Infra Red Absorption Spectra of Viruses. (19763)

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Infra red (IR) spectrometry has been of use primarily in the analysis of organic compounds and is thus being applied extensively as a quantitative and qualitative method(1). Within recent years IR spectrometry has been used in the study of biological problems. as exemplified in the study of urinary ketosteroids(2). porphyrins(3) and dextran(4). Reports(5,6) indicate that bacteria exhibit different IR absorption spectra which may eventually provide a means for their rapid identification.

Since viral agents may be less complex in their chemical constitution than bacteria, and perhaps thereby provide absorption spectra of more distinctive nature, such agents have been processed to relative purity and examined for their IR absorption characteristics. This type of examination might provide a rapid method of viral identification and delineation of their interrelationships.

Methods. Viruses, as follows, were processed and examined for their IR absorption spectra: meningopneumonitis (MP) (Francis strain).* ornithosis (P-4 strain),* mumps (Enders strain)* and Newcastle disease virus (NDV) (California strain).†

Embryonated chicken eggs were inoculated into the chorio-allantoic cavity with 0.1 cc of 10^{-1} dilution of the viruses noted above. Ornithosis and meningopneumonitis agents were inoculated into 9-day-old embryos and chorio-allantoic fluids (CAF) were harvested 3 days later from the dead embryos. NDV was inoculated into 10-day-old embryos and CAF were harvested from the dead embryos 2 days later. Mumps virus was inoculated into 7-day-old embryos and CAF were collected 5 days later. In this manner, all of the CAF specimens came from 12-day-old incubating embryos. The fluids were each centrifuged at 3000 rpm for 30 minutes and the clear supernates were then centrifuged at 40,000 rpm‡ (140,000 x G) for one hour. The sedimented pellets were then resuspended in double distilled water to 1/10 of the original CAF volume. The slightly turbid suspensions were tested for concentration by microscopic examination of the MP and ornithosis viruses and by hemagglutination titration of the NDV and mumps viruses. All of the virus preparations were sealed in glass ampoules and stored in the CO_2 box.

In preparing specimens for IR spectrometry, the virus suspensions were spread evenly with a rubber policeman over the surface of Ag S-coated silver chloride plates§ (1 inch square x 1 mm thick). Approximately 0.2 ml was applied to each plate. The specimen was then desiccated in a 37°C dry incubator and stored overnight in a desiccator jar containing "drierite." Each dry specimen was then covered with a blank AgCl plate and both were sealed together at the edges with plastic ("Scotch") tape. The plates were then wrapped in paper and stored with "drierite" until examined. Two different lots of each virus, in duplicate, were examined.

The IR analyses were determined in a Baird Model B, double beam, recording infra-red spectrophotometer. The silver chloride slides were mounted in fiberboard carriers with an aperture of 13 mm. Carrier dimensions were approximately 7 x 15 cm. The blank was composed of silver chloride sheets sandwiched together as in the preparation of the specimens and mounted on a similar carrier.

Results. Fig. 1 demonstrates the IR absorption spectra obtained with the 4 viruses noted above. Between the wave lengths of 8 to 12 μ, the curves of mumps and MP viruses appear

* Obtained from the Viral and Rickettsial Registry of the American Type Culture Collection.

† Obtained from Dr. Raymond Bankowski, School of Veterinary Medicine, University of California, Davis.

‡ Spinco Ultracentrifuge, Specialized Instruments Co., Belmont, Calif.

§ Purchased from the Harshaw Chemical Co., Cleveland, Ohio.

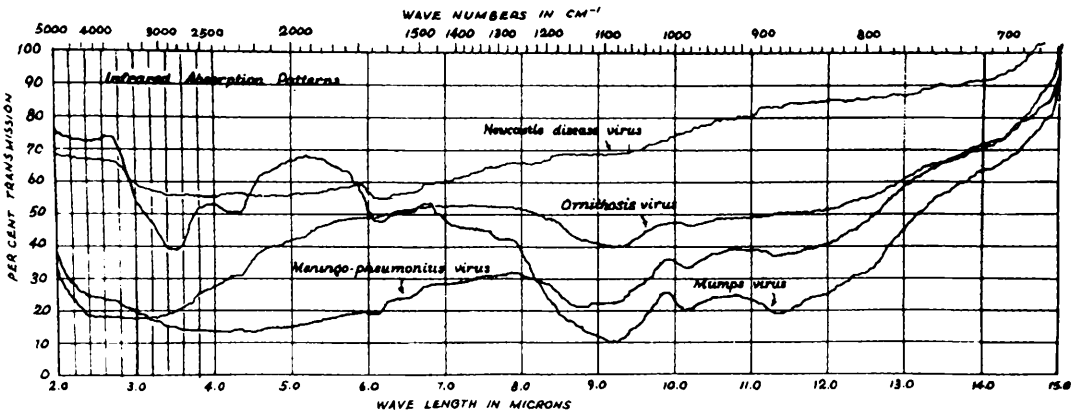

FIG. 1.

to be similar. Between these two points ornithosis virus tends to resemble the above two viruses, whereas NDV is entirely different. Between the wave lengths of 3 and 8 μ, mumps virus demonstrates an absorption pattern which is distinctly different from that of the 3 other agents. MP and ornithosis viruses have some slight similarity between these points. The NDV curve shows a slight dip between 6 and 7 μ, otherwise, it bears little resemblance to any of the other 3 viruses. All of the preparations of each virus examined were essentially the same.

Discussion. In general, there appears to be some similarity between the absorption spectra of MP and ornithosis viruses, whereas the spectra of mumps and of NDV viruses can be distinctly differentiated. MP and ornithosis viruses are similar morphologically and possess antigenic properties which are difficult to differentiate. This close relationship may explain the similarity of the IR absorption spectra demonstrated for these two agents. Mumps virus has no detectable morphological or antigenic relationship to MP and ornithosis viruses and appears to be distinctly different from the latter two in its IR absorption spectrum. NDV is unrelated to the other 3 viruses in morphology, in antigenic properties and apparently in its IR absorption spectrum (Fig. 1).

Since all of the CAF specimens were collected from embryos of the same age, this factor might be ruled out as an explanation for the divergence of absorption spectra. The fact that mumps virus was collected from live embryos, whereas the other three came from dead embryos, might introduce a significant variant that should warrant further study.

The curves depicted in Fig. 1 may not be definitive tracings since the viral preparations were still relatively crude. More precisely refined agents may produce IR absorption spectra of analytical value. From evidence thus far examined, this method of studying viral agents may be useful eventually both for their identification and for their classification.

Summary. Infra red absorption spectra have been determined of meningopneumonitis, ornithosis, mumps, and Newcastle disease viruses. The spectra of mumps and Newcastle disease are each distinctly different from that of meningopneumonitis and ornithosis, whereas the spectra of the latter two agents seem to indicate a close interrelationship.

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