formic acid and CO_2, and that of glyoxylic acid to equimolecular amounts of formic acid and oxalic acid with evolution of CO_2 if, as seems likely, oxalic acid is the direct product of the enzyme-catalysed oxidation of glyoxylic acid. In the presence of sufficient catalase to stop the secondary reaction between glyoxylic acid and H_2O_2, no formic acid should be formed. As previously suggested, the most likely product under these circumstances would be oxalic acid. Nord & Vitucci (1947) have shown that certain fungi convert glycollic to oxalic acid with the postulated intermediate formation of glyoxylic acid.

In the present work the amounts of added catalase were such as to give a catalase activity of the same order as that of 3 ml. of tobacco-leaf sap. In view of the results obtained it appears that when untreated sap is used to catalyse the oxidation of lactate and glycollate its catalase activity would be sufficient to prevent the non-enzymic oxidation of pyruvate and glyoxylate by H_2O_2. Results with α-hydroxyacid oxidase preparations made from the sap would depend on the catalase activity of such preparations.

The influence of catalase on the oxidations catalysed by α-hydroxyacid oxidase provide an explanation of at least some of the variable O_2 uptakes and CO_2 outputs obtained by Clagett *et al.* (1949), Tolbert *et al.* (1949) and Tolbert & Burris (1950).

SUMMARY

1. Hydrogen peroxide is produced during the oxidation of lactate, glycollate and glyoxylate catalysed by plant α-hydroxyacid oxidase. This has been shown by the effect of catalase on the oxygen uptake.

2. The oxidation products depend on the amount of catalase present. In the absence of catalase, the enzyme-catalysed reactions are followed by non-enzymic reactions between hydrogen peroxide and pyruvate or glyoxylate, with the formation of acetate or formate respectively.

3. It is suggested that oxalate is the product of the enzyme-catalysed oxidation of glyoxylate.

REFERENCES

Agner, K. (1938). *Biochem. J.* **32**, 1702.
Clagett, C. O., Tolbert, N. E. & Burris, R. H. (1949). *J. biol. Chem.* **178**, 977.
Clift, F. P. & Cook, R. P. (1932). *Biochem. J.* **26**, 1788.
Dixon, M. (1943). *Manometric Methods*, 2nd ed. p. 64. Cambridge University Press.
Hatcher, W. H. & Holden, G. W. (1925). *Trans. roy. Soc. Can.* (3), **19**, 11.
Kenten, R. H. & Mann, P. J. G. (1952). *Biochem. J.* **52**, 125.
Kolesnikov, P. A. (1948*a*). *Dokl. Akad. Nauk. S.S.S.R.* **60**, 1205. Cited in *Chem. Abstr.* (1948), **42**, 7373*f*.
Kolesnikov, P. A. (1948*b*). *Biokhimiya*, **13**, 370. Cited in *Chem. Abstr.* (1948), **42**, 8889*b*.
Negelein, E. & Brömel, H. (1939). *Biochem. Z.* **300**, 225.
Nord, F. & Vitucci, J. C. (1947). *Arch. Biochem.* **14**, 229.
Pirie, N. W. (1946). *Biochem. J.* **40**, 100.
Pucher, G. W., Vickery, H. B. & Wakeman, A. J. (1934). *Industr. Engng Chem.* (Anal. ed.), **6**, 140.
Singer, T. P. & Kearney, E. B. (1950). *Arch. Biochem.* **29**, 190.
Sumner, J. B. & Somers, G. F. (1943). *Chemistry and Methods of Enzymes*, p. 171. New York: Academic Press Inc.
Tolbert, N. E. & Burris, R. H. (1950). *J. biol. Chem.* **186**, 791.
Tolbert, N. E., Clagett, C. O. & Burris, R. H. (1949). *J. biol. Chem.* **181**, 905.

The Biochemistry of the Nitrifying Organisms

1. THE AMMONIA-OXIDIZING SYSTEMS OF *NITROSOMONAS*

By H. LEES
Department of Biological Chemistry, University of Aberdeen

(*Received* 27 *December* 1951)

Micro-organisms of the genus *Nitrosomonas* are autotrophs, typically found in soil, which oxidize ammonia to nitrite. Since the energy yielded by this oxidation is apparently the only primary energy source available to the organisms, no cell growth or proliferation can take place without ammonia oxidation. On the other hand, Nelson (1931) showed that ammonia oxidation could take place in the absence of carbon dioxide, i.e. under conditions that excluded the possibility of cell growth or proliferation, the energy released being (presumably) dissipated as heat instead of being used, in part at least, for carbon dioxide assimilation. It therefore follows that while any compound that inhibits ammonia oxidation by the organisms must inhibit their proliferation, the fact that a com-

pound inhibits proliferation cannot be taken as evidence that such a compound is inhibiting ammonia oxidation. A great deal of the confusion and contradiction that exists about the inhibitory effects of various compounds on 'nitrification by *Nitrosomonas*' can be traced to a failure to characterize the type of inhibition studied. The inhibitory actions of various compounds have, for instance, commonly been studied by means of experiments in which parallel inoculations of *Nitrosomonas* were made into two batches of culture medium, similar in all respects except that one batch contained some compound 'X' while the other did not. After some considerable period, usually days or weeks, the amount of nitrite formed in one culture was compared with the amount formed in the other. If the amount formed in the presence of 'X' was less than in its absence, 'X' was said to 'inhibit *Nitrosomonas*' or to 'inhibit nitrification by *Nitrosomonas*'. Clearly such experiments could give no indication how 'X' acted. If 'X' directly inhibited ammonia oxidation, then of course less nitrite would be formed in the presence of 'X' than in its absence. But if 'X' had no effect on ammonia oxidation and yet inhibited cell proliferation in some other way, the neogenesis of ammonia-oxidizing enzymes would be less rapid in the presence of 'X' than in its absence; as a consequence, nitrite formation in the presence of 'X' would be less than in its absence. In the present work the actions of various compounds on the formation of nitrite from ammonia by *Nitrosomonas* have been studied directly in experiments lasting not more than 3 hr.; since cell proliferation during this period must have been very small, it follows that inhibitions of nitrite formation noted under these conditions were predominantly due to an inhibition of ammonia oxidation. To the best of the author's knowledge, no such experiments have been performed before.

MATERIALS AND METHODS

Nitrosomonas was cultured from an Aberdeenshire garden soil (see below) by methods essentially similar to those already described (Lees, 1951). Minor modifications were, however, made in the design of the culture vessels and in the method of preparation of the inorganic culture medium.

Description of the organisms. The organisms so isolated, which were used throughout the present work, conformed precisely to the description of *N. europaea* given by Meiklejohn (1950). On silica gel, characteristic 'starfish colonies' (Meiklejohn, 1950) were obtained. It is therefore considered that the organisms were a normal strain of *N. europaea*.

Purity of the cultures. The commonly accepted criteria for the purity of *Nitrosomonas* cultures are (*a*) they should not form nitrate since the formation of nitrate from nitrite would indicate the presence of *Nitrobacter*, (*b*) they should show no growth on nutrient agar since growth on nutrient agar would indicate the presence of organisms other than *Nitrosomonas*. There is usually little difficulty in freeing the cultures of *Nitrobacter*, because *Nitrobacter* is inhibited by ammonia, which must, of course, always be present in growing cultures of *Nitrosomonas*. The important criterion of purity is therefore the lack of growth on nutrient agar; which, being a negative criterion, is of rather doubtful reliability (e.g. Nelson, 1931; Grace, 1951). The cultures used in the present work were not only pure by the two negative criteria mentioned, but had a high free-energy efficiency (Baas-Becking & Parks, 1927). The determinations of free-energy efficiency will form the subject of a future publication (Hofman & Lees, 1952). Since the presence of contaminants could have had no effect on the free-energy efficiency of the cultures except to lower it, it is considered that the high free-energy efficiency of the present cultures is a further indication of their purity.

Preparation of the medium. Although the medium originally described for the isolation of the organisms (Lees, 1951) proved satisfactory in most respects, it was necessary for the conduct of experiments on the free-energy efficiency of the cultures (Hofman & Lees, 1952) that the amount of adventitious organic material in the medium should be kept as low as possible. Since most laboratory chemicals seemed to be contaminated with specks of organic detritus, the medium used in the present work was prepared by precipitation from three solutions, each being filtered before use. Solution *A*: $CaCl_2$ (100 g.), $MgCl_2.6H_2O$ (100 g.), KH_2PO_4 (50 g.) dissolved in 1 l. of water slightly acidified with HCl to assist solution. Solution *B*: $FePO_4.2H_2O$ (1 g.) dissolved in 25 ml. N-H_2SO_4. Solution *C*: $Na_2CO_3.10H_2O$ (500 g.) dissolved in a minimum of warm water. Solutions *A* and *B* were mixed and added to solution *C*. The resulting precipitate of phosphates, sulphates, and carbonates of Ca, Mg, and Fe, was washed at the centrifuge with distilled water, until the washings were free of chloride, and finally suspended in 1 l. of distilled water. The medium on which the organisms were grown consisted of 10 ml. of this suspension, 1 ml. M-$(NH_4)_2SO_4$, and 89 ml. of distilled water. The principal difference in composition between this medium and the original medium (Lees, 1951) was that the new medium contained $MgCO_3$ while the original medium did not. The $MgCO_3$ raised the initial pH of the medium from 7·5 to 8·5, which is the optimal pH for nitrite formation by *Nitrosomonas* (Meyerhof, 1916).

Culture of the organisms

Cultures were grown in the dark at 28–30° in culture vessels whose design is shown in Fig. 1.

Sterilization of culture vessels. The 250 ml. glass jar containing 100 ml. of culture medium was plugged with cotton wool and sterilized in a small autoclave for 20 min. at 125°. Despite its alkalinity, the medium lost only a negligible amount of its NH_3 during this sterilization; the normal practice of sterilizing the $(NH_4)_2SO_4$ separately (e.g. Meiklejohn, 1950) would thus seem to be unnecessary. The rubber bung with its associated aerator and sampling assembly was not sterilized *in situ*, but was wrapped in paper, placed upside down in a beaker, and sterilized separately in the same autoclave. This procedure was used because when the bung was sterilized *in situ*, red dye from it sometimes seeped into the medium in the jar; *Nitrosomonas* would not grow in a medium so contaminated. After sterilization, the component parts of the culture vessel were assembled and inoculated with approximately 0·5 ml. of a culture of actively nitrifying *Nitrosomonas*, the vessel was

then attached to the air line through which air, sterilized by passage through two gas washers (Lees, 1949) (5% (w/v) $CuSO_4$ plus 5% (v/v) H_2SO_4), was supplied. From time to time, samples of approximately 2 ml. volume were withdrawn from the vessel via the sampler tube and analysed for NH_3-N, NO_2-N, and occasionally NO_3-N (see below). When the concentration of NH_3-N fell below 50 μg./ml. fresh NH_3 was added as sterilized $(NH_4)_2SO_4$ solution (if the pH of the sample, tested by B.D.H. (British Drug Houses Ltd.) Universal Indicator, was above 7·0) or as sterilized $(NH_4)_2CO_3$ (if the pH of the sample was below 7·0). It should be understood that the pH of the cultures must fall during growth of the organisms because of replacement of NH_4^+ by NO_2^-. The course of nitrite formation, commonly used as an indication of growth of *Nitrosomonas* cultures (Stephenson, 1949), in a typical culture used in the present work is shown in Fig. 2. Subcultures into fresh medium were made when the parent culture had formed 200–300 μg. NO_2-N/ml.

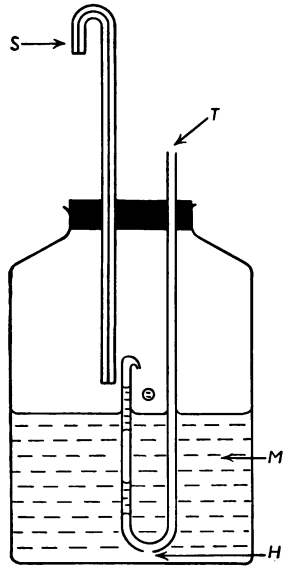

Fig. 1. Culture vessel. Sterile air is forced into the apparatus at *T*. In passing round the U-tube the air entrains bubbles of liquid medium *M* at the pin-hole *H*. The bubbles are carried by the air stream up the U-tube and expelled on to the surface of the liquid medium. The air escapes through the capillary tube *S*. The tube *S* can be dipped into the medium for the withdrawal of samples.

Incubation experiments

When the NO_2-N concentration of a growing culture of *Nitrosomonas* had risen above 200 μg. NO_2-N/ml., approximately 50 ml. of the culture were expelled through the sampler tube (*S*, Fig. 1) into a 100 ml. centrifuge tube, and centrifuged to bring down the precipitate of Ca and Mg carbonates to which the organisms adhere (Winogradsky, 1890*a*, *b*; Meiklejohn, 1950). The precipitate, with its adherent organisms, was washed two or three times at the centrifuge with water or with 0·9% NaCl solution to remove nitrite and NH_3, and finally resuspended in distilled water. Samples (2 ml.) of this suspension were incubated at 30° with solutions of the substance under test, both in the presence and absence of $(NH_4)_2SO_4$ solution, the final volume of suspension with all additions being 4 ml. Incubation was carried out in 50 ml. conical flasks, over the bottom of which the 4 ml. solution formed a thin, well aerated, layer. After the requisite period, the contents of the flasks were suitably diluted and centrifuged in conical centrifuge tubes to remove the inorganic carbonate precipitate. The NO_2-N in 5 ml. of the supernatant liquid was then determined (see below). In the present work, no nitrite was formed in the absence of NH_3, i.e. no compound tested, other than NH_3, gave rise to nitrite. No nitrite was formed if $(NH_4)_2SO_4$ solutions were incubated with boiled suspensions of *Nitrosomonas*. No appreciable amount of nitrite was formed if the incubation was carried out *in vacuo* or if the air in the flask was replaced by N_2. It therefore follows that the formation of nitrite under the experimental conditions used in this work was an aerobic, biological process, and that the nitrite formed was formed from NH_3.

Determination of NH_4-N. A 1 ml. sample of culture fluid was treated with approximately 2 ml. saturated borax

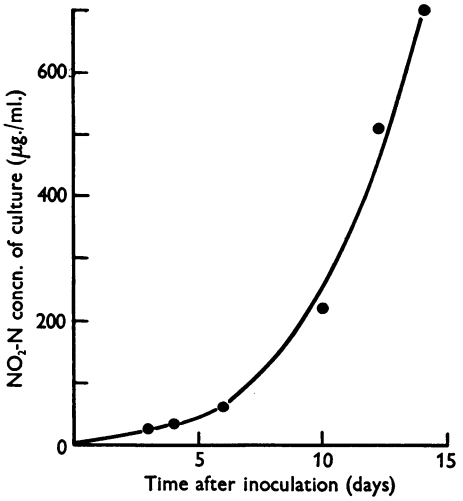

Fig. 2. The course of nitrite formation in a growing culture of *Nitrosomonas*. Culture volume, 100 ml.; inoculum volume, 0·5 ml.

solution and distilled from the Markham microdistillation apparatus (Markham, 1942) into 2 ml. 1% boric acid containing 0·25% of Conway and O'Malley's indicator (Conway & O'Malley, 1942). After about 5 ml. of distillate had been collected, the contents of the receiver were titrated with 0·007 N-HCl from a 1 ml. burette.

Determination of NO_2-N. A suitably diluted sample (5 ml.) of culture fluid (or fluid from an incubation experiment) was treated with 1 ml. of sulphanilic acid reagent followed by 1 ml. 1-naphthylamine reagent; the reagents were prepared according to Snell & Snell (1936). After 30 min. the light absorption of the treated sample was determined in an EEL colorimeter (Evans Electroselenium Ltd.) fitted with the 404 green filter supplied with the instrument. The NO_2-N content of the sample was obtained by reference to a standard curve constructed from the measured light absorptions of nitrite solutions of known strength, and

treated in the same way. The maximum amount of NO_2-N that could be used for a single estimation was about 2·5 μg., the minimum amount about 0·1 μg.; readings were repeatable to within 0·02 μg. within these limits.

Determination of NO_3-N. This was carried out according to the method used by Lees & Quastel (1946), except that the H_2O_2 oxidation was omitted. Under these conditions nitrite did not interfere with the estimation of nitrate by the phenoldisulphonic acid reagent (at least up to concentrations of 700 μg. NO_2-N/ml. sample).

Table 1. *Nitrite formations in washed suspensions of* Nitrosomonas *incubated for* 180 *min. in the presence of various compounds and* 10^{-3} M-$(NH_4)_2SO_4$

(All compounds were used at a concentration of 5×10^{-3} M except where otherwise stated. Final volume of washed suspension + additions = 4 ml.)

Compound tested	NO_2-N formed during incubation (μg.)
None (water control)	3·1
Glycine	3·2
L-Alanine	3·2
DL-Valine	3·1
L-Leucine	3·0
L-Isoleucine	3·0
L-Serine	3·0
L-Threonine	3·0
L-Aspartic acid	3·0
L-Glutamic acid	3·0
L-Lysine	3·0
L-Arginine	2·0
L-Histidine	0·0
L-Cystine ($2·5 \times 10^{-3}$ M)	3·0
DL-Methionine	3·0
L-Phenylalanine	2·5
L-Tyrosine	2·7
DL-Tryptophan	3·0
L-Proline	3·1
L-Hydroxyproline	3·0
L-Glutamine	3·0
L-Asparagine	3·0
DL-Methionine sulphoxide	2·6
Creatinine	2·6
Glycocyamine	2·6
2:4-Dinitrophenol	3·0
Ethylurethane	1·0
D-Mannose (5×10^{-2} M)	3·0
Streptomycin (Ca salt; 250 p.p.m.)	3·0

Soil percolation

Samples (10 g.) of garden soil were percolated at 28–30° according to the normal technique (Lees, 1949). The soil was a sandy loam from Milltimber, Aberdeenshire; pH, 6·8; organic matter, determined by the method of Bremner (1949), was 6%.

RESULTS

The effects of all the common amino-acids on the oxidation of ammonia by *Nitrosomonas*, together with the effects of various other compounds selected for reasons that will appear in the Discussion are shown in Table 1. The lack of any effect of streptomycin at a concentration of 250 p.p.m. is interesting, since streptomycin inhibits growth of the organisms at far lower concentrations (Fig. 3).

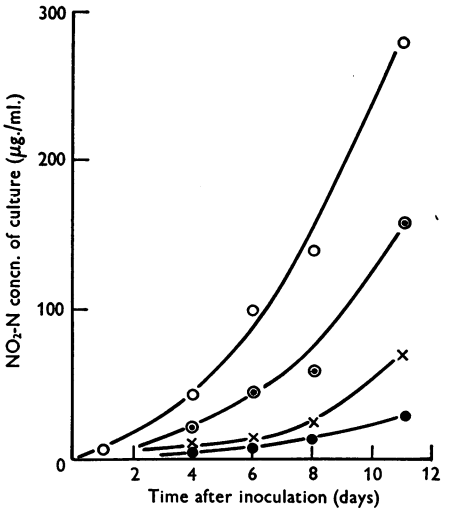

Fig. 3. The effect of streptomycin on nitrite formation in growing cultures of *Nitrosomonas*. Culture volumes, 100 ml.; volumes of inocula, 0·5 ml. (○—○), untreated medium; (●—●), streptomycin 0·4 p.p.m. in medium; (×—×), streptomycin 0·2 p.p.m. in medium; (⊙—⊙), streptomycin 0·1 p.p.m. in medium. Streptomycin was added to the medium as the Ca salt at the start of the experiments.

Table 2. *Nitrite formations in washed suspensions of* Nitrosomonas *incubated for* 90 *min. in the presence of various concentrations of* $(NH_4)_2SO_4$ *and a single concentration of inhibitor*

(Final volume of washed suspension + additions = 4 ml. Bracketed figures are nitrite formations expressed as percentages (to the nearest 10%) of the 'control' nitrite formation.)

$(NH_4)_2SO_4$ concn. (M)	NO_2-N (μg.) formed during incubation in presence of: No inhibitor (control)	L-Histidine $2·5 \times 10^{-4}$ M	Guanidine $1·2 \times 10^{-4}$ M	Allylthiourea 5×10^{-7} M
$2·5 \times 10^{-3}$	1·00	0·60 (60)	0·36 (40)	0·46 (50)
$1·2 \times 10^{-3}$	0·70	0·40 (60)	0·28 (40)	0·35 (50)
$6·2 \times 10^{-4}$	0·50	0·32 (60)	0·20 (40)	0·24 (50)
$3·1 \times 10^{-4}$	0·30	0·24 (80)	0·16 (50)	0·15 (50)
$1·6 \times 10^{-4}$	0·16	0·08 (50)	0·08 (50)	0·08 (50)
Nil	Nil	Nil	Nil	Nil

It has already been shown (Lees, 1946, 1948) that chelating agents inhibit nitrification in soil; the inhibitory effects of various chelating agents on ammonia oxidation by *Nitrosomonas* are shown in Fig. 4; histidine is included among the chelating agents since Maley & Mellor (1950) have shown that it is outstanding among the amino-acids in its power to bind metal ions. Further experiments with three of these inhibitors failed to reveal any evidence that the inhibitions produced were competitive with respect to ammonia, since the percentage inhibition of ammonia oxidation caused by one concentration of inhibitor was independent of ammonia concentration when this was varied over a fairly wide range (Table 2).

Table 3. *The breakdown of* DL-*methionine percolated as* 100 *ml. of approx.* 10^{-2} M *solution through* 10 *g. Aberdeenshire garden soil*

(The presence of volatile sulphur compounds in the air issuing from the percolator was detected by smell.)

Time from start of percolation (hr.)	Nitrogen (μg./ml.) present in percolate as NH_3-N (*a*)	Amino N (*b*)	(*a*) + (*b*)	Volatile sulphur compounds in air issuing from percolator
6	0	131	131	+
24	7	132	139	+ + +
48	27	97	124	+ +
96	55	80	135	–
144	77	60	137	–

Quastel & Scholefield (1949, 1951) have shown that low concentrations of DL-methionine inhibit soil nitrification as studied by a percolation technique. Percolation experiments carried out in this laboratory on the effect of percolated DL-methionine on soil nitrification have confirmed Quastel & Scholefield's finding, but, since the results in Table 1 afforded no indication that DL-methionine inhibited ammonia oxidation by washed suspensions of *Nitrosomonas*, it seemed possible that the inhibitions observed by Quastel & Scholefield, and confirmed here, might have been due to products formed from the percolated methionine by the soil heterotrophs. The breakdown of DL-methionine percolated through soil was therefore studied. The results of these experiments, given in Table 3, showed that deamination of percolated methionine was rapid. From an early stage in the percolation, the air issuing from the percolators had a foul smell reminiscent of hydrogen sulphide or mercaptans which indicated some breakdown of the methionine. Paper chromatograms carried out on samples of the percolate by Dr T. Hofman showed a gradual disappearance of the methionine, but no detectable production of methionine sulphoxide which Quastel & Scholefield (1951) suggested might be formed from percolated methionine. It thus seemed likely that the results of any experiments carried out with methionine under percolation conditions would be most difficult to interpret unequivocally and soil experiments on these lines were not continued.

DISCUSSION

Some of the compounds which the present results have shown to inhibit ammonia oxidation by *Nitrosomonas* have already been shown to inhibit either soil nitrification or the oxygen uptake of suspensions of *Nitrosomonas*. These compounds are guanidine (Meyerhof, 1916), allylthiourea, potassium ethyl xanthate, salicylaldoxime, and sodium diethyldithiocarbamate (Lees, 1946, 1948), and thiourea (Quastel & Scholefield, 1949, 1951). Inhibitions by L-histidine and 8-hydroxyquinoline have not, to the writer's knowledge, been previously reported. All these inhibitors can be subsumed under the heading 'chelating agents', and the simplest explanation of their effects is therefore that they inhibit the action of some metal enzyme. This is in agreement with the suggestion (Lees, 1946, 1948) that some metal enzyme is intimately concerned in ammonia oxidation by *Nitrosomonas*. Quastel & Scholefield (1951) have advanced an alternative theory to account for the inhibitory effects of guanidine, urethane, and thiourea; they suggest that 'It seems likely that the affinity of the N atom in the structure R.NH.C≡ for the ammonia-oxidizing enzyme is primarily involved.' There are, however, a number of objections to this theory. The inhibition of nitrite formation caused by 5×10^{-3} M-urethane in the present work was 65% (Table 1); Quastel & Scholefield (1951) noted a 45% inhibition of the oxygen uptake of a soil sample treated with ammonium chloride and 5×10^{-3} M-urethane, compared with the oxygen uptake in the absence of urethane. The agreement between these two results is good, but the degree of inhibition is of quite different order from the inhibition of nitrite oxidation brought about by chelating agents (Fig. 4), the weakest of which (L-histidine) was completely inhibitory at a concentration of $2{\cdot}5 \times 10^{-3}$ M. Moreover, as Quastel & Scholefield admit, arginine and creatine should, on their theory, inhibit ammonia oxidation by *Nitrosomonas*, whereas their own results failed to reveal any inhibition by these compounds. The 'affinity of the N atom in the structure R.NH.C≡' seems to imply competition between ammonia and R.NH.C≡ for some active site on the enzyme, yet inhibitions by guanidine and allylthiourea were apparently not competitive (Table 2); this finding is difficult to harmonize with Quastel & Scholefield's theory, but is consistent with the 'chelation' theory, which does not involve any concept of competition. In addition, the distribution of the points in Fig. 4 is what would be expected if all

the compounds used in determining the points had inhibited ammonia oxidation by a common chelation mechanism, since the slopes of the lines relating percentage inhibition with log. (inhibitor concentration) were all the same. Moreover, the weakest chelating agent, L-histidine, gave the weakest inhibition, while the stronger chelating agents gave correspondingly stronger inhibitions. There thus seem to be reasonable grounds for retaining the original 'chelation' theory, which has the merit of accounting for rather more facts.

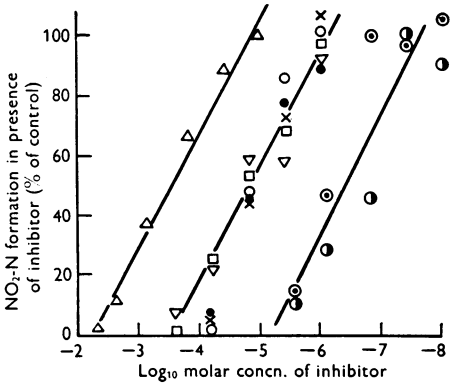

Fig. 4. The effects of various inhibitors on nitrite formation in washed suspensions of *Nitrosomonas* incubated for 180 min. in the presence of the inhibitor and 10^{-3}M-$(NH_4)_2SO_4$. Nitrite formations are given as percentages of the nitrite formation in similar washed suspensions, containing 10^{-3}M-$(NH_4)_2SO_4$, but without the addition of any inhibitor. (△—△), L-histidine; (▽—▽), guanidine; (●—●), 8-hydroxyquinoline; (□—□), salicylaldoxime; (○—○), potassium ethyl xanthate; (×—×), sodium diethyldithiocarbamate; (◑—◑), thiourea; (⊙—⊙), allylthiourea.

Jensen (1950) showed that D-mannose (6×10^{-3} M) inhibited growing cultures of *Nitrosomonas* studied over periods of several days, whereas the other common monosaccharides did not. The present work failed to reveal any direct effect of D-mannose within 3 hr. on nitrite formation by *Nitrosomonas* even at concentrations 8 times greater than those used by Jensen. This suggests that D-mannose inhibits some essential growth process in *Nitrosomonas* other than ammonia oxidation.

Despite the wide variety of nitrogenous compounds tested in the present work, none, except ammonia, was converted to nitrite by *Nitrosomonas*. This is in agreement with the generally accepted theory that ammonia is the obligatory primary oxidation substrate of *Nitrosomonas*. Although the stages by which the ammonia is converted to nitrite remain largely obscure there is now some evidence (Lees, in preparation) that hydroxylamine may be involved, as suggested by Kluyver & Donker (1926). If hydroxylamine, either free or as a bound hydroxamic acid (Kermack & Lees, 1952), is in fact formed during ammonia oxidation by *Nitrosomonas*, then the metabolism of *Nitrosomonas* would resemble in some respects the metabolism of *Azotobacter vinelandii*, where hydroxylamine appears to be involved both in the reduction of nitrate to ammonia and in ammonia oxidation (Virtanen & Jarvinen, 1951). The role of hydroxylamine in ammonia oxidation by *Nitrosomonas* is now being investigated in this laboratory.

SUMMARY

1. Nitrite formation by *Nitrosomonas* has been studied by means of short-term incubation experiments on washed suspensions of the organisms.

2. Despite the wide variety of nitrogenous compounds tested, only ammonia gave rise to nitrite under these conditions.

3. All chelating agents tested inhibited the formation of nitrite from ammonia by *Nitrosomonas*.

4. In a discussion of the results it is suggested that some metal enzyme is intimately concerned in ammonia oxidation by *Nitrosomonas*.

I am indebted to Prof. W. O. Kermack, F.R.S., for advice, help, and encouragement throughout this work. I also wish to thank Glaxo Laboratories Ltd., for a gift of streptomycin.

REFERENCES

Baas-Becking, L. G. M. & Parks, G. S. (1927). *Physiol. Rev.* **7**, 85.
Bremner, J. M. (1949). *Analyst*, **74**, 492.
Conway, E. J. & O'Malley, E. (1942). *Biochem. J.* **36**, 655.
Grace, J. B. (1951). *Nature, Lond.*, **168**, 117.
Hofman, T. & Lees, H. (1952). *Biochem. J.* **52**, 140.
Jensen, H. L. (1950). *Tidsskr. Planteavl*, **54**, 62.
Kermack, W. O. & Lees, H. (1952). *Sci. Progr.* **40**, 44.
Kluyver, A. J. & Donker, H. J. (1926). *Chem. Zelle*, **13**, 134.
Lees, H. (1946). *Nature, Lond.*, **158**, 97.
Lees, H. (1948). *Biochem. J.* **42**, 534.
Lees, H. (1949). *Plant & Soil*, **1**, 221.
Lees, H. (1951). *Nature, Lond.*, **167**, 355.
Lees, H. & Quastel, J. H. (1946). *Biochem. J.* **40**, 803.
Maley, L. E. & Mellor, D. P. (1950). *Nature, Lond.*, **165**, 453.
Markham, R. (1942). *Biochem. J.* **36**, 790.
Meiklejohn, J. (1950). *J. gen. Microbiol.* **4**, 185.
Meyerhof, O. (1916). *Pflüg. Arch. ges. Physiol.* **166**, 240.
Nelson, D. H. (1931). *Zbl. Bakt.* (2. Abt.), **83**, 280.
Quastel, J. H. & Scholefield, P. G. (1949). *Nature, Lond.*, **164**, 1068.
Quastel, J. H. & Scholefield, P. G. (1951). *Bact. Rev.* **15**, 1.
Snell, F. D. & Snell, D. T. (1936). *Colorimetric Methods of Analysis*. London: Chapman and Hall.
Stephenson, M. (1949). *Bacterial Metabolism*. London: Longmans Green and Co.
Virtanen, A. I. & Jarvinen, H. (1951). *Acta chem. scand.* **5**, 220.
Winogradsky, S. (1890*a*). *Ann. Inst. Pasteur*, **4**, 257.
Winogradsky, S. (1890*b*). *Ann. Inst. Pasteur*, **4**, 760.