Enhancement by Inositol of the Nodulation of Isolated Bean Roots

Abstract. The percentage of isolated bean roots nodulated and the number of nodules per root were increased by the addition of mesoinositol to the agar medium into which the bases of bean roots were inserted. The following were without marked effect: glycine, thiamine, pyrodoxine, niacin, indoleacetic acid, gibberellic acid, kinetin, adenine, adenosinetriphosphate, biotin, riboflavin, calcium pantothenate, and folic acid.

With a modified method of aseptic isolated root culture (*1*) it has been possible to obtain rather widely fluctuating percentages of nodulated roots (averaging 50 percent) with 1.9 to 3.3 nodules per nodulated root (*2*). The object of the experiments reported here was to increase nodulation and to improve the consistency of the results, with the final aim of gaining a better understanding of the factors involved in nodule formation.

Isolated roots of *Phaseolus vulgaris* L., var. Pencil Pod, black wax bean were grown and inoculated as described earlier (*2*), except that three roots instead of one were grown per Petri dish. Each dish carried three agar-containing vials and 50 g of washed silica sand moistened with 10 ml of the inorganic salts of medium "0" (*2*). The cut end of each root was inserted into the agar medium in a 12 by 35-mm shell vial, and the vial was laid on its side in the Petri dish. The vials contained a basal medium consisting of 10 percent sucrose and glycine, thiamine, pyridoxine, and niacin at the levels indicated for the organic constituents of medium "0," or 20 times higher. Thus, the roots were nourished by the inorganic salts medium in the sand and by other materials furnished from the base of the root in contact with the agar. The roots were maintained in the dark at 26°C. Fifteen to 20 days after inoculation with a mixture of the Nitragin Company's strains 3I6C11, 3I6C13, and 3I6C17 of *Rhizobium phaseoli* Dangeard, the roots were examined for nodules.

Experiments were run in which the medium in the vials was altered to determine the inference on nodulation. When glycine, thiamine, pyridoxine, and niacin were increased to a level 20 times higher than that in medium "0," no significant increase in nodulation occurred. Similar negative results were obtained with indoleacetic acid ($10^{-10}M$) and gibberellic acid (50 and 250 mg/lit.).

Since nodulation was enhanced by the addition of a mixture of $Ca(NO_3)_2 \cdot 4H_2O$ (300 mg/lit.), KNO_3 (80 mg/lit.), kinetin (0.05 mg/lit.), *d*-biotin (0.05 mg/lit.), riboflavin (0.05 mg/lit.), folic acid (0.5 mg/lit.), calcium pantothenate (5 mg/lit.), and mesoinositol (100 mg/lit.), the effects of the nitrates of kinetin, and of the five vitamins were tested separately. The results presented in Tables 1 and 2 indicate that kinetin did not enhance nodulation—a result confirmed by other experiments (not shown in the tables) in which this substance was inactive at concentrations of 0.025, 0.05, 0.1, and 0.5 mg/lit., either by itself or in combination with adenine (10 mg/lit.), or sodium adenosinetriphosphate (100 mg/lit.). On the other hand, the results shown in Tables 1 and 2 indicate enhancement by nitrate and by the vitamin mixture; Tables 2 and 3 show that the effect of the vitamin mixture can be attributed to mesoinositol.

The results obtained with nitrate confirm and extend previous work (*2*) which showed that nitrate, while inhibitory when added to the medium in the dish, did not inhibit nodulation if it was included with the nutrients supplied in the vial. It is interesting to note that the beneficial effect of nitrate apparently does not depend merely on the amount of nitrogen added (43 mg/lit.), for no effect was obtained by increasing glycine 20 times (an increase of 56 mg of N per liter). On the other hand, it seems improbable that the effect can be attributed to the cations Ca and K, for both are abundant in the inorganic medium. Although this point needs further clarification, it seems that the stimulation noted results from better utilization of nitrate than of glycine.

The striking promotion of nodulation by mesoinositol was unexpected, for mesoinositol has never been associated with nodule formation or function. Furthermore, it apparently is not required by the rhizobia. It is included in several vitamin mixtures used in plant tissue cultures (*3*), but its specific role in such mixtures has received little study. Jacquiot (*4*) found that it favors bud formation by elm cambial tissue, and there are several reports of its effect on cell division [see, for example (*5*)]. While the present experiments were in progress, Braun (*6*) reported that fully altered plant tumor tissue will grow in White's basal culture medium but that

Table 1. Effect of omitting one component at a time from the basal medium supplemented with nitrate, kinetin, and the mixture of five vitamins. Columns A, percentage of roots nodulated; columns B, mean number of nodules per nodulated root.

Expt.	Complete mixture		Complete mixture					
			Minus nitrate		Minus kinetin		Minus vitamins	
	A	B	A	B	A	B	A	B
1	71	5.5	53	5.1	69	6.6	43	3.9
2	65	8.8	65	7.9	65	8.1	59	3.9
3	77	9.0	64	7.0				
4	88	10.0						

Table 2. Effect of omitting one vitamin at a time from the basal medium supplemented with the other four vitamins and nitrate. Columns A, percentage of roots nodulated; columns B, mean number of nodules per nodulated root.

Expt.	Basal medium		Basal medium + nitrate		Complete mixture: basal medium + nitrate + vitamins		Complete mixture									
							Minus inositol		Minus biotin		Minus riboflavin		Minus Ca pantothenate		Minus folic acid	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
5					88	10.0	77	6.6	92	10.6	87	9.5	94	13.7	88	12.1
6	68	3.6	83	5.4	94	8.6	85	4.9	93	9.0	93	10.0	90	10.1	93	9.1

Table 3. Effect of adding each one of the vitamins to the basal medium supplemented with nitrate. Columns A, percentage of roots nodulated; columns B, mean number of nodules per nodulated root.

Expt.	Basal medium supplemented with nitrate									
	Plus inositol		Plus biotin		Plus riboflavin		Plus Ca pantothenate		Plus folic acid	
	A	B	A	B	A	B	A	B	A	B
7	84	9.5	73	5.8	73	5.2	70	6.6	67	4.5

cells with lesser degrees of neoplastic change have more complex requirements, mesoinositol being prominent among the required components.

An evaluation of the significance of the present findings regarding the promotion of nodulation by mesoinositol must await further experimentation (7).

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Fibrillation and Potassium Influx

Abstract. Absolute influx and efflux of potassium-42 have been measured in isolated rabbit atria during acetylcholine-induced fibrillation. The efflux of potassium was increased three to four times; influx was not changed. The data are interpreted as indicating that an inhibition of active K uptake is not involved in the initiation of fibrillation, and that the process results from a marked increase in Na permeability.

Fibrillation has been induced by stimulating, at high frequency, isolated rabbit atria suspended in low potassium (K) media (1) in the presence of acetylcholine. Ion-exchange studies revealed that fibrillation began when the rate of net loss of K and gain of sodium (Na) exceeded critical values (2). Isotope investigations showed that with the onset of fibrillation the efflux of K reached a rate three to four times that of the spontaneously beating preparation (3). Net losses occurring under the conditions of the experiment prevented an accurate determination of influx. Therefore, we were unable to ascertain the nature of the permeability change involved in the process.

Recently a method has been devised (4) which permits an estimation of K^{42} influx with the onset of fibrillation. Absolute rates of influx were calculated by methods described by Keynes and Lewis (5). Influx is given by the product of the initial rate of entry of K^{42} to the tissue, the sensitivity of the counter, and the volume–to–surface-area ratio of the atrial fibers (6). The initial rate of entry can be obtained from the following relation:

$$\left(\frac{dy}{dt}\right)_{t=0} = \frac{Y}{T}\left(\frac{kt}{1-e^{-kt}}\right)$$

where Y is counts in the tissue after time T and k is the specific transfer coefficient obtained from efflux. During fibrillation, k was estimated to be of the order of 7.5 to 8.0×10^{-4} sec^{-1} (3).

Table 1 is a summary of our findings. First, it should be noted that acetylcholine increases both efflux and influx of K, whereas during fibrillation only an increase in efflux is obtained. Influx remains essentially unchanged. Thus, the changes induced by acetylcholine result from an increase in membrane permeability to K, while those that occur during fibrillation cannot be so interpreted. Earlier studies on the effects of temperature on efflux during fibrillation and acetylcholine treatment also suggested that different mechanisms were involved (7). A marked increase in Na permeability will explain the findings during fibrillation: Potassium leaves the tissue in exchange for sodium. This is in keeping with an earlier finding that the rate of entry of Na^{24} to atria was markedly increased (15 to 20 times) during the arrhythmia (7). These data suggested that the quantity of Na entering the tissue exceeded that of K which was lost. This would indicate that there was a sudden release of an anion in the tissue or, more probably, that membrane permeability to chloride is increased.

It should be noted that the mechanism proposed for the permeability change accompanying the onset of fibrillation is similar to that postulated for excitation and conduction in nerve (8) but differs in that K permeability is not increased. This is probably one of the factors responsible for the observed differences between the electrical properties of heart muscle and nerve (9).

Finally, it should be pointed out that the normal or slightly increased rate of influx during the early phases of fibrillation indicates that a depression of active transport is not a factor in the initiation of the arrhythmia, as was recently suggested by Goodford (10).

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Table 1. Effects of acetylcholine and fibrillation on the transmembrane flux of potassium. Fibrillation was induced by stimulating at 1200 count/min for 1 min. Atria were suspended in Ringer's solution containing 1.35 mmole of K^+ in the presence of acetylcholine (6.4×10^{-3} mole).

No. of observations	Experiment	Absolute flux (pmole cm^{-2} sec^{-1})	
		Influx	Efflux
8	Control	1.15 ± .08	4.32 ± .14
10	5 min after addition of acetylcholine (6.4×10^{-3} mole)	2.22 ± .13	7.88 ± .62
5	5 min after onset of fibrillation	1.20 ± .11	12.7 ± .48

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Failure of Nicotine to Affect Development of Offspring When Administered to Pregnant Rats

Abstract. Administration of nicotine to rats at any point in pregnancy has no apparent effect upon completion or duration of pregnancy, or upon body development, litter size, weight, or mortality of offspring. These results differ sharply from the effects in mice reported by others. The possible etiologic significance of anoxia in the malformations reported in mice is discussed.

Nishimura and Nakai recently reported (1) the development of a variety of skeletal anomalies, predominantly of the limbs, in the offspring (sacrificed at term, or examined at midpregnancy) of mice injected with a 0.1-percent aqueous solution of nicotine (0.025 mg/g) sometime between the 5th and 15th days of pregnancy. The percentage of congenital malformations, the number of pregnancies undergoing complete resorption, and the lethal effects of the drug upon the embryo were greatest when the drug was administered daily on days 9, 10, and 11 of pregnancy, although any or all of these effects could be produced, though to a considerably lesser