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-

cause of these last two properties, and the additional facts that it may be separated from living organisms, and that it has a direct effect on a specific chemical reaction (respiration), the factor is classifiable as a respiration coenzyme. For convenience it has been designated as coenzyme R, the R referring to respiration. Whether it is related to any of the active fractions in other organic extracts capable of stimulating growth or respiration, variously called bios, rhizopin, pnein, auximones, certain vitamins, Euler's activator, Z complex, etc., is still an open question. It is certainly not identical with bios, since its addition to a synthetic medium essentially free from bios resulted in a growth of yeast negligible compared with the heavy growth obtained where bios was present. Work now in progress on the purification of the coenzyme involves the determination of its more strictly chemical properties; its possible relation to the other active factors mentioned; the possibility of further fractionation; and whether it affects a fermentative step or a strictly oxidative step (involving oxygen gas) in the normal respiration of the organism.

The test organism used to the greatest extent in these studies was the red clover root nodule organism (*Rhizobium trifolii*). With this organism the rate of respiration increases from a small value in the presence of a trace of the factor to as high as 1,000 cmm O_2 per mg dry weight per hour ($Q_{O_2} = 1000$) at 31° C. in its presence. The determinations were made by means of the Warburg[1] apparatus. This value is somewhat higher than for most organisms. Carbon dioxide production is ordinarily affected to the same approximate extent as oxygen consumption, that is, the respiratory quotient remains practically constant. This holds both under aerobic conditions and under conditions of partial oxygen deficiency. Less extensive studies with a number of other species of legume nodule bacteria show that the factor is also essential for the alfalfa, pea and bean nodule bacteria, while the responses with soybean and cowpea bacteria were less striking, due probably to the slower rate of growth of these organisms. It is not, however, specific for legume bacteria, since some of the other bacterial species tested gave similar responses. Reducing substances, such as cystine, thio-glycollic acid and glutathione, do not in any degree act as substitutes for coenzyme R. The same is also true of active iron preparations such as synthetic humate iron, provided that they are free from the respiration factor. Such active iron, which so markedly stimulates the rate of growth (not of respiration) of Azotobacter[2] growing in a synthetic medium, has a less marked effect on Rhizobia and then only in case the coenzyme is also added. This is true because these organisms need very much less iron than does Azotobacter. Natural humates, however, contain both the coenzyme and available iron, hence they greatly increase the growth of both Rhizobia and Azotobacter. In legume symbiosis the host very probably furnishes the bacteria, living on the roots, with an adequate supply of the respiration factor just as it is known to furnish carbohydrates and mineral matter.

Special reference should be made to the behavior of Azotobacter. These organisms make a normal growth on a medium essentially free from all traces of the respiration factor. This is interesting, in view of the fact that Azotobacter has substantially the highest rate of respiration ($Q_{O_2} = 5000$) possessed by any organism. A study made of *Azotobacter vinelandii* showed that the respiration coenzyme is synthesized by this organism and given off into the medium in considerable quantities. Older cultures (5 days) contain considerably more of it per unit dry weight of the organism than do younger cultures.

The source of the material usually used in our chemical work has been commercial cane sugar, but undoubtedly yeast, cane molasses, natural humic acid, crude egg albumen and many plant extracts would be more concentrated but more impure sources. The indications are that the coenzyme is widely distributed throughout the plant and animal kingdoms.

It may be of interest to those workers engaged in the study of legume nodule bacteria to state that these organisms ordinarily make a growth on a sugar-mineral medium, containing a suitable source of nitrogen and the respiration factor, that is substantially as good as in the presence of yeast extract. The most suitable form of nitrogen, whether nitrate, ammonia, asparagin, urea or some other, will vary with the bacterial strain. These facts show that, aside from furnishing readily available nitrogen, the chief rôle of the yeast water in the case of the nodule bacteria is to supply a source of the essential respiration factor.

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[1] *Jour. Phys. Chem.*, 34: 1183, 1930, fig. 4.
[2] SCIENCE, 74: 522–524, 1931.

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