cells required a 12-ml 0.5% aseptic supplement of tetrazolium red (2,3,5-triphenyltetrazolium chloride). All cultures were harvested after 21 hr of incubation, prior to which they were treated for 45 min with 1% (by volume) β-propiolactone, a fast-acting germicide (Curran and Evans, J. Infect. Diseases **99**:212, 1956). The bacteria contained in all three flasks were then combined, centrifuged, washed, recentrifuged, and suspended in 40 ml of 0.5% phenol-saline. The preparation was stored in amber bottles and maintained at temperatures below 5 C.

Numerous infectious diseases are characterized by the appearance of antibodies in the infected host, which may be of clinical significance. This method suggests the use of color-coded composites of heterologous genera for the multiple-screening of suspected serum samples.

GROWTH INHIBITION BY BIOTIN IN A STRAIN OF *RHIZOBIUM JAPONICUM*[1]

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The root-nodule bacteria vary in their response to vitamins. This is particularly true for biotin. This vitamin has been reported as a requirement for, or a stimulant to, the growth of some strains of *Rhizobium trifolii*, *R. phaseoli*, *R. meliloti*, *R. lupini*, and *R. leguminasarum* (West and Wilson, J. Bacteriol. **37**:161, 1939; J. Bacteriol. **38**:110, 1939; Enzymologia **8**:152, 1940; Wilson and Wilson, J. Bacteriol. **43**:329, 1942). These same authors indicated that biotin had no apparent effect on the slow-growing organisms of the cow pea or soybean group. These observations were recently confirmed by Graham (J. Gen. Microbiol. **30**:245, 1963), who studied the vitamin requirements of 68 strains of rhizobia including 10 strains of *R. japonicum*. He reported considerable difference in vitamin response and found no growth stimulation due to biotin in any *R. japonicum* strains.

In the course of determining a minimal synthetic medium for several strains of *R. japonicum*, it was found that strain 508 (University of Wisconsin strain 508; obtained from O. N. Allen, Department of Bacteriology) would not grow in two previously reported, biotin-supplemented, defined growth media (Bergerson, Australian J. Biol. Sci. **14**:349, 1961; Nicholas et al., Biochim. Biophys. Acta **56**:632, 1962). To observe further this inhibition, a chemically defined, vitamin-free liquid growth medium was prepared which would support growth of strain 508. When this medium was supplemented with biotin at a level normally supplied to biotin-requiring strains (0.01 μg/ml), almost complete growth inhibition resulted (Table 1).

Since the above findings were quite unexpected, the following experiment was conducted. DeLong flasks containing 10.0 ml of the basal medium were supplemented with B vitamins and B vitamins less biotin and thiamine. In this experiment, the B vitamins were deleted two at a time, which

TABLE 1. *Inhibition of Rhizobium japonicum strain 508 by biotin in a chemically defined medium**

Biotin level	Optical density†
μg/ml	
None	0.69
0.01	0.07

* The basal medium contained (mg/ml): K_2HPO_4, 1; KH_2PO_4, 1; NaCl, 0.2; $MgSO_4$, 0.18; $CaSO_4$, 0.13; NH_4Cl, 0.5; sodium glutamate, 0.2; and mannitol, 5. All media were adjusted to pH 6.8, sterilized by Millipore filtration, and held 3 days before inoculation.

† The optical density was measured after 7 days of incubation on a rotary shaker at 30 C. Measurements were made at 420 mμ in a Bausch & Lomb Spectronic-20 colorimeter.

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is why both biotin and thiamine happened to be omitted here. Reversal of this growth inhibition did not occur when other pairs of B vitamins were similarly deleted. Further growth media were prepared substituting 0.1% Norit-treated, vitamin-free, casein hydrolysate (Nutritional Biochemicals Corp., Cleveland, Ohio) for glutamate, and this medium was supplemented by the addition of 0.01 μg/ml of biotin. The media were sterilized by Millipore filtration after being adjusted to pH 6.8, and were held for 3 days before inoculation with 0.01 ml of a culture having an optical density of 0.1 at 420 mμ (Table 2). A marked growth reduction occurred in the presence of the B vitamins. These data show that casein hydrolysate is superior to glutamate as an amino acid source, but, in spite of the better growth, the biotin inhibition was not reversed. Although not clearly shown in Table 2, it has been found that some of the other vitamins stimulate growth but do not overcome the inhibition by biotin.

TABLE 2. *Effect of other nutrients on biotin inhibition of Rhizobium japonicum strain 508**

Supplement	Optical density
Glutamate (0.2 mg/ml)	0.37
B vitamins	0.07
Glutamate and B vitamins†	0.16
Glutamate and B vitamins other than biotin and thiamine	1.69
Vitamin-free casein hydrolysate (0.1%)	1.69
Vitamin-free casein hydrolysate and biotin (0.01 μg/ml)	0.48

* The basal medium was the same as in Table 1 except that glutamate was omitted. Optical density was determined as in Table 1.

† Unless otherwise indicated, the following vitamins (in μg/ml) were included: thiamine, 0.1; riboflavine, 0.1; pyridoxal phosphate, 0.1; nicotinic acid, 0.1; folic acid, 0.1; calcium pantothenate, 0.1; biotin, 0.01; and inositol, 4.8.

TABLE 3. *Effect of biotin from three commercial lots on the growth of Rhizobium japonicum strain 508 in a chemically defined medium**

Biotin lot no.	Biotin added (μg/ml)			
	0	0.001	0.01	0.1
503913†	0.69	0.07	0.03	0.03
1831‡		0.05	0.05	0.03
9419‡		0.07	0.05	0.06

* The medium and methods were the same as in Table 1. Results are expressed as optical density.

† Calbiochem.

‡ Nutritional Biochemicals Corp.

The possibility existed that an impurity in the commercial biotin reagent might be responsible for the inhibition. To determine this, other lots of biotin were checked for their ability to inhibit the growth of this rhizobial strain. These biotin sources were found to produce the same inhibitory effect over a 100-fold range of concentration (see Table 3).

It is believed that this is the first report of biotin inhibition of growth of a microorganism, although thiamine has been reported inhibitory to yeast strains (e.g., Rabinowitz and Snell, Federation Proc. **8**:240, 1949). No mechanism for this inhibition can be offered at this time, but further study is being continued in the hope that this system may contribute to our understanding of the physiological role of biotin.

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INORGANIC POLYPHOSPHATE OF HIGH MOLECULAR WEIGHT FROM *AEROBACTER AEROGENES*

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Massive accumulation of inorganic polyphosphate, giving rise to the volutin granules of cytology, has been reported in many microorganisms. The experiments described below had