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Metabolic fate of [8-^{14}C]adenine and [8-^{14}C]hypoxanthine in nodules and root tissues of soybean plants

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Approximately 30% of radioactivity from [8-^{14}C]adenine and more than 60% of that from [8-^{14}C]hypoxanthine were recovered from the degradation products of purines, i.e. ureides and CO_2, in nodules and roots of soybean plants. The nodules seem to have greater ability to degrade ureides. The nodules also possess a greater capacity for salvaging purine bases than do the roots.

AFTER it was revealed that ureides (allantoin and allantoic acid) are the predominant forms of organic nitrogen produced by nitrogen-fixing soybean root nodules[1-3], the biogenesis of ureides has been studied in nodules of various leguminous plants, such as soybean and cowpea. Current evidence indicates the involvement of purine synthesis *de novo* and the subsequent oxidation of purines in the biogenesis of ureides[4]. However, little is known about the salvage of purines, i.e., the reutilization of preformed purines for the synthesis of nucleotides and nucleic acids in these nodules. The present study compares the metabolic fate of purine bases supplied exogenously to the nodules and to the roots of the same soybean plants.

Plant materials were obtained from one-month-old soybean plants (*Glycine max* cv. Yukimusume), inoculated with *Rhizobium japonicum* (strain IAM 12608) and grown in a nitrogen-free culture medium[5]. Nodules were separated from roots with a razor blade as indicated in Figure 1. The labelled compounds were administered as described earlier[6]. Briefly, samples of approximately 150 mg of nodules and roots were sliced with a razor blade and incubated in 2.0 ml of 30 mM potassium phosphate (pH 5.7) that contained 10 mM sucrose and 0.2 ml (37 kBq) [8-^{14}C]adenine (specific activity 2.29 GBq mmol^{-1}) or 0.2 ml (37 kBq) [8-^{14}C]-hypoxanthine (specific activity 1·96 GBq mmol^{-1}) in a 30 ml-Erlenmeyer flask with a centre well that contained 0.1 ml of 20% KOH. After a 4-h incubation at 27°C, samples were washed with water, extracted, and analysed for labelled metabolites as described earlier[6].

Table 1 shows the uptake and metabolism of exogenously supplied [8-^{14}C]adenine and [8-^{14}C]hypoxanthine by samples of nodules and roots. Similar rates of uptake of [8-^{14}C]adenine were found in nodules and in roots. Nevertheless, the extent of incorporation of [8-^{14}C]adenine into the salvage compounds, i.e., nucleic acids plus free nucleotides, was higher in nodules than in roots. In both roots and nodules, approximately 30% of the radioactivity from [8-^{14}C]adenine taken up by the cells was distributed in the degradation products of purines (i.e., allantoin, allantoic acid and CO_2). The amount of $^{14}CO_2$ released was nearly three times higher in nodules than in roots, but the distribution of radioactivity into ureides was higher in roots than in nodules.

Contrary to the results with adenine, the extent of uptake of [8-^{14}C]hypoxanthine by root tissues was only half of that by nodules. The ability to salvage hypoxanthine was higher in nodules than in roots. However, in agreement with the results obtained from several other plant materials[6-8], the rate of salvage of hypoxanthine was much lesser than that of adenine in both nodules and roots of soybean plants. More than 60% of the radioactivity from [8-^{14}C]hypoxanthine was recovered from the degradation products formed during a 4-h incubation. It is noteworthy that more than 40% of radioactivity recovered from [8-^{14}C]hypoxanthine was found in the CO_2 fraction from the nodules, but nearly 50% of the radioactivity remained as ureides in the roots.

Although it has been stressed that the nodules of soybean plants are the site of ureide biogenesis[4], the present results clearly indicate that nodules also have the machinery necessary for the salvage of purines for the synthesis of nucleotides. They possess somewhat greater capacity for salvaging purine bases than do the roots. In addition, the ability to degrade ureides also seems to be quite considerable in the nodules. The present results suggest that no particular qualitative difference exists with respect to the metabolism of purines between the soybean nodules and the roots. To

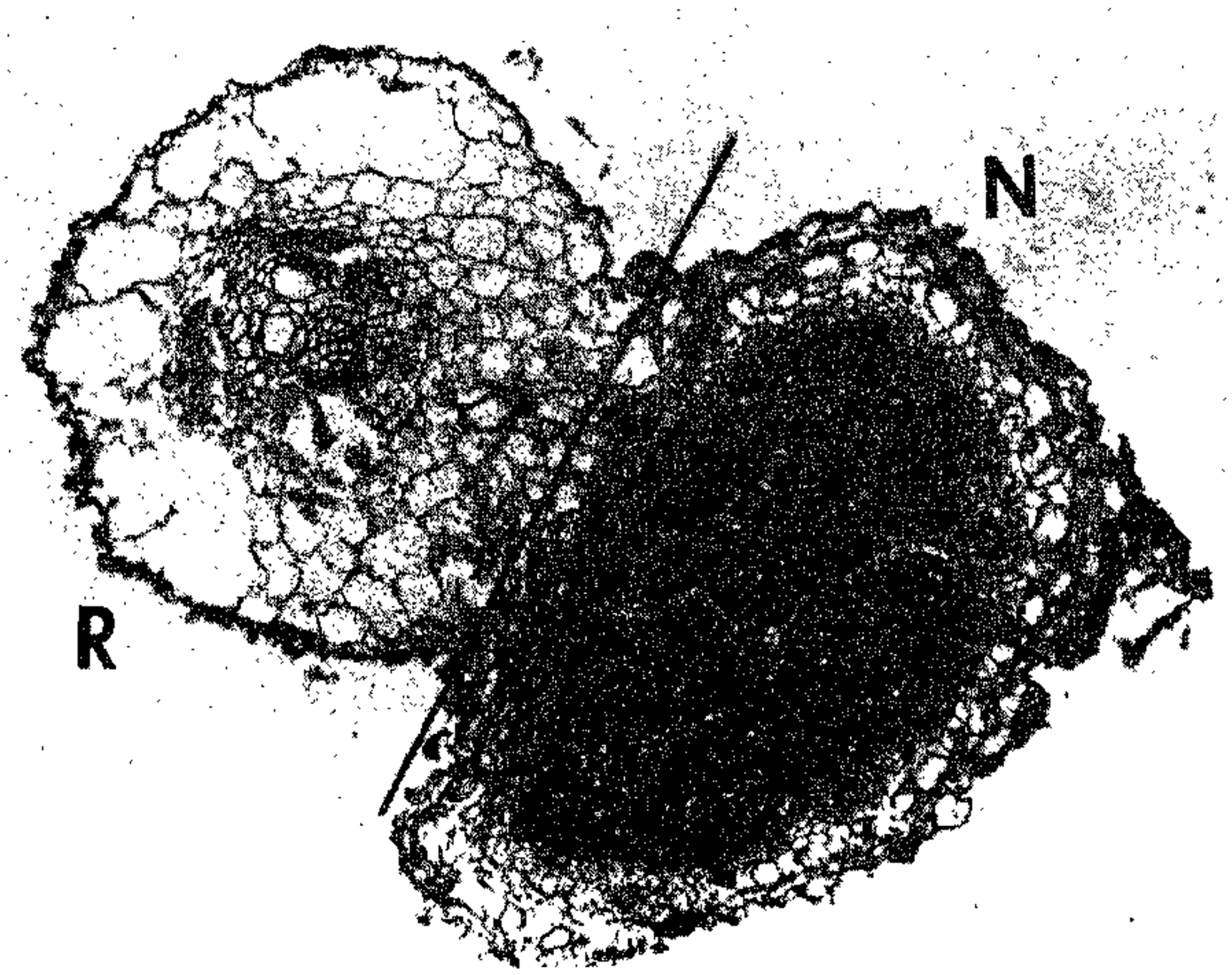

Figure 1. Micrograph of a cross-section of a nodulated root from a one-month-old soybean plant infected with *Rhizobium japonicum*. Nodule and root were separated as indicated by the solid line (N, nodule; R, root).

Table 1. Uptake and metabolism of [8-^{14}C]adenine and [8-^{14}C]-hypoxanthine exogenously supplied to the nodules and the roots of soybean plants

	[8-^{14}C]Adenine			[8-^{14}C]Hypoxanthine		
Precursor	Nodules	Roots	(N/R)	Nodules	Roots	(N/R)
Total uptake (nmol/100 mg fr. wt/4 h)	2.10	2.07	(1.0)	2.00	0.99	(2.0)
Incorporation (% of total uptake)						
RNA	21.2	14.8	(1.4)	17.6	8.5	(2.1)
DNA	1.4	2.4	(0.6)	2.2	1.7	(1.3)
Nucleotides	22.5	12.1	(1.9)	4.3	1.6	(2.7)
Nucleosides	13.6	11.1	(1.2)	3.6	7.3	(0.5)
Purine bases	10.6	30.6	(0.3)	7.3	16.6	(0.4)
Ureides	14.0	21.4	(0.7)	19.6	48.7	(0.4)
CO_2	15.2	5.7	(2.7)	43.7	14.2	(3.1)
Others	1.2	1.8	(0.7)	1.4	1.3	(1.1)

Amounts of adenine and hypoxanthine taken up by the samples are expressed as nmol per 100 mg material per 4 h. The rates of incorporation of radioactivity from [8-^{14}C]purines are expressed as the percentage of radioactivity taken up by the material. These values are averages of the results from duplicate samples. The ratio N/R, i.e. the ratio of the value in the nodules to that in the roots is also shown in parentheses for each class of compounds.

our knowledge, this is the first report of an examination of the salvage of purine bases in the nodules of leguminous plants.

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Major *Entamoeba histolytica* (200:NIH) immunogen

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Antigenic analysis of axenic *Entamoeba histolytica* (200:NIH) antigen, by preparative polyacrylamide slab gel electrophoresis, reveal the presence of 13 distinct protein fractions. Major antigenicity was found in fraction 4 by indirect haemagglutination test. Fraction 4 on electrophoresis under non-denaturing conditions yielded a single band.

AMOEBIASIS is an important world wide disease resulting in an annual mortality of 10,000 to 40,000 and