BRAIN TO BRAIN TRANSMISSION OF THE SUBMAXILLARY GLAND VIRUS IN YOUNG GUINEA PIGS

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PLATES 20 AND 21

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Peculiar cellular structures in the duct cells of the submaxillary gland of guinea pigs were first noted by Jackson (1) in 1920. Shortly thereafter, these structures were shown by Cole and Kuttner (2) to be nuclear inclusions associated with a virus occurring, apparently, without lethal effect on the host. In this way, a virus of lower animals was discovered, which interestingly demonstrates a natural biological balance between parasite and host. The body responds, however, to the presence of the virus, as pointed out by Kuttner (3) who observed that uninfected guinea pigs are susceptible and infected animals are resistant to the artificial administration of the virus by intracerebral inoculation. Andrewes (4), furthermore, has demonstrated by in vivo and tissue culture experiments that immune bodies are in the serum of infected guinea pigs.

That the virus is lethal when cerebrally injected into uninfected guinea pigs is significant as showing that it is not innocuous under artificial conditions and this introduces the possibility that the virus may have natural potentialities other than those of harmless residence in the submaxillary gland. Brain to brain transfer in the guinea pig suggested itself as a possible means to enhance the virulence of the virus so that it might assume under experimental conditions a more important rôle toward its host. Kuttner and Andrewes have independently reported their inability to transmit the virus in this manner with lethal effect. The former author, however, records that transmission was accomplished when massive doses or multiple injections were used and that under such conditions there was only mild mor-
SUBMAXILLARY GLAND VIRUS IN GUINEA PIGS

bidity and no mortality. It seemed to us that the question has sufficient interest to warrant further experimentation and this paper presents the results of our studies on the virus, especially in regard to the successful brain passage in young guinea pigs.

The problem of increasing the virulence of a non-lethal virus has additional interest in connection with the possible significance of cellular inclusions in the salivary glands of man (Ribbert (5), Wilson and Dubois (6), and Farber (7)).

EXPERIMENTAL

The submaxillary glands of six groups of guinea pigs bought in the open market were used as the source of virus. Five of these groups (4 to 6 in each lot) were adult animals, each weighing from 500 to 800 gm. The sixth group was composed of 3 young guinea pigs, 14 days old and averaging 120 gm. in weight.

The age of the animals employed for the intracerebral inoculations ranged from 7 to 32 days. Because of the difficulty in injecting and anesthetizing the very young animals and the danger of encountering spontaneous infections in older ones, we found guinea pigs 2 to 3 weeks old to be the most satisfactory for transmission purposes.

Technic.—The submaxillary glands were removed from the animals of a source group; one gland of each animal was fixed for staining and study, and the others were ground together in a mortar. The emulsion was then centrifuged and a part of the supernatant fluid passed through a Berkefeld N filter. Young guinea pigs were inoculated intracerebrally, each with 0.1 cc. of either the supernatant fluid (hereinafter designated "emulsion") or the filtrate. Brain to brain passage was attempted by injections of 0.1 cc. of emulsion or filtrate of the brain removed aseptically from an animal when moribund or recently dead.

The diluting fluid for tissue emulsions was either sterile water or saline; and the results did not show any advantage of one diluent over the other. The amount of diluting fluid for each gland was 2 or 3 cc., and for each cerebral hemisphere 8 cc.

Bacteriologic examinations, under both aerobic and anaerobic conditions, were made of all gland and brain emulsions and filtrates. Ordinary saprophytic bacteria were grown from the emulsions of two of the six groups of glands, but they were not responsible for death in the cerebrally injected animals in the passage series. This was shown by negative postmortem cultures of the brains. Brain emulsions and all gland and brain filtrates were sterile upon culture.

Glands and brain tissues were fixed in Zenker's fluid and paraffin sections were stained in a variety of ways, principally by Giemsa's method, eosin-methylene blue, and hematoxylin-eosin.

1 All cerebral inoculations were made under complete ether anesthesia.
The available evidence for the presence of the virus is the finding of cellular changes in the epithelial cells of the ducts. These changes consist essentially in the hypertrophy of the epithelial cell, the appearance of an eosinophilic body within the nucleus, and the frequent occurrence of basophilic strands which bridge the unstained space surrounding the inclusion and extend to the nuclear membrane or to the irregular chromatin masses that lie upon it. In addition, the extranuclear bodies emphasized by Pearson (8) and termed “cytoplasmic inclusions,” are often noted (Fig. 7).

Scott and Pruett (9) associate increase in size of the inclusion with the duration of infection. They further conclude that the presence of cytoplasmic inclusions is evidence of a more protracted infection. Our observations on the development of the specific cellular changes in the gland are that: (1) cytoplasmic inclusions occur more frequently in the naturally infected glands than in glands of cerebrally inoculated animals that die; (2) nuclear inclusions appear to be smaller and the affected cells more irregular in the artificially infected guinea pigs; and (3) the nuclear inclusions in adult animals spontaneously infected frequently retain more of the basic dye and seem to be more dense (Figs. 7 and 8).

As the source of virus, the submaxillary glands of 27 guinea pigs, divided into six groups, were removed aseptically and the animals allowed to live. From these six groups, the virus was transmitted in four instances to young guinea pigs by intracerebral inoculation. On the other hand, inclusion bodies were found in the glands of members of only three groups. It appears that the virus is present in the submaxillary glands more frequently than is indicated by inclusion bodies. Serial sections might have disclosed a higher proportion of positive glands. In these experiments, 4 to 10 sections of each gland were examined by the use of a mechanical stage, the fewer number being searched in positive cases. In all, sections from 8 guinea pigs showed cellular inclusions, making about 30 per cent positive. This proportion may be compared with the incidence of 54 per cent found by Jackson, 84 per cent by Cole and Kuttner, and 32 per cent by Andrewes.
It has been stated by various authors that the virus is present in a certain proportion of guinea pigs that have reached the age of 3 weeks or a month. Our sources of virus were adult animals much over a month old, except in one instance when the glands of 3 guinea pigs 14 days old were employed in an attempted control experiment. The virus was successfully transmitted from these young animals and when the sections were prepared, the gland of one was found to be positive. We regard this as a rare instance but of interest in the question of natural incidence of the infection.

The cellular inclusions occurring in the spontaneously infected animals were seen only in the serous portion of the submaxillary gland. We found none in the mucous portion, contrary to the reports of other investigators. The nuclear inclusions were most often in the epithelial cells of the ducts of the magnitude of 10 to 12 cells in circumference. The differing incidence of the inclusions in the positive cases is demonstrated by the observation of a single inclusion in a duct in one case and 133 inclusions in 79 ducts in another, 5 sections being examined in each instance.

**Transmission**

Serial brain to brain transmissions were attempted in six experiments briefly outlined below. The results of microscopic examination of the source submaxillary glands are presented first in each instance, although actually they were determined after the experiment was under way.

1. Sections of the submaxillary glands of 4 adult guinea pigs showed no typical inclusions. Brain to brain inoculations were fatal to the second transfer, with inclusions demonstrable in the meningeal exudate and the submaxillary glands in the animal of the first brain to brain passage, and in the submaxillary gland of the second fatal transfer.

2. Sections of the submaxillary glands of 4 adult guinea pigs showed no inclusions in the epithelial cells. Cerebral injections were negative. No subinoculations were made.

3. Sections of the submaxillary glands of 4 adult guinea pigs exhibited no inclusions. The 2 animals inoculated intracerebrally with the filtrates died after 17 and 26 days. Brain sections had no meningeal exudate or inclusions, but typical inclusions were found in the submaxillary glands of each. No subinoculations were made.
4. Sections of the submaxillary glands of 6 adult guinea pigs showed very few inclusions in 2 animals. Cerebral injections of gland emulsion and filtrate into 5 guinea pigs resulted in the death of one which had inclusion bodies in the meningeal inflammatory cells and in the submaxillary gland. The attempted brain to brain transfer was unsuccessful, the animal dying the next day.

5. Examination of the sections of the submaxillary glands of 6 adult guinea pigs disclosed affected cells in the ducts of 5 animals. In some cases the cellular involvement was severe. Emulsion and filtrate injections into the brain of 3 young guinea pigs were all fatal, and sections of their brains demonstrated inclusion bodies in the meningeal inflammatory cells. Brain subinoculations from 2 of these animals were lethal in 3 of 6 cerebrally injected guinea pigs, with the inclusions demonstrated in each instance in the cells of the meningeal exudate, and in the submaxillary gland of one animal. The brain emulsion or filtrate from 2 of these animals caused death in 4 of 5 guinea pigs that were cerebrally injected; only one of the 4 had inclusion bodies in cells of the meningeal inflammation and in the submaxillary gland. Further brain to brain inoculations were negative (Table I and Figs. 2–4).

6. Submaxillary gland material from 3 young guinea pigs, 14 days old, was used in an attempted control experiment. Examination of submaxillary sections disclosed a positive gland from one animal. The 3 animals cerebrally inoculated with this material died, and the brain and submaxillary gland of one were positive for inclusion bodies. No subinoculations were attempted.

The above data show that in three experiments brain to brain injections were not done, either because no infection was manifest in the originally inoculated animals or because those fatally inoculated died at night some hours before being found. In the three experiments in which brain to brain inoculations could be attempted, successful passage of the virus was attained in two series. As proof of transmission, we accept the rather rigid requirements of death with the demonstration of nuclear inclusions in a meningeal exudate. This type of evidence is present in one series to the second generation and in the other to the third. Failure of further transmission depended on the same factors as obtained in the negative experiments referred to above. Although gland filtrates, as well as emulsions, were frequently
infective, no proved passage of the virus by filtrates of brain substance was accomplished, suggesting that there may be a quantitative factor in brain to brain inoculations. Apparently, the virulence of the virus was not increased by cerebral passage. Guinea pigs dead 1 or 2 days after inoculation were usually found to have died of cerebral trauma and such results were considered negative. The most pertinent results were in Experiment 5 which is outlined in Table I.

In the three experiments in which transfer of the virus was attempted, there were 23 guinea pigs injected from brain to brain. 10 of these died from 7 to 17 days after inoculation, and meningeal exudate with inclusions in the inflammatory cells was found in 5 of them. On the other hand, 8 of the 10 fatalities manifested cerebral nervous symptoms before death, and a 9th was found dead in the cage without showing symptoms but yielding the specific cell changes in the cerebral sections. It appears that a microscopic meningeal manifestation occurs less frequently than do symptoms and death resulting from the injection of the virus. Furthermore, the virus may be present in a microscopically negative brain, since from such a source it has been fatally transferred to another brain with positive microscopic results.

**Findings in the Cerebrally Inoculated Guinea Pigs**

Symptoms often appeared 1 or 2 days before the collapse of the inoculated animal and persisted until its death; at other times, however, the acute signs had an early onset and lasted only a few hours. The symptoms were predominately nervous in character. Early evidences of infection in young guinea pigs were muscular weakness and inability to rise when placed on the side. Somewhat later, there were observed local and general tremors spasmodically occurring, escape movements when on the side, opisthotonos, and nervous involvement of the bladder. Terminal symptoms were notably those of exhaustion and marked respiratory difficulty. The interval between inoculation and death ranged from 7 to 17 days.

Significant temperature reactions occurred in some of the animals on the 2nd day after the inoculation. In others, however, when the incubation period was prolonged to 15 days or more, the rise bore no discernible relation to the appearance of the symptoms or the time of
### TABLE I

**Experiment 5. Transmission of Submaxillary Gland Virus from Brain to Brain**  
*Source of Virus, Glands of 6 Adult Guinea Pigs*

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<thead>
<tr>
<th></th>
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<tr>
<td>No. 34</td>
<td>7 days</td>
<td>Pos.</td>
<td>Pos.</td>
</tr>
<tr>
<td>Brain inoculum</td>
<td>Emul. No. 38</td>
<td>Filt. No. 41</td>
<td>Filt. No. 44</td>
</tr>
<tr>
<td>Guine pig inoculated</td>
<td>1 day</td>
<td>9 days</td>
<td>7 days</td>
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<tr>
<td>Survival or days to death</td>
<td>Neg.</td>
<td>Pos.</td>
<td>Pos.</td>
</tr>
<tr>
<td>Typical cerebral pathology</td>
<td>—</td>
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<tr>
<td>No. 55</td>
<td>12 days</td>
<td>Pos.</td>
<td>Neg.</td>
</tr>
<tr>
<td>Brain inoculum</td>
<td>Emul. No. 51</td>
<td>Filt. No. 57</td>
<td>Filt. No. 56</td>
</tr>
<tr>
<td>Guine pig inoculated</td>
<td>17 days</td>
<td>6 days</td>
<td></td>
</tr>
<tr>
<td>Survival or days to death</td>
<td>Pos.</td>
<td>Neg.</td>
<td>Neg.</td>
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<tr>
<td>Typical cerebral pathology</td>
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death. Thermal elevations generally lasted not more than 24 to 48 hours.

At necropsy, the brains of animals succumbing to intracerebral inoculation with the virus showed no visible meningeal exudate. The dura was sometimes slightly thickened. Superficial cerebral vessels appeared congested and the cut surface of the brain revealed a mild hyperemia. Occasionally, there was evidence of hemorrhage in the cerebrum and spinal cord. No gross pathological changes were observable elsewhere in the body.

Microscopic examination of the brains discloses an irregular involvement of the meninges and superficial cortex, and in some instances of the subependymal tissue.

The changes consist in a mild thickening of the meninges and a variable degree of infiltration with mononuclear cells. These reacting cells are found in the meningeal spaces as well, and frequently invade the neighboring cortical tissue. The inflammatory reaction often follows the meninges and their accompanying blood vessels into the upper layers of the brain, where the proximal nervous tissue may be similarly involved (Fig. 1).

The meninges of a particular specimen are not uniformly affected and in negative areas even the perivascular tissue may be normal. However, where inflammation does occur in the meninges, the regions about the vessels are most intensely infiltrated. In the upper layers of the cortex, there is a mild perivascular infiltration of some vessels not directly associated with the meningeal folds. Other small blood vessels, on the other hand, may be entirely normal in this regard, irrespective of their location in the brain substance.

The invading inflammatory cells are mononuclear in structure, only an occasional cell being polymorphonuclear. The mononuclear cells vary in size, shape, and staining reaction. Large, weakly stained cells with vesicular nuclei are found together with smaller, more intensely stained cells. The specific cellular change consisting of nuclear inclusions occurs in a certain proportion of both large and small cells which are commonly irregular in shape. The inclusions are acidophilic bodies separated from the nuclear membrane by a narrow unstained zone (Figs. 2–4). A single inclusion occupies a nucleus; it is regular in outline, stains not so intensely as the corresponding structure in the submaxillary gland, and has a uniform appearance. Occasionally, large cells are seen that have more than one nucleus. In such a cell, there is sometimes an acidophilic alteration of the various nuclei, similar to the acidophilic change in the mononuclear cells already described. Phagocytosis is not a conspicuous feature in these sections and has been seen only a few times in the rare areas of hemorrhage and necrosis. It is noteworthy that cytoplasmic inclusions like those seen in the submaxillary duct epithelium are not
found in the inflammatory cells, and that the essential cells of the nervous tissue
were not found to contain inclusion bodies of either type.

Andrewes records the lesions seen by him in the spinal cord of
cerebrally inoculated guinea pigs. We have examined the cord of 5 of
the 10 animals dying after cerebral injections and find lesions in each
instance. The alterations in the spinal meninges were less severe but
resemble those of the cephalic meninges. There is infiltration about
the roots of the spinal nerves, and ganglion cells of both the anterior
and posterior horns show distinct signs of degeneration. Foci of
mononuclear cells and perivascular mantling are frequently observed
(Fig. 5).

Recent hemorrhage, though infrequent, is seen in single and mul-
tiple areas in both the brain and spinal cord. The hemorrhage varies
from a few cells lying in the region of a small vessel to large extravasa-
tions associated with necrosis. A few very large mononuclear cells,
which may or may not contain inclusion bodies, are found in areas of
extensive hemorrhage and occasionally are vacuolated and contain red
blood cells.

In one-half of 10 fatally inoculated animals, there was no evidence of
inclusions in the salivary glands; all of this negative group died 7 to 9
days after injection. On the other hand, typical nuclear inclusions
were found in the glands of the remaining 5 animals, in which 15 days
or more elapsed between inoculation and death. In general, the inclu-
sions were smaller and much more numerous in the glands of artifi-
cially infected animals than in those spontaneously infected (Figs. 7
and 8). In addition to the characteristic changes in the serous portion
of the submaxillary glands of this group, inclusions were also observed
in the mucous portion in one instance and in the parotid in three in-
stances (Fig. 6). We did not kill the guinea pigs yielding the source
virus and hence the parotid was not examined in spontaneously infected
animals; Kuttner observed that the parotid was not involved in the
natural infection.

SUMMARY AND CONCLUSIONS

The submaxillary gland virus of guinea pigs was serially transmitted
from brain to brain in young guinea pigs. The source of virus was the
submaxillary glands of six groups of stock animals. Brain to brain
transfer was effected in two series, in one to the second generation and in the other to the third. The transmission was evidenced by the presence of nervous symptoms and death and by a typical microscopic pathology of the brain. Only certain attempts were successful, ten of twenty-three brain to brain injections being fatal with the specific histopathology present in five. A few observations suggest that the virus may be present spontaneously in the gland and experimentally in the brain without cellular changes being demonstrable, or before they are evident.

While we were able to transmit the virus from brain to brain with fatal results by single injections of small doses, this was not readily accomplished and the transmission failed after two or three passages. We were unable to show any perceptible increase in virulence or adaptation of the virus to the brain tissue of the natural host.

The histopathology was that of a meningoencephalitis. The inflammatory reaction irregularly involved the meninges, the underlying brain substance, and the perivascular tissue of the meninges and upper cortical layer. These structures were infiltrated with mononuclear cells, many of which contained a typical acidophilic inclusion. Congestion of cerebral capillaries uniformly occurred and various degrees of recent hemorrhage were frequently found. Necrosis was noted only when associated with an occasional area of extensive hemorrhage. Similar changes were observed in sections of the spinal cord.

When sufficient time (15 days or more) elapsed between cerebral inoculation and death, typical cellular inclusions were seen in the salivary glands, whereas none was found in animals that died earlier (7 to 9 days). Under the first mentioned conditions, inclusions were demonstrated in the parotid and mucous portion of the submaxillary glands, although in spontaneously infected animals, we failed to find the mucous portion involved and other workers report that the parotid is spared.

About one-third of the stock guinea pigs examined showed cellular inclusions in both the nucleus and cytoplasm of epithelial duct cells of the serous part of the submaxillary gland. From an analysis of the results of brain to brain inoculations, it was evident that spontaneous infection and resistance to cerebral inoculation increased with age. The 3rd week of life is the period of choice for such experimentation.
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REFERENCES


EXPLANATION OF PLATES

PLATE 20

FIG. 1. Section of brain of fatally inoculated Guinea Pig 34 (Table I) showing inflammatory reaction in meninges, perivascular tissue, and adjacent cortex. Hematoxylin-eosin. × 150.

FIG. 2. Photomicrograph of meningeal exudate over brain of Guinea Pig 36, showing type of reaction and nuclear inclusion is a large mononuclear cell. Result of primary brain inoculation (Table I). Hematoxylin-eosin. × 1425.

FIG. 3. The same as Fig. 2, for Guinea Pig 45, inoculated with brain of No. 36 (Fig. 2). Hematoxylin-eosin. × 1425.

FIG. 4. The same as Fig. 2, for Guinea Pig 51, inoculated with brain of No. 45 (Fig. 3). Hematoxylin-eosin. × 1425.

PLATE 21

FIG. 5. Section of spinal cord of Guinea Pig 36, showing focus of mononuclear reaction. Hematoxylin-eosin. × 315.

FIG. 6. Section of parotid gland of Guinea Pig 51; fatal cerebral inoculation (Table I). Nuclear inclusions in epithelial duct cells. Eosin-methylene blue. × 1425.

FIG. 7. Nuclear and cytoplasmic inclusions in a duct cell of the submaxillary gland of an adult guinea pig naturally infected. Eosin-methylene blue. × 1425.

FIG. 8. Section of submaxillary gland of Guinea Pig 51, illustrating small size of nuclear inclusions in cerebrally inoculated animal; compare with Fig. 7. Hematoxylin-eosin. × 1425.
(Hudson and Markham: Submaxillary gland virus in guinea pigs)