when the dose was more than 3.0 g/day before the onset of hypomania (7.8 days). The greater incidence of hypomania when the dose of L-dopa was greater (adjusted for the exact number of days in each period) is statistically significant ($\chi^2 = 5.7$, $P < 0.05$).

These results suggest that the catecholamine precursor, L-dopa, induced typical hypomanic behaviour in patients with previous episodes of spontaneous mania. Increased motor activity and aggressiveness in rodents and in primates correlate directly with increased concentrations of catecholamines in the brain after administration of L-dopa[3], but whether dopamine, noradrenaline, a catecholamine metabolite or a competitive effect on the metabolism of serotonin or other amines is responsible for the behavioural effects of L-dopa remains controversial[31-36].

In man, several reports have suggested that mania is triggered by some drugs known from animal studies to increase functional brain catecholamines[22,24] and suppressed by the catecholamine synthesis inhibitor, α-methyl-p-tyrosine (our unpublished results). A regular increase in urinary catecholamine excretion and a decrease in rapid eye movement (REM) sleep occur in the 24 h preceding the onset of full manic behaviour[25]. Similar increases in urinary dopamine and homovanillic acid (HVA) excretion, increases in cerebrospinal fluid HVA, and decreased REM sleep, together with augmentation effects on visual cortical evoked responses, were observed in these patients, and suggest that both central and peripheral catecholamines were altered by L-dopa[20,21].

The hypomanic response reported here may prove to represent a combination of an excitant or stimulant effect of L-dopa plus a prerequisite susceptibility for mania which is found in bipolar manic-depressives. Whether this susceptibility represents a specific sensitivity to an L-dopa load in amine metabolic pathways, or a less specific psychological or other biological sensitivity to the psychomotor activating effects of L-dopa, remains to be determined.

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Medical and Ecological Considerations of L-Dopa and 5-HTP in Seeds

IT is well known that 5-hydroxy-L-tryptophan (5-HTP) occurs in low concentration in mammalian brain serving as the precursor of 5-hydroxytryptamine (5-HT, serotonin). Furthermore, 5-HTP but not 5-HT can pass readily across the blood-brain barrier so that the physiological effects associated with an increase of 5-HT in the brain may be induced in rats and dogs by the interperitoneal injection of 5-HTP[1,2]. A recent report indicated that 5-HTP produced improvement in a patient suffering from the Parkinson-like symptoms of chronic manganese poisoning after treatment with L-3,4-dihydroxyphenylalanine (L-dopa) had aggravated his condition[3].

In mammalian tissues the rate-limiting step in the biosynthesis of 5-HT is the hydroxylation of L-tryptophan and 5-HTP does not accumulate where the enzyme balance favours its decarboxylation rather than its biosynthesis. This particular balance of hydroxylase and decarboxylase activity is not common to all living systems, however, and 5-HTP accumulates in high concentration (6–10% fresh weight) in the mature seeds of *Griffonia simplicifolia*, a West African legume of reputed physiological activity[4-6].

Quickening interest in the use of L-dopa in the treatment of Parkinsonism[7] leads us to point out that comparable differences in the relative activities of the enzymes responsible for the synthesis and decarboxylation of this amino-acid also exist between animals and plants. Like 5-HTP, L-dopa is rapidly decarboxylated in mammalian tissues and indeed the liberation of 3,4-dihydroxyphenylethylamine (dopamine) from this compound after it has entered the brain probably accounts for its beneficial effects in Parkinsonism[7]. The same ratio of hydroxylase : decarboxylase activity is not necessarily found in plants, however, and the accumulation of free L-dopa in certain species has long been known[8-10]. The richest source of this amino-acid so far reported is the seed of *Mucuna pruriens* from which Damodaran and Ramaswamy[10] isolated L-dopa in amounts equivalent to 1.5% of the seed weight. We now report that the seeds (excluding the seed coats) of six species of *Mucuna* (including those of *M. pruriens*) which we have analysed contain between 5.9 and 9.0% of free L-dopa.

Table 1 L-Dopa Content of *Mucuna* Seeds

	Species	Weight of whole seed (g)	L-Dopa in seed (excluding seed coat) (%)
1	*M. andreana* *	6.0	6.3
		7.6	8.9
		9.4	6.9
2	*M. pruriens* †	0.4	6.4
		0.4	5.9
3	*M. mutisiana* ‡	7.4	6.8
		7.3	6.3
4	*M. holtoni* §	6.8	6.4
		6.2	7.5
5	*M. urens* §	6.3	6.4
		4.8	7.4
6	*M. sloanei* ‖	2.8	8.7
		3.5	9.0

* Collected 15 miles north of Puntarenas on Pan American Highway, Puntarenas Province, Costa Rica, February 13, 1970. The range of values obtained for 20 seeds of *M. andreana* showed that whilst the two smallest seeds contained the smallest percentages of L-dopa, the seeds with the largest amounts of the amino-acid were medium sized. The largest seed measured contained less L-dopa than the medium sized seeds.

† Collected on the Isla Providencia, Colombia, August 24, 1969.

‡ Collected on the Isla Providencia, Colombia, August 25, 1969.

§ Gift of Dr W. H. Tallent, USDA Northern Utilisation Research and Development Division, Peoria, Illinois.

‖ Gift of Mrs J. Morton, Morton Collectanea, University of Miami, Florida.

The seed embryos from which the seed coats had been removed were finely ground and 100 mg of each extracted three times with 2 ml. ethanol/0.02 M HCl (1 : 1) containing 1% ascorbic acid. After centrifugation, an aliquot of the combined extracts was diluted with buffer solution (0.38 M sodium citrate adjusted to *p*H 2.2) and the L-dopa content determined by comparison with standard solutions using a Beckman 120 C amino-acid analyser (10 cm column of Beckman resin PA 35 at 32.5° C; buffer solution (*p*H 4.25) (0.38 M Na^+) flowing at 50 ml./h). The hard pigmented seed coats, which did not contain L-dopa, accounted for 15–20% of the whole seed weight in representative samples of *M. andreana*, *M. holtoni* and *M. pruriens*, 20–30% in samples of *M. mutisiana* and *M. urens* and 30–40% in samples of *M. sloanei*.

Analytical values showing the content of three seeds of *M. andreana* and of each of the other five species are given in Table 1. Confirmation of the identity and configuration of the amino-acid has been obtained by isolating it from seeds of *M. mutisiana* using a method which will be described elsewhere.

Although our immediate purpose here is to draw attention to a rich source of L-dopa which may be of use in medicine, the high and surprisingly uniform concentration of the amino-acid in the seeds of all five species of *Mucuna* raises the interesting problem of its significance in the seed. When the distribution of an unusual amino-acid is limited to a number of species that are closely related in other respects, then it is probable that these species are all derived from a common ancestral form in which the genome controlling the synthesis of the unusual amino-acid first arose; the presence of the unusual amino-acid thus serves as a good phylogenetic marker. The original mutant strain of *Mucuna*, whose seeds contained a high concentration of L-dopa, would only have replaced existing strains, however, if this trait conferred some net advantage on the individuals displaying it. A concentration of 6–9% L-dopa in the seed embryo represents a principal commitment both of those metabolic resources available to the parent plant for reproduction, and of the seed's storage potential. Yet it is a commitment made by all species analysed. The presence of a large concentration of any compound in a seed suggests storage, and it is likely that the seedlings of these *Mucuna* species metabolize L-dopa during their development. If, however, storage is the only function of this amino-acid, it is difficult to understand why selection has not favoured a genotype in which one of the precursor "protein" amino-acids such as phenylalanine or tyrosine is stored, for L-dopa requires additional enzymes for its synthesis and degradation.

The freedom from insect and small mammal attack exhibited by large legume seeds generally[11], and *Mucuna* seeds specifically[12], suggests an additional protective role for the L-dopa. Of the many hundreds of bruchid beetles that attack legume seeds, only the larvae of *Caryedes brasiliensis* and a closely related species can feed successfully on *Mucuna* seeds, and during the initial stages of this coevolution, there must have been a strong selection favouring the evolution of a bruchid genotype capable of metabolizing or detoxicating L-dopa. All three of the medium-sized seed-eating mammals (*Sciurus*, *Dasyprocta*, *Cuniculus*) that live in areas of Central America where *Mucuna* species grow reject the opened seeds after eating a small amount (0.5 to 1 g) when first offered them. These same animals feed readily on immature seeds of *Mucuna* and on other large legume seeds that are protected from insects by other than chemical means[12].

The narrow range of L-dopa concentrations found in all the seeds is consistent with a protective role; the seeds of genotypes producing lower concentrations would be susceptible to attack by a wider range of insects and animals (even now, large animals can eat the seeds as a purgative or diuretic[13]). Seedlings from seeds with excess concentrations of L-dopa would, on the other hand, be at a disadvantage when in competition with seedlings using a higher proportion of their seed reserves for normal vegetative purposes. No alkaloids were detected when chromatograms of seed extracts were developed with Dragendorff's reagent, and although several unidentified compounds were detected when the chromatograms were developed with ninhydrin, the areas and intensities of the unknown "spots" were very much less than those of L-dopa.

These findings strengthen our belief that the relative immunity of these seeds to insect attack may be due, in part at least, to the high concentrations of L-dopa which they contain. We also feel that this type of ecological relationship may have many parallels. The accumulation of canavanine (an arginine antagonist in many organisms[14]) in seeds of *Canavalia* and *Dioclea* may well be another example of such a system and it is of interest that the host-specific bruchids that feed on these seeds are also in the genus *Caryedes* (J. M. Kingsolver, personal communication). Direct evidence for this hypothesis is being sought by means of feeding experiments with a variety of insect larvae.

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