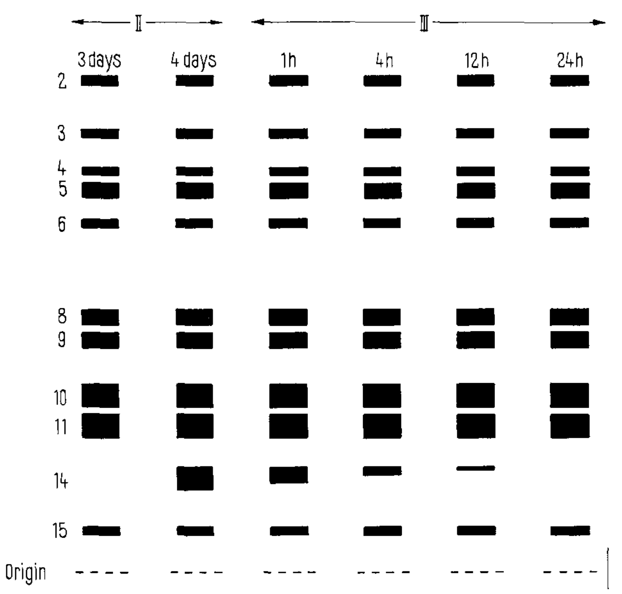
until after 12 h it is present only as a trace, and after 24 h is absent. This protein band is found to be strongly present in the late embryo and again in the first instar. Its behaviour in succeeding instars is similar to that shown in the Figure for the 3rd instar. It has not been found to be present in immature or mature adults.

None of the other bands have been found to show this cyclical behaviour; however, around the period of the moult, digestion of starch at the point of insertion of the sample suggests the occurrence of carbohydrases at this time.

Discussion. The only comparable study is that of STEINHAUER and STEPHEN[7] on *Periplaneta*. These workers using paper electrophoresis detected only three distinct bands, and they found them to be present in all stages of cockroach development. It is of interest to note that their band 2 was the most variable in its behaviour, showing distinct similarity with band 14 in the present investigation. Unlike band 14, however, which reached a peak of concentration at the time of the moult and disappeared soon after this, STEINHAUER and STEPHEN[7] record their band 2 as absent only for a short time in the intermoult period. Moreover, while band 14 was absent in all the adult locusts examined, STEINHAUER and STEPHEN[7] found band 2 continued to be present for 3–4 weeks in adult male cockroaches, and for some days in adult females. It would therefore appear probable that the band 2 of STEINHAUER and STEPHEN[7] corresponds to a number of the bands appearing on the starch electropherogram of which band 14 is but one. An electrophoretic study of the eluted band 2 of STEINHAUER and STEPHEN[7] would be of considerable interest. MISSELIJN et al.[8], in an agar gel electrophoretic study of the hemolymph of three species of *Triatoma*, both mature and immature, record no differences with respect to age in the individuals which they examined. Similarly, COLES[9] noted no large protein changes associated with moulting in *Rhodnius*. No record of a similar moulting protein fraction occurring in holometabolous insects has been found. The absence of other records is perhaps understandable, for it is only by regular sampling throughout the entire developmental stages of the insect that cyclical changes such as this become apparent.

It would appear probable that the band is intimately associated with moulting in some way, and further attempts are being made to investigate this. In this context, it is of interest to note the similarity in the cyclical behaviour of band 14 in the locust hemolymph and in the mitotic activity of the prothoracic gland[15] in view of the suggestion by WILLIAMS[16] that the secretion of the prothoracic gland may correspond to, or be associated with a protein fraction.



Diagrammatic representation of starch electropherograms of locust hemolymph samples made during late second and early third instars. The intensity of staining of the bands is represented by the relative thickness of the bands in the diagram.

Résumé. Une étude électrophorétique de l'hémolymphe aux divers stades de développement du criquet, *Locusta migratoria migratorioides*, révèle la présence d'une fraction protéique dont le comportement semble être lié au cycle de la mue.

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Growth of Some Chemoautotrophic Bacteria at Different Oxygen Tensions

In aerobic chemoautotrophic bacteria, molecular oxygen acts as a final electron acceptor in the oxidation of the inorganic substrates; the oxygen thereby being reduced to water. A maximum amount of energy is produced in these processes on which growth and other energy-consuming processes are dependent.

It is well known that most chemoautotrophic bacteria, despite their obvious aerobic nature, are difficult to culture on solid media although they might grow readily in liquid cultures where the diffusion of oxygen is much slower. This discrepancy has sometimes been ascribed to a harmful effect of the agar or other gelling agents. Such an explanation seemed unlikely and an investigation was initiated to find out what effects oxygen might have on the growth and substrate oxidation in some chemoautotrophic bacteria, viz. *Nitrosocystis oceanus*, *Nitrosomonas europaea*, *Nitrobacter agilis*, and *Thiobacillus thiooxidans*.

The organisms were spread by means of a glass rod on the surface of mineral agar media using Oxoid Ion-agar no. 2 as solidifying agent. The substrates were 1.0% $Na_2S_2O_3$ (*T. thiooxidans*), 0.2% NH_4Cl (*N. oceanus*), 0.25% $(NH_4)_2SO_4$ (*N. europaea*) and 0.03% $NaNO_2$ (*N. agilis*). As a carbon source, excess $CaCO_3$ was used for the two ammonium-oxidizers and 0.15% $KHCO_3$ for *N. agilis*. For *T. thiooxidans* 10% CO_2 was added to the gas mixtures. Each plate contained exactly 20 ml of medium. The cultures were incubated at room temperature in evacuated jars refilled with the desired oxygen-nitrogen-CO_2 mixtures. Following incubation the agar surfaces were first examined under the microscope using a low magnification lens and the media were then analyzed chemically. Sulfate was determined gravimetrically as $BaSO_4$, and nitrite colorimetrically by the standard Griess-Ilosway method.

Results. No oxidation of ammonium or nitrite occurred when the nitrifying bacteria were exposed to a concentration of 90% oxygen (Table) and, as could consequently be expected, there was no growth on any of the plates. Moreover, cells of *N. oceanus* exposed to 90% oxygen for three weeks would not grow or nitrify with a further incubation under lower oxygen tension. In air, substrate oxidation by *N. oceanus* or *N. agilis* was good, but only a trace of nitrite was produced by *N. europaea*. There was some growth of *N. oceanus*; microcolonies consisted of about 30–50 cells or less after three weeks of incubation. In some cases, the latter organism would not grow at all. At a low oxygen tension (2.3%), substrate oxidation by *N. europaea* was more rapid than in air, but at a slower rate than that found with the two other nitrifiers. Because the cultures of *N. europaea* and *N. agilis* were both contaminated with morphologically similar heterotrophic bacteria, the growth of these nitrifiers could not be determined with certainty. On the other hand, growth of *N. oceanus* on solid medium incubated at the lower oxygen tension was abundant when compared to plates incubated in air. Most microcolonies consisted of at least 200 cells, some colonies were even larger. The colonies seemed to be rather loose, since they were easily ruptured when a coverglass was put on the agar surface. The single cells thus set free were all motile. No cyst formation was observed on any plate.

In another experiment *N. europaea* and *N. agilis* were inoculated on agar media containing ^{14}C as $Na_2{}^{14}CO_3$. Although substrate oxidation had the same trend with respect to the amount of oxygen in the gas phase as shown in the Table, ^{14}C incorporation was greatest at the lower oxygen concentration. *N. europaea* had most ^{14}C uptake in 2.3% oxygen and *N. agilis* in air.

With *T. thiooxidans* the higher oxygen tension also had a marked effect, only small amounts of sulfate were produced and growth was poor. In some experiments no growth could be detected, only scattered cells from the inoculum being present. In air *T. thiooxidans* grew fairly well but not as well as with a lower oxygen tension. Elementary sulfur precipitated in and around the colonies under both sets of conditions, but there was considerably more precipitate in the plates exposed to low oxygen tension, particularly in the areas within the colonies. In a later experiment the greatest amount of substrate oxidation occurred in air followed by 2.3% and 0.1% oxygen concentrations.

As judged from the above results, molecular oxygen seems to have a dual effect on nitrifying bacteria and *T. thiooxidans*. The oxidation of the inorganic substrates is generally enhanced when the partial pressure of oxygen is increased, whereas growth is depressed. The fact that almost no nitrite was produced, or oxidized in the case of *N. agilis*, in 90% oxygen must reflect the absence of growth and not of an inhibition of substrate oxidation per se. That substrate oxidation really is stimulated by increased oxygen tension has been demonstrated using Warburg techniques with resting cell suspensions of *N. oceanus*[1]. A doubling of oxygen consumption during the first hour was obtained in 90% oxygen as compared with cells respiring in air. Oxygen uptake was approximately 20% lower in a concentration of 2.3% oxygen in the gas phase than in air.

A stimulating effect of elevated oxygen tensions on substrate oxidation by *N. europaea* and *N. winogradskyi* (with subsequent lethal effect!) was demonstrated by MEYERHOF many years ago[2]. It has also been observed that vigorous aeration of liquid cultures of *N. agilis* at the beginning of the growth period prolonged the lag phase[3]. Recently SCHÖN[4] demonstrated that the growth of *N. winogradskyi* was inhibited when the organism was exposed to 95% oxygen, whereas the oxidation of nitrite would proceed linearly.

A discussion, including some thermodynamical aspects for simultaneous substrate oxidation and assimilation in aerobic chemoautotrophic bacteria, will be published separately[5,6].

Substrate oxidation by nitrifying bacteria and *Thiobacillus thiooxidans* at different oxygen concentrations. Incubation time was 3 weeks for the nitrifiers, 10 days for *T. thiooxidans*

Oxygen %	*Nitrosocystis oceanus*	*Nitrosomonas europaea*	*Nitrobacter agilis*	*Thiobacillus thiooxidans*
	μg NO_2-N/plate produced		μg NO_2-N/plate consumed	mg SO_4-S/plate produced
90	10	0	40	12.6
21 (air)	840	2	1190	56.0
2.3	630	72	580	84.2

Résumé. Les bactéries suivantes: *Nitrosocystis oceanus, Nitrosomonas europaea, Nitrobacter agilis* et *Thiobacillus thiooxidans* ont été cultivées en milieu solide dans une atmosphère dont on a varié la teneur en oxygène. On a trouvé que la croissance de toute ces bactéries a été arrêtée en présence d'un excès d'oxygène (90%). D'autre part l'oxydation de l'ammonium du nitrite et du thiosulfate n'a pas été inhibée par oxygène.

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