lay estrus in female dogs. 17α-acetoxyprogesterone successfully inhibited estrus during 70-wk treatment period. When incorporated in dog feed and fed *ad lib.*, a minimum dose of 2.5 mg kg was effective. When administered once daily in suspension form, a dose of 4 mg kg was required. Histopathological examination of ovaries of treated dogs revealed inhibition of development of vesicular follicles. Bitches previously treated with 17α-acetoxyprogesterone returned to cycle, were bred and whelped normal, healthy litters of puppies. It was suggested that bitches be bred no sooner than 6 mos after cessation of treatment to insure maximum fertility. Drug was judged completely safe for dogs.

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Calcium Requirements of Various Species of *Azotobacter*.* (25997)

G. L. BULLOCK, J. A. BUSH AND P. W. WILSON
Department of Bacteriology, University of Wisconsin, Madison

In a reexamination of the trace metal requirements of *Azotobacter vinelandii* O, Esposito and Wilson(1) demonstrated that under defined conditions a quantitative difference existed in level of calcium ion necessary for its growth on molecular and on ammonium nitrogen. The effect of deficient calcium was primarily on length of the lag phase rather than total growth and was definitely associated with the phosphorus metabolism of the organism(2). When it was later established (3) that this requirement could be met by acetate, ethanol or malate, a survey was undertaken to determine how general these findings were among the Azotobacteriaceae. Over a period of 3 years about 25 strains have been examined with the results summarized in this report.

Materials and methods. Esposito and Wilson(1,2,3) have furnished the essential details of technics employed including the special methods for providing a medium with known concentrations of the calcium ion. Further information is given in the thesis of J. A. Bush(4). In most trials urea was used as a source of combined nitrogen to eliminate the complication of change in pH which accompanies utilization of ammonium salts by many of the strains. These are listed in Table I. The inoculum was grown on the modified Burk's medium(1) with no or 20% of Ca^{++} normally added. Size of inoculum was usually 0.1% so maximum calcium carryover was 1.2×10^{-7} M in comparison with the 6×10^{-4} M $CaSO_4 \cdot 2\ H_2O$ (23.4 ppm Ca^{++}) used in the complete Burk's medium.

Results. Calcium requirements of different strains. To present the results obtained with all the strains listed in Table I is neither practical nor necessary; typical findings observed with a single strain of 3 of the species are

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TABLE I. Strains of *Azotobacter* spp. Tested.*

Species	Strain and source
A. vinelandii	O (Wis.); KS-4 (J. O. Harris); 7484, 7496 (ATTC); C-1 (J. P. Voets); B (D. Burk); 3-A (D. B. Johnstone)
agilis	S-1, S-3, S-4 (Wis.); 144 (D. B. Johnstone)
chroococcum	A-3, A-5, VTI (J. P. Voets); 44 (R. L. Starkey)
beijerinckii	B-1, B-2, B-3, B-5 (J. P. Voets)
macrocytogenes	(H. L. Jensen)
indicus	(R. L. Starkey)

* Because of disagreement over taxonomy of Azotobacteriaceae(5,6) nomenclature used by the research workers who kindly furnished us with the indicated cultures has been retained.

illustrated in Fig. 1-3. These data together with those of the other strains may be summarized:

Azotobacter vinelandii (Fig. 1). Although all cultures tested showed an absolute Ca^{++} requirement for growth with atmospheric nitrogen, limited growth was noted in some strains after a lag of 100 to 200 hr. Growth occurring after the lag period was not consistent either between strains or with the same strain. Omission of the calcium ion in the combined nitrogen medium resulted in a slightly extended lag period and a reduction in total growth. The extent of these effects also varied between strains.

Azotobacter chroococcum. Of all species tested, *A. chroococcum* consistently had the most marked requirement for calcium ion when fixing gaseous nitrogen. Cultures without added calcium did not grow even after 2 weeks incubation, and if growth then occurred the cells differed morphologically from those of the inoculum. The old cells were heavily vacuolated, swollen, and variable in their gram reaction. In the combined nitrogen medium without added calcium, results were similar to those obtained from the *A. vinelandii* strains, namely, a slightly extended lag phase and reduced total growth.

Azotobacter beijerinckii. The organisms in this group were the only ones which required addition of calcium ion for growth in both forms of nitrogen media. Cultures with no added calcium did not begin to grow for at least 60 hr while some strains tested required 80-120 hr to initiate growth. Addition of 2.4×10^{-6} M calcium to the combined nitrogen medium shortened the lag period and allowed limited growth, while 2.4×10^{-5} M calcium resulted in a normal growth curve. In the nitrogen free medium 1.2×10^{-5} M added calcium was stimulatory and 2.4×10^{-4} M was necessary for normal growth.

Azotobacter macrocytogenes. Omission of calcium from both forms of nitrogen resulted in an extension of the lag phases similar to results obtained with *A. beijerinckii.* However, *A. macrocytogenes* would begin growth in media without calcium before *A. beijerinckii.* The growth on combined nitrogen without calcium ion was characterized by heavy polysaccharide production.

Azotobacter agilis (Fig. 2). Strains of this species exhibited a consistent growth pattern, in that addition of calcium to either form of nitrogen media allowed only better total growth while omission of the ion did not affect length of lag period or rate of growth.

Azotobacter indicus (Fig. 3). Lag phases of up to 80 hr on both nitrogen-free and combined nitrogen media, were observed with the 0.1% inoculum used in this study. The calcium ion was found to be inhibitory to growth of this organism on both forms of nitrogen. Final pH of the cultures was between 5.0 and 5.5 as compared with 6.8-7.2 for the other species.

Replacement of calcium requirement. Three strains of *A. vinelandii* (O, 7484, 7496) were tested for their ability to use acetate and ethanol in place of calcium when fixing N_2. Check for possible calcium contamination in the acetate was made by adding concentrated HCl to an aliquot of the stock solution of sodium acetate, boiling to remove acetic acid, neutralizing with NaOH and making up to original volume with deionized water. The residue solution was added to control flasks at the same level as were the acetate solutions. Acetate does replace, at least in part, the calcium requirement of these strains (Fig. 4, Table II). No growth occurred in the medium to which acetate residue was added, or in medium with no added calcium, indicating that the medium and acetate stock solutions

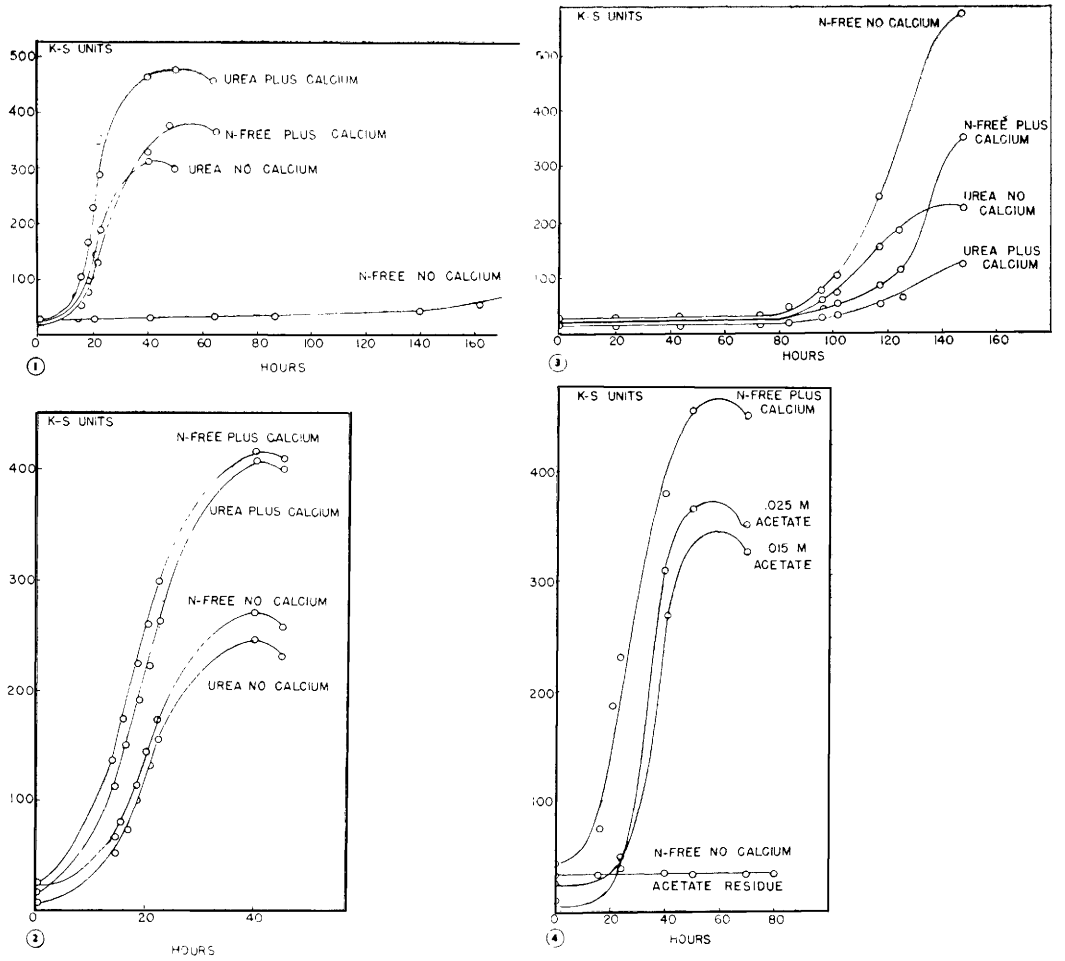

FIG. 1. Effect of calcium on growth of *Azotobacter vinelandii* KS4 in nitrogen-free and nitrogen containing media. Legends in Fig. 1-3 are: N-free + calcium = Burk's medium. N-free, no calcium = Burk's medium minus added calcium. Urea + calcium = Burk's medium + .015 M urea. Urea, no calcium = Burk's medium minus added calcium + .015 M urea.

FIG. 2. Effect of calcium on growth of *Azotobacter agilis* S-3 in nitrogen-free and nitrogen containing media.

FIG. 3. Effect of calcium on growth of *Azotobacter indicus* in nitrogen-free and nitrogen containing media.

FIG. 4. Effect of acetate as a calcium replacing factor in *Azotobacter vinelandii* 7484. N-free + calcium = Burk's medium. N-free, no calcium = Burks' medium minus calcium. .025 M acetate = Burk's medium minus calcium + 1.0 ml 2.5 M acetate/100 ml. .015 M acetate = Burk's medium minus added calcium + 1.0 ml 1.5 M acetate/100 ml. Acetate residue = Burk's medium minus added calcium + 1.0 ml of 2.5 M acetate residue solution.

contained insignificant amounts of calcium ion. Since final pH of the cultures to which acetate had been added was between 8.5-9.0 and since some strains of *A. vinelandii,* not here reported, showed replacement with lower acetate final concentrations (0.0075 and 0.0015 M), but only limited replacement with higher acetate final concentrations (0.025 and 0.015 M), it is possible that the pH rise caused by liberation of sodium ions was inhibitory to growth at the higher acetate concentrations. Ethanol also replaced the calcium ion for nitrogen fixation, although less total growth and lower fixation levels were obtained.

Discussion. The results in Fig. 1-3 suggest that species of the azotobacter may be associated into 3 groups according to their re-

TABLE II. Effect of Acetate and Ethanol as Calcium Sparing Factors on Nitrogen Fixation by *Azotobacter vinelandii*.

Media	Final nitrogen, μg/ml Strain 0	Strain 7496
Burk's medium	280	265
Burk's medium minus added calcium	25	20
Idem plus .025 M acetate	275	230
Burk's medium minus calcium plus 1.3×10^{-4} M ethanol	135	

quirements for Ca^{++}. Group I consists of those strains in which a definite quantitative difference is required for growth on free and combined nitrogen. It is represented in this study by strains of *A. vinelandii* and *A. chroococcum* for which the difference is marked and by those of *A. beijerinckii* (and possibly *A. macrocytogenes*) in which the difference observed was only one order of magnitude. Group II represented by strains of *A. agilis* are those in which no requirement for added calcium was demonstrable when grown on either free or fixed nitrogen, *i.e.*, the purified medium contained sufficient Ca^{++} for their needs on either source. Group III consists of the single strain of *A. indicus* tested for which the calcium ion is definitely inhibitory independent of source of nitrogen. Whether these differences are sufficiently consistent among the various species to be useful for taxonomic purposes cannot be determined in view of the relatively small number of strains used. Some investigators(5), however, have urged creation of a new genus (*Beijerinckii*) for *A. indicus* because of its physiological differences, including inhibition by Ca^{++}, from the other species of the Azotobacteriaceae.

While these studies were in progress Norris and Jensen(7,8) published results of a similar survey that included several of the strains used in this investigation. Although there is a large area of agreement among the findings from the two laboratories, two discrepancies deserve comment. First, Norris and Jensen were unable to confirm our results of replacement of calcium by acetate(3) when a small inoculum was used. In our original study we used 2% of a 24 hr culture containing about 50 μg N/ml so that initial concentration was approximately 1 μg N/ml. We have verified this finding using 0.1% inoculum (0.05 μg N/ml) using 3 strains of *A. vinelandii* (Fig. 4, Table II) and have also confirmed it with strain O using only 0.01% inoculum.

Probably of greater significance was the inability of these workers to detect a quantitative difference in requirement for Ca^{++} on N_2 and NH^{+}_4 –N for strains of *A. vinelandii* including our strain O. This strain has been repeatedly and independently tested by a number of workers in our laboratory over a period of several years with essentially the same results—about the only difference we have noted has been the level of calcium necessary to obtain normal growth with respect to lag, turbidity, or total nitrogen. This will vary as the experimental conditions change, but always a range can be found in which a marked difference in response on free and combined nitrogen is observed. For example, when size of the inoculum is varied from 0.01 to 2%, length of the lag on both sources of nitrogen markedly increases, but a range of Ca^{++} concentration is readily established in which growth on combined nitrogen is essentially normal whereas during this same period of time, little if any growth occurs on N_2. The obvious criticism that, in spite of all efforts to purify the medium, contaminating calcium plays a role, misses the point that a medium can be prepared with its level of calcium so controlled and other conditions (size of inoculum, temperature and time of incubation) so defined that marked differences in growth response of this strain on free and combined nitrogen can be repeatedly and consistently obtained.

Summary. Calcium ion requirements of strains representing 5 species of the Azotobacteriace have been studied. Quantitative differences in growth response on molecular nitrogen and on urea-N were readily detected with strains of *A. vinelandii* and *A. chroococcum* and less definitely with *A. beijerinckii* and possibly *A. macrocytogenes*. No specific requirement for calcium was detected with strains of *A. agilis,* and the ion inhibited growth of *A. indicus* on either source of nitrogen. Acetate and ethanol replaced the cal-

cium required for nitrogen fixation by 3 strains of *A. vinelandii* tested.

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Effect of Epinephrine on Experimental Ectopic Ventricular Tachycardias.* (25998)

DAVID SCHERF, DOGAN TANER AND MUHTAR YILDIZ
Dept. of Medicine, N. Y. Medical College

One is occasionally faced with the problem of a patient requiring administration of catecholamines while at the same time exhibiting ectopic cardiac rhythms. The most striking example is seen in shock accompanying acute myocardial infarction and not infrequently associated with ventricular extrasystoles or tachycardia. The investigations on action of epinephrine during chloroform anesthesia(1) as well as later reports on epinephrine-induced ventricular fibrillation while heart was under the influence of benzol, cyclopropane or halogenated hydrocarbons have warned the clinician not to use catecholamines in the course of certain ventricular arrhythmias, the danger being appearance of ventricular fibrillation. At symposium on action of catecholamines on the heart, held in Burlington, Vt., we mentioned the effects of intravenous injections of epinephrine in dogs during ventricular tachycardias(2). This is the report on these experiments.

Method. Twenty-nine dogs were anesthetized with intraperitoneal injections of Nembutal (18 mg/kg) and morphine (8 mg/kg). Morphine was given to increase vagal tonus thus preventing a sinus tachycardia interfering with ectopic rhythms. For the same reasons the vagi were left intact. Artificial respiration was instituted and the heart was exposed. The electrocardiogram was registered in lead 2. Ventricular arrhythmias were provoked by sub-epicardial injection of either 0.05 cc of a 20% solution of sodium chloride, a 3.8% solution of sodium oxalate or application of a few crystals of delphinine to the surface of one of the ventricles. Epinephrine hydrochloride was injected into the superior caval vein in the amount of 0.01 cc/kg as soon as a ventricular tachycardia with a constant rate appeared.

Results. The data from these experiments are shown in Table I. In none of these experiments did ventricular fibrillation occur after intravenous injection of epinephrine nor did epinephrine produce any of the dangerous prefibrillatory arrhythmias. In the tachycardias provoked by sodium chloride in the right ventricle the rate increased after epinephrine in 13 of 25 experiments; it remained unchanged 6 times and decreased in 6 experiments. Data from the left ventricle are similar. In 14 experiments the rate increased 10 times, it was unchanged in 2 experiments and became slower twice after intravenous injection of epinephrine. When sodium oxalate was used to create ventricular tachycardia, the rate increased after epinephrine injection 4 times, decreased once and remained unchanged 3 times. In 5 experiments epinephrine was injected during a ventricular tachycardia elicited by topical application of

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