

olive oil it was 133.5 ± 10.2 sec. (variation coefficient 7.5 per cent), in the control group 245.0 ± 7.3 sec. (variation coefficient 7.3 per cent), that is, 65 per cent longer than in the fat-treated group. The time of survival at the altitude of 10,000 m., again 4 hr. after the ingestion of fat, was 156.0 ± 11 sec. (variation coefficient 22.3 per cent); in the control group 272.8 ± 30 sec. (variation coefficient 33.3 per cent), that is, 60 per cent longer. The difference is highly significant at the 1 per cent level. The significance of the difference of survival times disappears at an altitude of 9,500 m., 24 hr. after the administration of oil or starch.

The results presented in this report show a decreased tolerance of anoxia in mice 4 hr. after the application of a single dose of olive oil. Furthermore, they provide evidence for the participation of the above-mentioned relative hypoxia of the tissues after a single load of fat in the different unfavourable changes of the circulatory system due to an increased demand of oxygen in the tissues or due to an altered mechanism of oxygen transport. The different factors are fully analysed in further experiments which will be published in detail elsewhere.

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Lævan-forming Halophilic Bacteria

NON-HALOPHILIC bacteria which are able to form lævan from sucrose have been described within several genera, for example, *Bacillus*¹, *Pseudomonas* and *Xanthomonas*², *Streptococcus*³, *Aerobacter*⁴, as well as *Corynebacterium*⁵. However, lævan-forming bacteria occurring among the halophiles offer special interest from the enzymological point of view and, in addition, are of considerable economic importance.

Strains of bacteria which produce a viscous slime on nutrient agar containing six per cent sucrose and eight per cent sodium chloride were isolated from 'ropy' herring brine. These bacteria are non-spore-forming, non-motile, Gram-negative rods. They are obligate halophiles of the moderate type, showing maximal growth at 1–2 *M* sodium chloride, within the temperature range 20–30° C., and around pH 7. Neither acid nor gas is formed from carbohydrates. The bacteria are aerobic, but are able to grow under anaerobic conditions in the presence of nitrate which is reduced to nitrite and gas. Provisionally, they have been classified within the genus *Achromobacter*.

Neither lithium nor potassium can be substituted for the sodium ions, but bromide, sulphate and phosphate may partially replace the chloride ions.

The bacteria may be grown in synthetic media, supplemented with thiamine. Glutamic and aspartic acids are utilized as sole sources of carbon, energy and nitrogen. In the presence of ammonium salts, pyruvic, citric, succinic, fumaric and malic acids as well as α -alanine support growth. No other amino-acid tested could be used as sole carbon source, in spite of the fact that the readily water-soluble amino-

acids are all oxidized rapidly by washed cells in the Warburg apparatus. None of the carbohydrates tested, for example, arabinose, xylose, glucose, mannose, galactose, fructose, sucrose, maltose, lactose, raffinose and starch, can be used as carbon and energy source; nor are these substances oxidized in the Warburg apparatus. Thus, these bacteria do not attack carbohydrates, with the one exception that sucrose is transformed to a slime if the bacteria are at the same time provided with some source of carbon and energy which they can utilize, for example, certain amino-acids, pyruvic acid, or intermediates of the tricarboxylic acid cycle.

The slime formed from sucrose was precipitated by the addition of two volumes of 96 per cent ethanol. It was found to contain less than 0.05 per cent nitrogen. After hydrolysis⁶ by heating in 0.1 *N* oxalic acid for 45 minutes at 70° C., neutralization with calcium carbonate and centrifuging, the hydrolysate gave only one spot on the paper chromatogram. By means of its *R_F* value and colour reaction with naphthoresorcinol, this spot was identified as fructose. The results indicate that the slime substance is a polysaccharide of the lævan type and the enzyme active in its formation a lævansucrase.

For the study of the enzyme activity, acetone-dried preparations of the bacteria were used. The relative increase of viscosity, caused by the acetone-dried cells in a solution containing 0.08 *M* sucrose, 8 per cent sodium chloride, and 0.1 *M* phosphate buffer, pH 7.0, was taken as a measure of the enzyme activity. The lævansucrase was found to be constitutive and bound to the cells, showing little or no activity in solutions without added sodium chloride, with maximal activity in the range 1–3 *M*. Very low activities were obtained when the bacteria were washed several times with lithium or potassium chloride solutions before the acetone treatment, and determinations of the activity were made in solutions containing either of these chlorides instead of sodium chloride. It appears that the lævansucrase of these bacteria is specifically activated by sodium ions.

Whereas non-halophilic lævan-forming bacteria form slime from raffinose as well as from sucrose, the bacteria studied in the present work are only able to use sucrose as substrate. Other sugars, added to the sucrose-containing solution, inhibit the slime formation to varying degrees, the strongest effect being obtained with lactose.

Although the lævansucrase is a constitutive enzyme, the growth conditions of the bacteria seem to affect the amount of enzyme formed or its activity. From bacteria grown in aerated media or in the presence of nitrate, acetone-dried preparations of much higher activity were obtained than from cells grown under limited oxygen supply.

A detailed report of the results will be given elsewhere.

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