TABLE I. A Comparison of the Antibody Levels in Sera from Individuals Exposed to Influenza Virus for Several Months and a Few Days (Control Group).

| Test group | | | | Control group* | | | |
|---|---|---|---|---|---|---|---|
| Serum No. | PR 8 | Lee | FM-1 | Serum No. | PR 8 | Lee | FM-1 |
| | | | Final titer | | | | |
| 1A | 160 | 640 | 40 | 9A | 80 | 320 | 320 |
| B | 1280 | 5120 | 320 | B | 80 | 320 | 320 |
| C | 1280 | 2560 | 320 | C | 80 | 320 | 320 |
| 2A | 80 | 160 | 160 | 10A | 160 | 640 | 160 |
| B | 640 | 2560 | 1280 | B | 160 | 320 | 80 |
| C | 640 | 2560 | 1280 | C | 320 | 320 | 160 |
| 3A | 160 | 160 | 160 | 11A | 320 | 640 | 640 |
| B | 1280 | 640 | 640 | B | 320 | 640 | 640 |
| C | 1280 | 640 | 640 | C | 320 | 640 | 640 |
| 4A | 320 | 160 | 160 | 12A | 80 | 640 | 160 |
| B | 10240 | 1280 | 640 | B | 80 | 640 | 160 |
| C | 10240 | 5120 | 640 | C | 160 | 640 | 160 |
| 5A | 640 | 640 | 640 | 13A | 80 | 80 | 80 |
| B | 1280 | 1280 | 1280 | B | 40 | 80 | 80 |
| C | 2560 | 1280 | 1280 | C | 40 | 80 | 80 |
| 6A | 320 | 160 | 2560 | 14A | 320 | 640 | 160 |
| B | 2560 | 640 | 2560 | B | 160 | 640 | 80 |
| C | 5120 | 640 | 2560 | C | 160 | 320 | 160 |
| 7A | 160 | 320 | 160 | 15A | 80 | 320 | 80 |
| B | 2560 | 5120 | 5120 | B | 80 | 320 | 80 |
| C | 2560 | 5120 | 5120 | C | 80 | 320 | 80 |
| 8A | 80 | 320 | 160 | 16A | 320 | 1280 | 640 |
| B | 2560 | 2560 | 5120 | B | 320 | 1280 | 320 |
| C | 2560 | 2560 | 2560 | C | 640 | 1280 | 640 |

* Eight of 57 students.

these strains are unable to cause a typical clinical picture in man after several years of animal or chick embryo passage.

The reason for the high titers against the Lee strain is obscure. This observation, however, has been made previously(3,5). There are several possible explanations: (1) sporadic inapparent infections with Influenza B occurring in the population previous to this study, and (2) residual titers from a previous infection with this virus.

*Summary.* Data are presented which suggests that inapparent infections with influenza viruses may occur after continued exposure for relatively long periods of time.

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## Distribution of Mumps Virus in the Experimentally Infected Monkey.* (18561)

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With the recent development of technics for the identification of mumps virus by serological tests(1), and for its propagation

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1. Enders, J. F., and Cohen, S., PROC. SOC. EXP. BIOL. AND MED., 1942, v50, 180.

in the embryonated egg(2,3), considerable information has been obtained on the distribution of this agent in the human host in the course of natural infection. Mumps virus has been isolated from saliva(4), spinal fluid(5), blood(6), testis(7), parotid gland (8), and either pancreas or ovary(8). It is apparent that this agent can exhibit on occasion pantropic characteristics in the course of natural infections in man. Although Johnson and Goodpasture showed as early as 1934(9) that the monkey could be experimentally infected via Stensen's duct, relatively little is known about the distribution of mumps virus in this host following this procedure. The agent has been isolated from the parotid gland(2,3) which as yet is the only organ in which complement fixing antigen has been demonstrated. More recently Coons, *et al.*(10) have microscopically localized mumps virus antigen in this organ by means of homologous antibody labelled with fluorescein. The present paper deals with a more extensive search for mumps virus in the various organs of one experimentally infected monkey. The individual tissues were first examined for the presence of mumps virus antigen by means of fluorescent microscopy and specific antibody labelled with fluorescein. The same tissues were then tested for the presence of infectious virus by the classical method of inoculation of the embryonated egg.

*Materials and methods.* The Enders strain of mumps virus was employed. A normal young adult male rhesus monkey (Macacca mulatta) was infected by the bilateral injection of approximately 0.5 ml-1 ml of a 1:40 suspension of infected parotid gland into Stensen's duct. At the end of 96 hours (before any glandular swelling or other gross evidence of infection had developed) the animal was sacrificed and autopsied. Individual tissues were frozen and stored in $CO_2$ cabinet until the time of testing.

*Use of fluorescein-labelled antibody in the search for mumps virus.* Convalescent anti-mumps monkey serum was concentrated, conjugated with fluorescein isocyanate and purified as previously described(10,11). Small portions of it were absorbed with mouse liver powder and used to localize mumps virus in tissue sections. These were prepared from quick-frozen tissue in a cryostat by the method of Linderstrøm-Lang and Mogensen as modified in this laboratory(12). It was found that exposure of such frozen sections to fluorescein-antibody solutions often resulted in the formation of a brightly fluorescent scattered precipitate unrelated to the presence of mumps virus; this was especially troublesome in the case of central nervous system tissues. Placing the sections attached to slides in acetone at room temperature for 15 minutes, and then allowing them to dry for 30 minutes at 37° abolished this non-specific precipitation. We have no ready explanation for this phenomenon. The sections, after treatment with fluorescein anti-mumps conjugate for 30 minutes, were washed, mounted, and examined under the fluorescence microscope. Control of specific staining was accomplished as follows. Companion sections were treated with normal monkey serum and anti-mumps monkey serum respectively (both unlabelled), followed by exposure of both sections to labelled anti-mumps serum. Fluorescence due to mumps virus was absent when inhibited or "blocked" under appropriate conditions by specific unlabelled antibody. This technic has been described in detail elsewhere(10).

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3. Levens, J. H., and Enders, J. F., *Science*, 1945, v102, 117.

4. Leymaster, G. R., and Ward, T. G., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 346.

5. Henle, G., and McDougell, C. L., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 209.

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9. Johnson, C. D., and Goodpasture, E. W., *J. Exp. Med.*, 1934, v59, 1.

10. Coons, A. H., Snyder, J. C., Cheever, F. S., and Murray, E. S., *J. Exp. Med.*, 1950, v91, 31.

11. Coons, A. H., and Kaplan, M. H., *J. Exp. Med.*, 1950, v91, 1.

12. Coons, A. H., Leduc, E. H., and Kaplan, M. H., *J. Exp. Med.*, 1951, v93, 173.

*Isolation of virus.* After frozen sections had been prepared for histochemical studies the remainder of the tissue fragment was ground with alundum in isotonic phosphate buffer solution to make a 10% suspension, and after light centrifugation, penicillin was added to the supernatant to give a final concentration of 500 units per ml. Embryonated eggs 7 or 8 days old were inoculated amniotically, and one week later the amniotic fluids were harvested individually and tested for the presence of hemagglutinins. Specific serological identification was carried out by the hemagglutin-inhibition technic as outlined by Robbins *et al.*(13). In the absence of hemagglutination two blind passages employing pooled amniotic fluids (and occasionally amniotic membranes as well) were usually carried out before the original material was considered to be negative.

*Results.* I *Identification of mumps virus antigen by homologous antiserum labelled with fluorescein.* The positive findings were limited to the parotid glands, the spinal cord, the medulla, and possibly the cerebrum and cerebellum. In both parotid glands specific antigen was demonstrated chiefly in the cytoplasm of the acinar cells. These findings have been described elsewhere(10). In the spinal cord the antigen (presumably for the most part active mumps virus) was found scattered in the white matter in small rather amorphous irregular globules. No clear cell outlines were visible, and whether it was intra or extracellular could not be determined. It was irregularly distributed. In the cervical cord a moderate amount of staining was found just lateral to one dorsal horn, with smaller scattered amounts in the opposite ventral and lateral fibertracts. In the lumbar enlargement rather more staining was visible, this time in the ventral white matter lying between the emerging motor fibers from ventral horn on each side. The overlying meninges showed no antigen.

In the medulla a trace of similar amorphous material was found just beneath the floor of the 4th ventricle, which seemed to be in the cytoplasm of cells.

The examination of the cortex and cerebellum was unsatisfactory because it was undertaken first. The scattered non-specific precipitate described above (Methods) made definite findings impossible. By the time it was found that treatment of the sections with acetone mitigated this difficulty, the supply of tissue was exhausted.

The meninges covering the cortex were repeatedly examined, without finding specific fluorescence. Kidney, liver, spleen, adrenal, lung, heart, muscle, testis, one lymph node, fat tissue, stomach, small bowel, large bowel and pancreas showed no staining specific for mumps antigen.

II *Virus isolation.* Mumps virus was isolated from 7 of the 22 tissues examined. Positive results were obtained in the case of: left parotid gland, right parotid gland, meninges, cortex, cerebellum, cervical cord and lumbar cord. No virus was isolated from the following organs: liver, adrenal gland, kidney, lung, spleen, lymph node, heart, skeletal muscle, pancreas, fat, stomach, small bowel, large bowel, testis and medulla. In all 7 positive cases hemagglutinins were detected in the amniotic fluid of the first egg passage: in 2 of the 7 the titers were high enough to permit serological identification without further passage. The virus was identified by means of the hemagglutation-inhibition test utilizing first egg passage material in the case of both parotid glands; second egg passage material in the case of cortex, meninges, cerebellum and lumbar cord; and third egg passage material in the case of cervical cord.

As regards the organs from which virus could not be isolated, three egg passages were carried out before the material was discarded, with the exception of the lung which was passed but once. These results are summarized in Table I.

*Discussion.* Of considerable interest was the demonstration of mumps virus in widely scattered areas of the CNS only 4 days after inoculation of the monkey by the injection of Stensen's ducts. During these 4 days no signs pointing to the involvement of the CNS were observed. Presumably the route of infection was the hematogenous one; unfor-

13. Robbins, F. C., Kilham, L., Levens, J. H., and Enders, J. F., *J. Immunol.*, 1949, v61, 235.

TABLE I. Detection of Mumps Virus Antigen and Isolation of Mumps Virus.

| Tissue | Detection of antigen | Isolation of virus |
|---|---|---|
| Left parotid gland | + | + |
| Right " " | + | + |
| Kidney | — | — |
| Liver | — | — |
| Spleen | — | — |
| Adrenal | — | — |
| Lung | — | — |
| Heart | — | — |
| Skeletal muscle | — | — |
| Pancreas | — | — |
| Lymph node | — | — |
| Fat | — | — |
| Stomach | — | — |
| Small bowel | — | — |
| Large bowel | — | — |
| Testis | — | — |
| Meninges | — | + |
| Cortex | ? | + |
| Cerebellum | ? | + |
| Medulla | + | — |
| Cervical cord | + | + |
| Lumbar cord | + | + |

tunately the blood was not examined for the presence of virus. Since, however, the virus could not be detected in the other organs examined (with the exception of the parotid glands) it seems unlikely that it was present in the blood in high concentration at the time the animal was sacrificed. Had the experiment been permitted to run longer than 96 hours the distribution of virus might have been different. The results are compatible with the clinical observation that the CNS of man may be invaded by the virus during the course of natural infections.

The data presented in the present paper furnish additional evidence as to the value of fluorescein labelled antibody as a histochemical tool. Fair agreement was produced by the two methods—virus isolation and demonstration of viral antigen—employed to investigate the distribution of mumps virus in the experimentally infected monkey. In 14 of the 22 tissues examined the presence of virus could not be demonstrated by either method. In 4 tissues both methods yielded positive results. Virus was isolated from 2 tissues (cortex and cerebellum) in which the application of labelled antibody yielded questionable results due to technical difficulties. One tissue (medulla) gave a positive reaction with labelled antibody, but no virus was isolated from it. Conversely, no mumps antigen could be detected in the meninges, although the virus was itself isolated from both samples of this tissue that were tested. Thus, of 22 trials the methods were in agreement 18 times. In two instances they yielded divergent results, while in the case of 2 tissues, the results could not be compared because of technical difficulties.

*Summary.* The distribution of mumps virus in one experimentally infected monkey 96 hours after inoculation has been investigated by simultaneous labelled antibody and virus isolation studies. The results furnished by the two methods were in fair agreement. Virus was demonstrated in CNS (brain and spinal cord) as well as in both parotid glands, although the animal had shown no neurological signs. No virus was demonstrated in the other organs examined.

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## Lack of Effect of Folic Acid on Scurvy in Guinea Pigs. (18562)

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The frequent association of scurvy with megaloblastic anemia in infants was pointed out by Zuelzer(1) in his initial description of this hematologic disorder. Scurvy was then considered only as a manifestation of the poor nutritional status of the patient and other-

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1. Zuelzer, W. W., and Odgen, F. N., *Am. J. Dis. Child.*, 1946, v71, 211.