes and their contents (particles, rods, bands, fibrils, and varicose tubules) are not likely to be undescribed normal components in the brains of Balb-C mice of the NIH specific-pathogen-free breed since none of these structures were seen in the brains of Balb-C control mice of the same breed following inoculation of brain material obtained from normal Swiss-NIH mice.

The particles and rods observed in some enlarged cell processes structurally resemble papova virus. Accordingly, it is significant that tests carried out in suckling mice and in mouse embryo cell cultures inoculated with suspensions prepared from the brain hemispheres that had been put aside specifically for use in virus isolation studies failed to reveal the presence in the study material of polyoma, or Kilham's "K" virus, or any other infectious agent capable of producing illness or death in suckling mice, or cytopathic

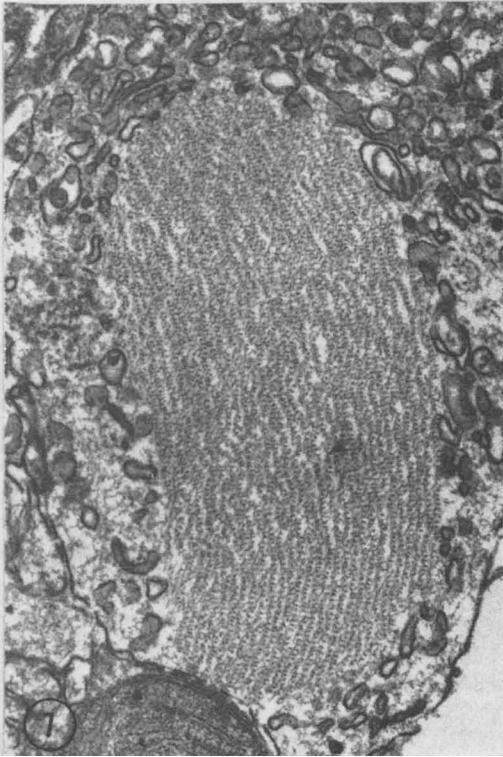
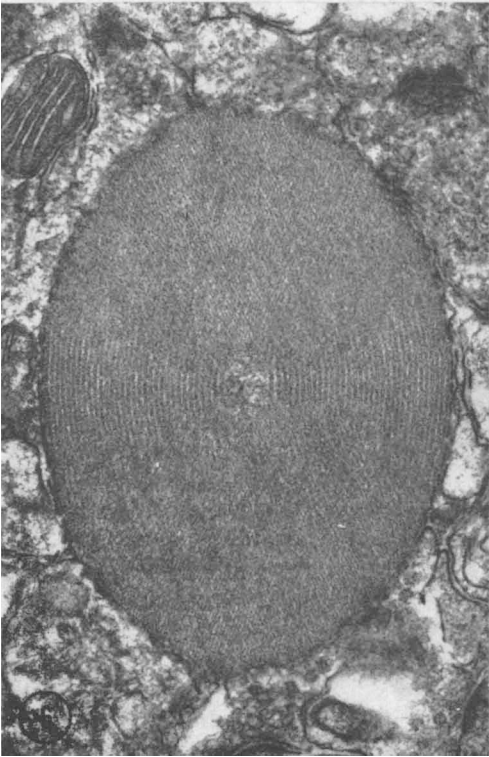


FIG. 5. Inclusion formed by parallel bands and fibrils bounded by a double membrane. 52,500 $\times$.

FIG. 6. Oval body containing parallel bands in a concentric arrangement. 35,000 $\times$.

FIG. 7. Aggregate of parallel fibrils to which are attached electron dense particles inside an unidentified cell. 34,500 $\times$.

change in mouse embryo cell cultures. There is not sufficient information available to determine whether or not these structures are related directly to the disease scrapie. It is entirely possible that they represent a latent virus encountered by chance in the Swiss-NIH mice which was transmitted in series as a contaminant along with the scrapie agent to the specific-pathogen-free Balb-C mice. If this is the case, it is of interest and of possible importance because the particles in our procedures failed to manifest their presence in the mouse by producing clinical disease or in mouse cell cultures by inducing cytopathic change. Another interesting aspect of their presence in the brains of scrapie infected mice is the absence of images in association with the particles and rods which could be interpreted as indications of virus replication. If these were conventional virus particles, one would have expected to find budding, a matrix, immature particles, or some other indication of virus synthesis.

It is difficult to believe that the particles and rods found in the brains of scrapie infected mice represent the scrapie agent. The biophysical and biochemical data lead one to believe that the size of the scrapie infectious particle is of the order of 25 Å (3).

It is emphasized that the enlarged pre- and postsynaptic cell processes and their contents could represent (a) degenerative lesions of normal brain components or (b) products resulting from a disturbance of some poorly understood physiologic mechanism induced by the scrapie agent or by the action of a latent virus. It is of interest that the enlarged processes containing varicose tubules described in the present work appear to be structurally identical to the agranular reticulum seen in liver cells of mice (10) and hamsters (11) following phenobarbital induced liver damage and in the cerebral cortex of a child with undifferentiated psychomotor retardation (12). A further interest was the report of the occurrence in the cerebral cortex of the same child (12) of structures morphologically iden-

tical to the parallel bands and fibrils which were encountered in the brains of scrapie infected mice. Structurally similar parallel bands and fibrils have been seen in the brain of man with amyotrophic lateral sclerosis (13). Conceivably, the varicose tubules, parallel bands, and fibrils represent a hypertrophy of these cell organelles which can be induced by any of a number of physiologic disturbances. In any event, these structures have not been reported to occur in cerebral cortex of normal mice.

Summary. Cerebral cortex obtained from scrapie infected mice was examined in the electron microscope. Three structures of unknown origin and significance were observed: (a) enlarged cell processes containing particles and rods, (b) enlarged cell processes occupied by varicose tubules filled with moderately dense material and (c) oval bodies formed by parallel bands and fibrils which were usually but not always, in a ring-like arrangement. These structures were generally associated in one way or another with synaptic terminals, but on rare occasions, they were found in unidentified cell processes. The possible significance of the presence of these structures in scrapie infected mouse brain is discussed.

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Interferon Production in Human Mumps Infections* (32681)

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Interferon is now accepted as one of the important defenses against viral disease in animals (1). We have been interested in assessing its specific role in human illness, especially with reference to the severity and clinical course of the disease. The various clinical forms of mumps infection in man have been well documented and range from inapparent infection to severe meningoencephalitis, orchitis, or pancreatitis. However, knowledge of the precise factors conditioning these different individual responses to the infection is largely lacking. One possibility is that interferon, acting during early cycles of virus replication in a given host, is a determinant of the form which the infection takes. In the winter of 1966-1967, a number of children and adults with clinical mumps were observed, and interferon was demonstrated in samples of serum and saliva. In this communication we describe the results of our preliminary studies.

Methods. Interferon titers were assayed in human foreskin fibroblasts with bovine vesicular stomatitis virus (VSV) challenge, as previously described (2). Serum samples were stored at 4°C and assayed within 3 weeks. Bovine serum albumin (BSA), 0.05%, was

added to urine samples which were stored at 4°C from within 1 hour after voiding and then dialyzed against Eagle's minimum essential medium (MEM) before interferon assay. After several studies demonstrated that only very low titers would be found, urines were concentrated by pressure dialysis 5-65X and then dialyzed against MEM. Previous studies with tissue culture interferon had shown this to be a satisfactory method of concentration.

In order to obtain samples of saliva, patients were asked to expectorate into sterile containers. Most specimens were collected within a few minutes, but a few were obtained over an interval of several hours. After collection, 0.05% BSA was added and the sample stored at 4°C. Before interferon assay, appropriate dilutions were centrifuged for 1 hour at 78,000g.

Most clinical specimens were obtained from subjects in the home. Diagnosis was based on the presence of parotitis, with or without other organ involvement, and an appropriate epidemiological history. If the diagnosis was questionable, as in cases of aseptic meningitis without parotitis or of parotitis occurring in the absence of exposure to mumps, paired sera were assayed for mumps complement fixation antibody titers.

Results. The results of studies on 64 sera, 12 urines, and 12 samples of saliva, obtained from 45 subjects, were analyzed. Figure 1 shows the range of serum levels found. Day 1 of illness is defined as that day on which any symptoms appeared, including fever, parotitis, or malaise. The highest titers occurred during the first 2 days of illness and then declined, although in a few patients very low or negative titers were noted on days 1 and 2.

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