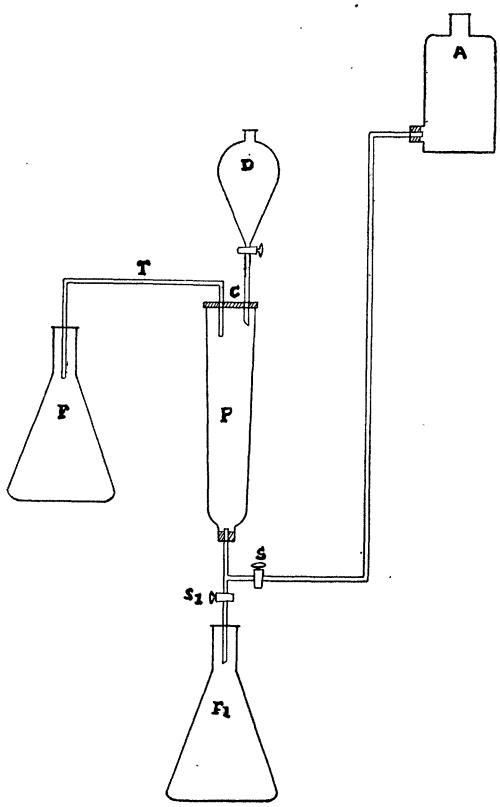


FIG. 1.

the petroleum ether flow was started, it produced a clouding effect at the point of entrance. However, this disappeared almost immediately. Upon further addition of the petroleum ether, the clouding increased gradually until the whole solution was uniformly cloudy, while the volume had increased materially. Evidently, this is the emulsification stage. As more petroleum ether was added to the solution or emulsion, a petroleum ether layer containing fat began to separate above. As still more immiscible solvent was added, the size of the petroleum ether layer increased, whereas that of the lower hydro-alcoholic layer diminished. At the same time the syphon began to function due to the air chamber formed in the top of the percolator. In this manner the excess of the petroleum ether layer was forced out. The bubbling up of petroleum ether through the lower hydro-alcoholic layer was continued until all the fatty oil had been removed. This point can be judged roughly when the petroleum ether that separates is no longer colored. Shortly after the separation of the petroleum ether layer, the lower hydro-alcoholic layer became clear. Upon the addition of more petroleum ether, the original hydro-alcoholic solution receded to a volume slightly below the volume introduced at first.

Continued extraction caused some of the material in the hydro-alcoholic extract, other than fat, to separate out on the walls of the percolator. Hence this method not only served as a means of avoiding emulsification, but also brought about a separation of a third substance, at least in this instance.

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# SPECIAL ARTICLES

## RELATIONSHIP OF THE VIRUSES OF VESICULAR STOMATITIS AND OF EQUINE ENCEPHALOMYELITIS

THE virus of equine encephalomyelitis, recovered by Meyer, Haring, and Howitt[1] from affected horses, is regarded by them to be distinct from the incitant of botulism, "forage poisoning," Borna's disease, poliomyelitis, and apparently, from that of enzootic encephalitis of the Moussu-Marchand type.[1,2] In view of the fact that the horse is the natural host for this disease and also for vesicular stomatitis, we undertook a comparison of the properties of the viruses obtained from both infections. We wish to thank Miss B. Howitt, of the George Williams Hooper Foundation of the University of California, for a specimen of the encephalomyelitis virus.

The following series of comparative tests were made:

*Intracerebral Inoculation*:[3] The intracerebral inoculation of guinea-pigs, white mice, *Macacus rhesus* and *cynomolgus* monkeys with either virus induces in each instance fatal encephalomyelitis,[4] characterized usually by the same period of incubation and set of symptoms. The rabbit, however, is much more resistant to the two viruses than the other animals mentioned, and 24 to 48 hour old chicks are unaffected by them. The viruses can be recovered from the submaxillary and parotid glands, blood, brain, spinal fluid, lung, spleen, liver and kidney of monkeys, guinea-pigs, and mice experimentally inoculated with either one. The gross and microscopic changes in the brain

[1] K. F. Meyer, C. M. Haring and B. Howitt, SCIENCE, 74: 227, 1931; *Jour. Am. Vet. Med. Assn.*, 79 (n. s. 32): 376, 1931.

[2] B. Howitt, *Proc. Soc. Exp. Biol. and Med.*, 29: 118, 1931; K. F. Meyer, *Ann. Int. Med.*, 6: 645, 1932.

[3] All operations were performed under ether anesthesia.

[4] For a description of experimental vesicular stomatitis pathology, see H. R. Cox and P. K. Olitsky, *Proc. Soc. Exp. Biol. and Med.*, 30: 653 and 654, 1933.

and cord are apparently identical, the same type of intranuclear inclusion body is found in the neurones, and similar tissue lesions occur in the liver and kidney of the monkeys, guinea-pigs and mice which have succumbed to either experimental encephalomyelitis or vesicular stomatitis.

*Pad Inoculation of Guinea-Pigs:* Both viruses injected into the pads of guinea-pigs induce vesicular reactions varying in degree and transmissible indefinitely in series from pad to pad. As a rule, the serous exudate within the vesicles resulting from the virus of encephalomyelitis is blood-tinged, while that from the virus of vesicular stomatitis is clear. The microscopic changes in the affected pads are identical and the epithelial cells show the same type of intranuclear inclusion bodies.[5]

Only the pad tissue of the dermal surface is uniformly susceptible to both viruses, and after five or six serial pad passages of the encephalomyelitic virus, the inoculated animals fail to exhibit signs of nervous involvement; such animals, after recovery, are resistant to intracerebral inoculation of the encephalomyelitic virus. Corresponding immunity reactions occur with the vesicular stomatitis virus.

*Inoculation of Mice:* The white mouse is highly susceptible to both viruses, whether inoculated intracerebrally or instilled intranasally. Tissue cultures of the viruses in dilutions of $10^{-6}$, mouse brain in dilutions of $10^{-7}$, and Seitz filtrates of affected guinea-pig pad, are all capable of inducing fatal infections characterized by the same set of symptoms and microscopic changes in the nervous system, liver and kidney.

*Immunological Reactions:* The results of repeated tests indicate that cross-immunity reactions do not occur between encephalomyelitis and stomatitis viruses.

*Other Properties:* The two viruses have been cultivated in tissue cultures composed of chick embryo tissue and Tyrode's solution,[6] for over forty generations, without loss of infectivity for guinea-pigs and white mice. The filterability through Seitz disks of both incitants is of the same degree: they pass the filters in a concentration of $10^{-5}$.

To summarize, the viruses of equine encephalomyelitis and vesicular stomatitis are similar in many but not all biological properties, and since the horse is the natural host for the two infectious agents, it is suggested that they may be generically related. However, inasmuch as cross-immunization does not occur, it follows that they are not identical. Just as there are at least three types of foot-and-mouth disease and two of vesicular stomatitis viruses, each immunologically distinct, the absence of cross-immunity does not exclude the possibility of a generic relationship.

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[5] For a description of experimental vesicular stomatitis dermatitis, see P. K. Olitsky and P. H. Long, *Proc. Soc. Exp. Biol. and Med.*, 25: 287, 1928; P. K. Olitsky, *Jour. Exp. Med.*, 45: 969, 1927.

[6] H. R. Cox, J. T. Syverton and P. K. Olitsky, *Proc. Soc. Exp. Biol. and Med.*, 30, 896, 1933.

## A RESPIRATION COENZYME

MANY studies have been reported in recent years dealing with the stimulating effect of various factors on the growth of lower organisms. There are extremely few instances on record, however, where it has been shown that increased growth was due specifically to an increased rate of respiration, that is, to an increased rate of oxygen consumption per unit dry weight of organism.

A factor not only specific but essential for respiration has been found at this laboratory. Certain organisms fail to reproduce or to make appreciable growth in the absence of this factor because of inability to respire. Its addition to the medium in sufficient quantity causes the rate of respiration per unit weight of organism to assume the normal maximum value within an hour or less, whereas the initiation of normal growth does not take place until several hours later. If relatively small quantities of the respiration factor are added the rate of respiration then assumed will vary according to concentration. The specificity of the factor for respiration, as distinguished from growth, has also been demonstrated by studying the behavior of the organisms under conditions where growth is impossible, for example, in a nitrogen-free medium.

Many properties of the respiration factor have been determined, even though it has not yet been isolated in chemically pure form. It is easily obtained in a reasonably concentrated form by extracting commercial sucrose with absolute alcohol. As little as 5 parts per million dry weight of such an extract (still consisting mostly of sugar) is sufficient to give a respiration rate approximately half the maximum. The same concentration will also stimulate the growth rate to almost as marked an extent during a growth period of some 3 or 4 days. The factor is soluble in water and in absolute alcohol, but insoluble in the ordinary fat solvents. Spectroscopic analysis did not show the presence of any inorganic element. Furthermore, active extracts lose their biological effect when ashed. The factor is readily dialyzable and heat stable, that is, it may be autoclaved repeatedly at 15 pounds pressure without appreciable effect on its activity. Be-