of the nitrifiers were due to a lack of oxygen supply. This view he tries to justify by referring to correct figures on oxygen consumption of nitrifying cultures and diffusion-rate of oxygen taken respectively from Stephenson and Höber.

It will be shown here that their application to the problem of getting pure cultures is not justified. The usual rate of nitritation ($2NH_3 \rightarrow N_2O_3$) in the static enrichment cultures is that 50 mgm. ammonium sulphate is oxidized in seven days. If diffusion alone is to supply oxygen, it would be necessary that all the bacteria should be present during the last 12 hr. of the culture in a layer of 1 mm. thickness at the surface of the culture. This could only be accomplished by swarming bacteria; but experience shows that this is not the case. Oxygen supply is therefore not by diffusion alone, but also by convection currents. This is not surprising, because in the incubators the bottoms of the culture flasks are heated and the surface of the culture liquid cools by evaporation, so there will be currents going up and down. Moreover, it can be shown that the rate of oxidation can be increased by adding a second portion of 50 mgm. ammonium sulphate at the end of the period of seven days. After this, the oxidation is completed in four days. It is therefore certain that, in enrichments to be used for plating, the nitrifying organisms never suffer from lack of oxygen. This was confirmed by the fact that in shaken cultures the rate of oxidation was usually the same as in the static cultures. Sometimes there was a slight acceleration by shaking, but this may have been due to the prevention of formation of zoogloea. The heterotrophic bacteria, however, are also stimulated by shaking, so that this is no improvement. This is to be expected, because an increase of autotrophic bacteria means more food for the heterotrophic bacteria, and therefore it seems very improbable that pure cultures will be obtained from enrichments by any method that will stimulate the growth of nitrifiers.

It is a fact that *Nitrosomonas* is stimulated by some metabolic products of the heterotrophic bacteria, for when *Nitrosomonas* is in a pure culture its growth is at first very slow. If the growth-rate of the enrichments were only determined by the oxygen supply, it would be expected that pure cultures would grow at least as rapidly as enrichments. It looks as if the heterotrophic bacteria produce some growth-factor for *Nitrosomonas* which is not strictly necessary or which they can learn to make themselves, for after some time the nitrification-rate becomes normal. The nitrification-rate of pure cultures is also accelerated by *Nährstoff*-Heyden[2]. This shows that the growth-rate of *Nitrosomonas* is not conditioned by the respiration process but by the assimilation process. As the minimum generation time of the nitrifying bacteria is about 5–6 hr., it is clear that in cultures inoculated with a small number of bacteria the nitrification-rate will always appear to be very slow, notwithstanding the nitrification-rate per bacterium is optimal.

It should be pointed out that for plating one needs only cultures with 100–500 bacteria per ml., hence it is quite unnecessary to have cultures with a high nitrification-rate with millions of bacteria per ml.

Dr. Lees's remarks about the amount of growth in Petri dishes are not correct. In the first place, the growth is not limited by the air space, but by the amount of ammonium sulphate. The capacity of a Petri dish of diameter 10 cm. and height 1·5 cm. is about 117 ml. Filling it with 25 ml. silica gel leaves an air space of 90 ml., which contains 18 ml. oxygen. In the gel there is 25 mgm. ammonium sulphate, for the oxidation of which 12·7 ml. oxygen is required, so there is plenty of oxygen. In the second place, a Petri dish is anything but closed. In the daytime the temperature in the incubators will fluctuate more or less, because they are often opened and closed and therefore there will be some replacement of air in the dish. Further, fresh air will be sucked in when oxygen is removed by the bacteria. To this may be added that Meyerhof found that the respiration-rate is first affected when the oxygen tension is lowered to half the tension of the oxygen of the air.

From all this it is clear that the problem of getting pure cultures[3] is not one of oxygen supply, and that it is very unlikely that the percolator method will give as a rule a pure culture. Notwithstanding this, the percolator method may be useful for obtaining nitrifying bacteria in quantities that otherwise would be difficult to get.

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[1] Lees, H., *Nature*, **167**, 355 (1951).
[2] Kingma Boltjes, T. Y., *Arch. Mikrob.*, **6**, 79 (1935). Hes, J. W., *Rec. Tr. Bot. néerlandais*, **34**, 234 (1937).
[3] Hanks, J. H., and Weintraub, R. L., *J. Bact.*, **32**, 653 (1936). Meiklejohn, J., *J. Gen. Microb.*, **4**, 185 (1950).

In the communication referred to by Prof. Kingma Boltjes, I was dealing explicitly with intensive nitrification. Prof. Kingma Boltjes, on the other hand, is considering rather low nitrification-rates, at which oxygen supply will be naturally less important. I therefore do not think that our respective calculations are exactly comparable.

I am, however, glad to have the opportunity of agreeing wholeheartedly with his remarks about the possible importance of growth-factors in the economy of *Nitrosomonas*; our own experiences have led us to suspect that one such factor may be biotin.

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Biochemical Role of Vitamin C

The interesting communication by Kalnenas[1] on the amounts of ascorbic acid in the plasmas of antarctic birds and mammals contains a statement that ought not to go unchallenged. He says, "Ascorbic acid is an indispensable hydrogen carrier in the metabolism of animals". Contrast this with the more realistic statement of Harris[2], "It has to be admitted . . . that the exact biochemical role of vitamin C is still obscure, although there is probably significance in the recent findings that in animal tissues it can catalyse the oxidation of the side chain of the . . . tyrosine".

Again, Damron *et al.*[3] have studied the *in vivo* oxidation of ascorbic acid to dehydroascorbic acid and then to diketogulonic acid and interpret their results to suggest that these reactions "represent pathways of decomposition rather than of normal metabolism in animal tissues".

Kalnenas is surely arguing in a circle. Only the primates and the guinea pig, so far as is known, cannot synthesize their requirements of vitamin C, as he correctly states; yet in all animals hitherto