In my opinion, therefore, there is no question of a segmental arrangement of the sympathetic ganglia in the sympathetic trunk.

It was a matter of no small surprise to me that I was able to demonstrate so few ganglion cells in the rami communicantes, for they have pointed the way to the original cells for their shifting; in a human embryo of 7 m.m. in length, they are still very rich in cells. (KEIBEL and MALL).

Microbiology. — *"Formation of pyruvic acid from malic acid by microbes"*. By Professor M. W. BEIJERINCK and Dr. T. FOLPMERS.

(Communicated in the meeting of January 29, 1916).

Of the organic acids malic acid seems the most easily decomposed by microbes. Then chinic acid might follow in the readiness of this decomposition, whereas the other acids are more difficult to split up.

The decomposition can take place by fermentation at exclusion of air, or by oxidation. Here oxidation will only be discussed.

As to the malates their oxidation is commonly a complete conversion into water, carbonate, and carbonic acid; it can be caused by a number of microbes. But among the bacteria numerous species occur which at the same time produce a less complete oxidation of the malates, whereby especially pyruvic acid is of importance.

The reaction evidently is:

$$\underset{\text{Malic acid}}{C_4H_6O_5} + O = \underset{\text{Pyruvic acid}}{C_3H_4O_3} + CO_2 + H_2O$$

or, as to calciummalate:

$$2C_4H_4CaO_5 + O_2 = C_6H_6CaO_6 + CaCO_3 + CO_2 + H_2O.$$

With potassiummalate the reaction has the same course. As a reagent on the pyruvic acid a ferric salt is used, such as ferric chlorid or ferric citrate, giving an intensive and characteristic orange yellow colour, which by the action of dilute hydrochloric acid first passes into red, then disappears.

The following experiment is simple and convincing.

Ordinary agar is diluted with once or twice its volume of pure 2% agar dissolved in water; to this mixture is added 1 or 2 % calciummalate, which does not quite dissolve, and a little ferric citrate or ferric chlorid as an indicator.

After pouring out and solidification a plate is obtained, still turbid by the not quite dissolved malate.

Canal water is now flowed over the plate and the excess removed so that isolated colonies can develop ; or on the surface of the plate streaks of various species of bacteria are made and the plate is then kept at 25° to 30° C. After a few days around most of the colonies or streaks the said reaction appears with great distinctness. Tartrates, citrates, succinates, glycolates, salts of volatile acids, and sugars do not give the reaction.

The experiment may be modified in different ways, for example, by using instead of broth agar a culture medium of the composition : 100 tapwater, 1 calciummalate, 0,1 ammoniumsulfate, 0.02 potassium-fosfate, with some ferric citrate as an indicator, and furthermore treated as above. The less favourable source of nitrogen is cause that on such a medium a smaller number of species of bacteria grow, but the reaction is as distinct, and the proportion of the active to the non-active germs on the ammoniacal medium even greater than on the broth-agar plate.

The species that cause the conversion may be arranged in the following order according to their intensity of action.

The most vigorous splitters are *B. fluorescens*, *B. fl. liquefaciens*, *B. calco-aceticus* and *B. pyocyaneus*. Then follow *B. aerogenes*, *B. viscosus* and *B. levans*, and some varieties of *B. violaceus*.

A little less active are *B. coli*, *B. proteus*, *B. prodigiosus*, *B. kieliensis*, and the vinegar bacterium *Acetobacter rancens*. Quite inactive are *B. termo*, *B. punctatus*, *B. devorans*, *B. ochraceus*, and the luminous bacteria ; nearly inactive are the vinegar bacteria *Ac. melanogenum*, and *Ac. pasteurianium*.

Nor do the moulds and yeasts examined up to now produce pyruvic acid, although many of them readily oxidise the malates to carbonic acid and water.

As some bacteria such as *B. pyocyaneus*, *B. aerogenes*, *B. levans*, *B. viscosus* and some moulds and yeasts are able also to oxidise the pyruvates themselves to carbonate and water, one might suppose that the species which appear not to produce pyruvic acid from malic acid, in fact do so, but only as a transition product, but what follows is in contradiction with this supposition. First, the pyruvates are less readily attacked than the malates, so that accumulation and not oxidation of the pyruvates would become probable. Secondly experiments prove that the ferric salt solution enters the microbic cells. As now the oxidation of the pyruvates like that of the malates undoubtedly takes place in the interior of these organisms, the presence of the pyruvic acid ought to cause a colouring of them, which is not observed.

With the active microbes the reaction is caused by a certain portion of the living protoplasm, or in other words, by an endoenzyme or an endooxidase, to which the name of malopyruvase might be given. It belongs to the complex of the different endoenzymes which together govern the respiration function, of which it is one of the factors in MENDELIAN sense.

From the levogyric or common malic acid and the inactive malic acid pyruvic acid results with the same readiness; from the dextrogyric malic acid, which we owed to Prof. BLANKSMA, it is produced with much more difficulty.

The said microbes which produce pyruvic acid from the malic acids do the same from fumaric acid:

$$C_4H_4O_4 + O = C_3H_4O_3 + CO_2$$

but not from maleinic acid. We used fumaric acid as lime salt, prepared from the free fumaric acid after its purification by washing with water. Hence, malate of lime was not present in our fumarate.

That the ferric reaction is in fact caused by pyruvic acid is corroborated by the following observations.

The conversion of the malate can very well occur in a strongly aerated culture liquid, for instance in an ERLENMEYER-flask containing a thin layer of: 100 tapwater, 2 calciummalate, 0.1 ammonium sulfate, 0.02 potassium phosphate, with or without ferric citrate as an indicator. By infection with *B. fluorescens* and when cultivated at 20 to 25° C., after a few days the pyruvic acid can be destilled with sulfuric acid and be recognised in the destillate, not only by the odour and ferric salts, but also by the two following reactions: with the sulfite of hydrazin a characteristic phenylhydrazon results, and with ammoniacal nitroprussidsodium a violet colouring is obtained, which by acetic acid passes into blue, with potash into red. [1])

Indirectly the correctness of our diagnose was proved by the fact that as well alanin as asparaginic acid with the fluorescent bacteria, and besides with *B. levans* and *B. viscosus*, produce the iron reaction, so that also from these substances, in accordance with our expectation, pyruvic acid results.

On the other hand, this acid cannot be obtained from asparagin, aminopropionic acid, and glycerin, nor, as has already been noted, from maleinic acid.

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[1]) ROSENTHALER, Der Nachweis organischer Verbindungen. P. 390, 1914.