

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  $31^\circ C$ . in its presence. The determinations were made by means of the Warburg<sup>1</sup> 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*<sup>2</sup> 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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<sup>1</sup> *Jour. Phys. Chem.*, 34: 1183, 1930, fig. 4.

<sup>2</sup> *SCIENCE*, 74: 522-524, 1931.