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BIOSYNTHETIC AND METABOLIC ACTIVITIES OF SOME ORGANELLES IN *PAPAVER SOMNIFERUM* LATEX

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Key Word Index—*Papaver somniferum*; Papaveraceae; poppy capsule; alkaloids; morphine metabolism; latex organelles.

Abstract—The biosynthesis of the five major alkaloids of the opium poppy (*Papaver somniferum* L.) from radioactive dihydroxyphenylalanine has been studied in the 1000 *g*, 10 000 *g*, 100 000 *g* pellets and the 100 000 *g* supernatant fractions of the capsule latex. A normal poppy variety as well as one which produces only traces of alkaloids were used. Definite evidence of biosynthesis was obtained for both varieties but only in the 1000 *g* pellet (as previously reported). None was found in the other fractions although electron microscopy showed that organelles, including vesicles, were present. The amounts of alkaloid biosynthesized however were very small relative to the amounts involved in the rapid changes already reported for the developing capsules. In contrast, all fractions of the latex were able to metabolize T-morphine *in vitro*, with the 100 000 *g* supernatant showing the highest activity and the amounts involved were also consistent with the changes found in the living plant.

INTRODUCTION

Previous work has shown that morphine is metabolized in *P. somniferum* plants into normorphine and other compounds [1] some of which are methanol-soluble. Others are methanol-insoluble but water-soluble polysaccharides to which morphine is bound by either weak (ionic?) or strong (covalent?) bonds. Still other metabolites are water-insoluble but yield alkaloid-like substances on vigorous treatment [2–4]. Accompanying these molecular changes is a translocation of the substances from the latex to the pericarp tissues and to the ovules and seeds [5]. *In vitro* work on isolated latex has shown that much metabolic change already takes place here [6, 7]; in addition biosynthesis from dihydroxyphenylalanine (DOPA) [6] or from tyrosine [8] to morphine has been demonstrated. Mitochondria, endoplasmic reticulum and possibly ribosomes are present, but the bulk of the organelles consist of alkaloidal vesicles [9, 13] which form a distinct pellet when the latex is centrifuged at 1000 *g*. This pellet contains 95–99% of the latex alkaloids and is capable of converting radioactive DOPA into morphine and other alkaloids [5]. Electron microscopy showed two types of vesicle: one with smooth or slightly granulated walls and the other with a distinct 'cap' which showed structural zonation. It was suggested that the latter was the site of biosynthetic activity while the comparatively smooth vesicles may function for storage only [9]. However, Roberts and Antoun [10] failed to detect the necessary enzymes such as methyltransferase and L-DOPA-decarboxylase in the 1000 *g* pellet: instead they were found in the supernatant. Even when this supernatant was further centrifuged at 4500 *g* and 10 000 *g* the L-DOPA decarboxylase activity still remained in the supernatant. However it is possible that some type of vesicle is still in this supernatant and that it is metabolically active; those used only for storage of alkaloids may be heavier and more easily deposited. We have therefore repeated our earlier work both on biosynthesis from radioactive DOPA and metabolism of radioactive morphine using fractions of latex produced at various values of *g*. Each has been examined by electron microscopy to check the type of organelle present.

RESULTS

Biosynthetic Activity

In the 1st experiment (Table 1) 2.4 g of latex was collected from the capsules of the Halle variety about 1 week after petal-fall and diluted with 2 ml of mannitol buffer and successively centrifuged to produce 1000 *g*, 10 000 *g* and 100 000 *g* pellets and 100 000 *g* supernatant. Each of the four fractions was diluted to the same volume as the final supernatant with mannitol buffer and to each L-3,4-dihydroxyphenyl-[3-^{14}C]-alanine (^{14}C DOPA) 11 000 × 10^3 dpm, was added and incubated for 3 hr. The reaction was stopped by adding 5% acetic acid and the filtrate basified and the alkaloids extracted into $CHCl_3$/isopropanol (3:1). After concentrating, the organic solvent layers were made up to volume and the individual alkaloids determined by HPLC. Aliquots were run on TLC and the morphine, codeine, thebaine, papaverine and noscapine eluted and their radioactivities determined. At this stage all the alkaloids were radioactive. However, after a second TLC run their radioactivities were greatly reduced. After a third purification only the alkaloids from the 1000 *g* pellet were radioactive and these were purified by a further one or two TLC runs, to constant radioactivity.

Table 1. Incorporation of DOPA. Radioactivities of alkaloids after feeding L-3,4,dihydroxyphenyl-[3-^{14}C]-alanine (11 000 × 10^3 dpm) to different fractions of isolated latex from *P. somniferum* Halle and Soma

Fraction	Alkaloid	Wt (mg)	Sp. act. (dpm/mM × 10^{-2}) after each successive purification				
			1	2	3	4	5
1st Experiment							
(a) 1000 *g* pellet	morphine	90.75	744	214	148	114	—
	codeine	7.83	3 812	1 235	200	185	—
	thebaine	19.60	1 406	383	187	196	—
	papaverine	6.70	3 065	1 200	258	227	—
	noscapine	13.00	3 155	405	153	83	—
(b) 10 000 *g* pellet	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
(c) 100 000 *g* pellet	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
(d) 100 000 *g* supernatant	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
2nd Experiment							
(a) whole latex	morphine	65.7	3 376	462	274	228	—
(b) 1000 *g* pellet	morphine	67.3	423	208	91	86	86
	codeine	7.5	3 436	2 771	505	347	299
	thebaine	5.2	1 247	1 238	914	840	—
	papaverine	4.7	2 834	539	637	430	397
	noscapine	5.1	2 758	123	—	—	—
(c) 1000 *g* supernatant	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
(d) 1000 *g* pellet, control	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
(e) 1000 *g* supernatant, control	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
3rd Experiment — (Soma latex)							
(a) 100 *g* pellet	morphine	6.4	7 125	812	208	185	194
(b) 100 *g* supernatant	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
(c) 1000 *g* pellet, control	no radioactivity in any alkaloid after 2nd (or 3rd) purification						
(d) 1000 *g* supernatant, control	no radioactivity in any alkaloid after 2nd (or 3rd) purification						

In the 2nd experiment 7.9 g of latex from capsules of the Halle variety about 1 week after petal fall were diluted with 6 ml mannitol buffer and the mixture divided into three roughly equal portions. The first (whole latex) was incubated directly with 11 000 × 10^3 dpm of ^{14}C-DOPA. The second portion was centrifuged at 1000 *g* for 1 hr and the supernatant was incubated with the same amount of ^{14}C-DOPA; the pellet was diluted to the original volume with buffer and also incubated similarly with ^{14}C-DOPA. The third portion (control) was separated into 1000 *g* pellet and supernatant but each was boiled for 30 min before adding the ^{14}C-DOPA. After a 3-hr incubation, the alkaloids were separated as already described. Once more only the 1000 *g* pellet alkaloids were radioactive after several purifications. After the first and second purifications even the 'controls' showed radioactivity.

In the 3rd experiment 3.9 g of latex from the Soma variety, which produces very little alkaloid, was used and, once more, only the 1000 *g* pellet showed biosynthetic activity.

Metabolic Activity

Young latex (collected 3–4 days after petal fall) and old latex (4 weeks after petal fall) were used in this work and for each sample incubation with radioactive morphine was carried out for 5 min (T_1) and for 4 hr (T_2). About 2 ml of latex was collected on each occasion and diluted with mannitol buffer and fractionated into 1000 *g*. 10 000 *g*, 100 000 *g* pellets and 100 000 *g* supernatant. Each fraction was made up to 4.0 ml with mannitol buffer and [1-*n*-^{3}H]-morphine hydrochloride, 8000 × 10^3 dpm, added. After 5 min, 0.5 ml was transferred to 5% acetic acid. The remainder was allowed to incubate for 4 hr and then transferred to 5% acetic acid. The acetic acid filtrates were basified with ammonia and shaken with $CHCl_3$–isopropanol (3:1) to separate the alkaloids. The radioactivities of the organic solvent and aqueous layers were determined and the morphine separated from the former and its radioactivity determined. The controls were prepared by boiling the fractions in buffer, for 30 min, before adding the radioactive morphine. The results in Table 2 show that in the young latex about 40% of the morphine had been metabolized in all fractions after 5 min and that at 4 hr metabolism had increased to 45% in the 1000 *g* pellet and to 58% in the 100 000 *g* supernatant. In the old latex only the 100 000 *g* pellet and supernatant showed significant metabolic activity of 20–51% against values for the control of 7–13%.

Electron Microscopy

Plate A represents a section of the 100 000 *g* pellet showing vesicles (V) surrounded by densely packed

Table 2. Metabolism of morphine. Radioactivities of morphine and non-morphine substances recovered from latex fractions after incubating with [1-*n*-3H]-morphine for 5 min (T_1 — 1000 × 10^3 dpm) and 4 hr (T_2 — 7000 × 10^3 dpm). Controls were fed 500 × 10^3 dpm at T_1 and 3500 × 10^3 at T_2. (a) Young latex samples, collected a few days after petal fall. (b) Old latex, 4 weeks after petal fall

Fraction	Time T_1 Morphine (dpm × 10^{-3})	Time T_1 Non-morphine (dpm × 10^{-3})	Time T_1 % Non-morphine to total recovered radioact.	Time T_2 Morphine (dpm × 10^{-3})	Time T_2 Non-morphine (dpm × 10^{-3})	Time T_2 % Non-morphine to total recovered radioact.
(a) Young latex						
1000 g pellet	609	285	31.9	3564	2939	45.2
10000 g pellet	512	360	41.3	3094	3311	51.7
100000 g pellet	544	376	40.8	2846	3594	55.8
100000 g supernatant	551	353	39.0	2691	3777	58.4
(b) Old latex						
1000 g pellet	810	74	8.3	5802	659	10.2
10000 g pellet	802	98	10.9	5439	1036	16.0
100000 g pellet	752	147	16.3	5089	1285	20.2
100000 g supernatant	653	227	25.8	3126	3307	51.4
(c) Control						
1000 g pellet	430	42	8.8	3160	260	7.6
1000 g supernatant	418	33	7.3	2953	437	12.9

cytoplasm largely composed of polysomes (PS). The vesicles were less numerous than those in the 1000 g pellet [9] and smaller, being on average about 0.5 μm against about 1 μm in the 1000 g pellet. Most had smooth to granular walls and it has been suggested [9, 13] that this granular material contracts and zonates to give the familiar capped vesicles, one or two of which can be seen.

Plate B represents the 10000 g pellet and again shows small vesicles but fewer polysomes and a few large vesicles, one of which has a distinct cap.

Plate C illustrates one of the capped vesicles from a 1000 g pellet. The zonation in the cap is very distinct with an inner region different from both lumen and cap. The pellet consisted mainly of large alkaloidal vesicles as previously reported [9]. The Soma variety (1000 g pellet) showed vesicles similar in shape and size to normal poppy plants but the caps seemed less dense.

DISCUSSION

Biosynthetic Activity

It is clear from the results of the 1st experiment (Table 1) that only the 1000 g pellet showed biosynthetic activity with a decreasing order of specific activities from thebaine to codeine to morphine consistent with the known biosynthetic pathway. No evidence for biosynthesis was found in the 10000 g or 100000 g pellet or 100000 g supernatant. In the 2nd experiment the biosynthetic activity of the 1000 g pellet was compared with that of the supernatant (without further fractionation) and of whole latex. Once more, no evidence for biosynthesis was found in the supernatant. The activity of the 1000 g fraction was similar to that found in the 1st experiment but both were less active than the whole latex, with the specific activities of the morphine being 114 and 86 against 228 (× 10^3 dpm/mM) in the whole latex. This confirms previous work on whole latex and pellet [5] and also confirms that the 1000 g pellet rather than the supernatant is a possible site of biosynthesis. This is further confirmed in the 3rd experiment (Table 1) where the 1000 g pellet from the low alkaloid strain 'Soma' produced radioactive morphine in contrast to the supernatant and controls. It is evident that this low alkaloid strain is as biosynthetically active as the alkaloid-rich strain (Halle); the low absolute amount of alkaloid produced may be partly due to the presence of fewer alkaloidal vesicles. The fresh latex is less viscous and less creamy in appearance than normal poppy latex: in one experiment we found 1 ml weighed 0.98 g whereas two 1-ml samples of Halle latex weighed 1.20 and 1.31 g and three samples of *P. bracteatum* latex, 1.10, 1.30 and 1.24 g. A further factor may be that in the Soma variety the vesicles are particularly active in metabolizing morphine so that less is accumulated (see below). Our results are not consistent with those of Roberts and Antoun [10] who have reported that some of the enzymes necessary for biosynthesis, especially DOPA-decarboxylase, only occur in the supernatant above 10000 g; there seems to be no explanation of this discrepancy at the moment.

One striking feature of the results in Table 1 is the high initial radioactivities of the alkaloids after solvent separation followed by TLC separation. This was equally true of all the samples, including controls, but only with those tabulated did the radioactivity persist after the 2nd or 3rd TLC separation. This may be due to the fact that the fed radioactive DOPA may be easily decarboxylated to dopamine which, being basic and phenolic, may have closely similar physical properties to those of the

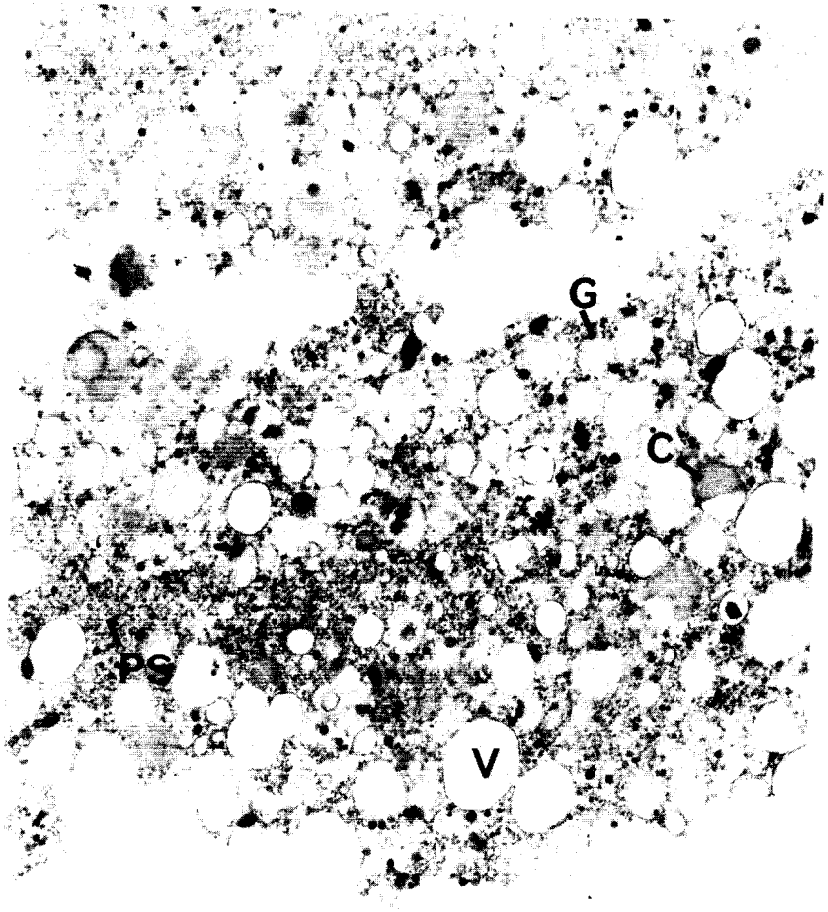

Plate A. Ultrathin section of 100 000 g pellet showing alkaloidal vesicles (V), some granulated (G) and some capped (C), surrounded by densely packed polysomes (PS). × 16 000.

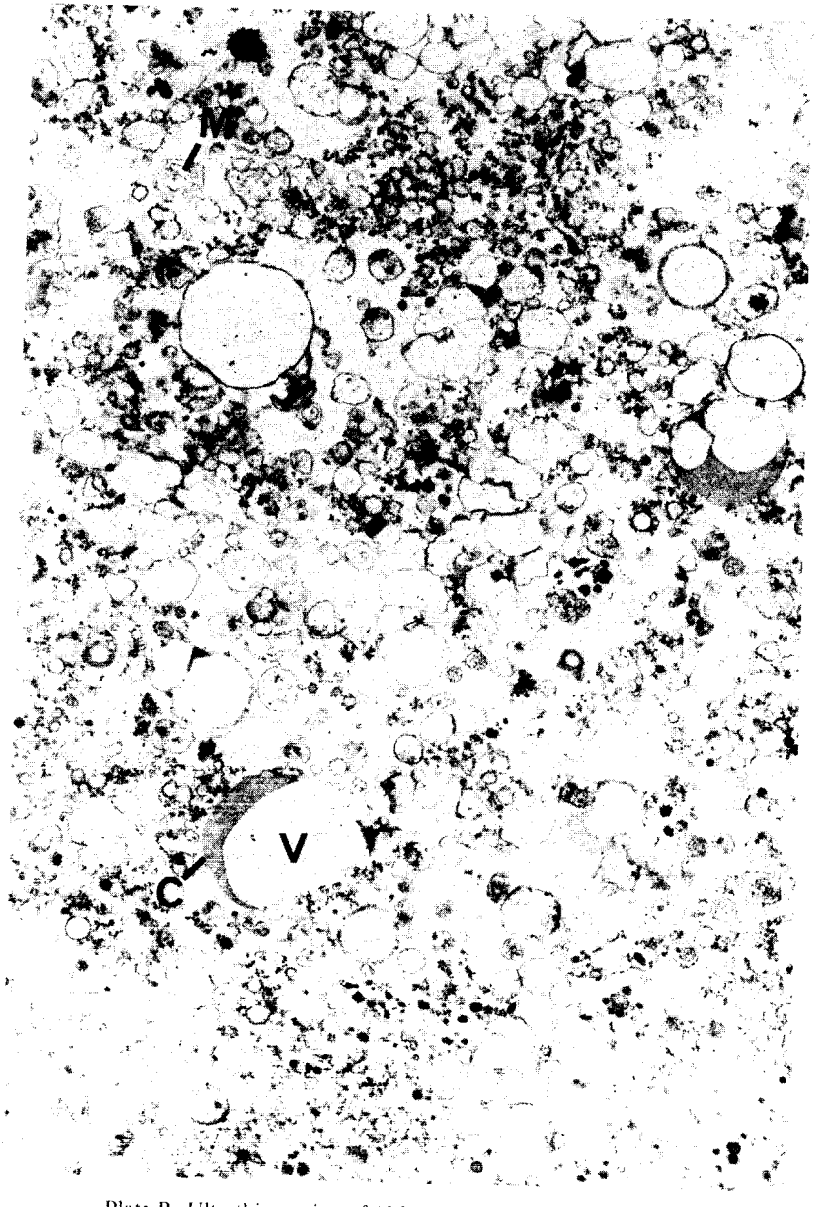

Plate B. Ultrathin section of 10 000 g pellet with some vesicles larger than those in Plate A. M = mitochondrion. × 16 000.

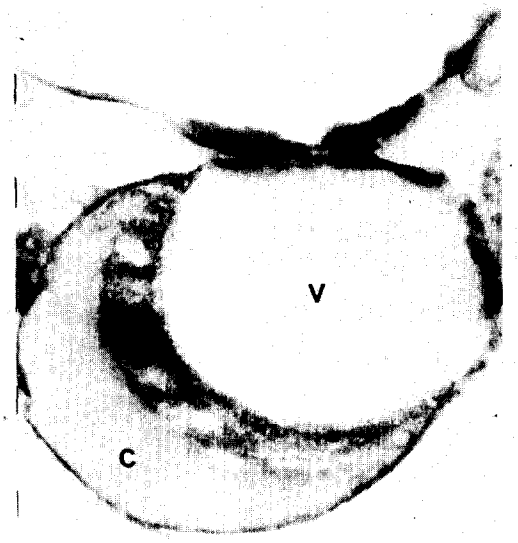

Plate C. Vesicle from an ultrathin section of 1000 *g* pellet, showing distinct zonation in the cap. × 86400.

alkaloids, so that the danger of contamination is particularly high when working with this precursor.

Metabolic Activity

In contrast to the biosynthetic studies all fractions of the latex seemed capable of metabolizing morphine with activity increasing from a 5 min incubation to a 4-hr period. Of considerable interest is the fact that the 100000 *g* pellet and supernatant were more active than the heavier fractions. Not surprisingly the young latex was significantly more active than the old; in fact in the latter only the supernatant was markedly more active than the controls. The apparent activity in the controls (boiled latex) may be due entirely to impurities or slight instability of the T-morphine. We have found that during manipulation, even in entirely *in vitro* conditions, about 10% of the radioactivity is lost. In the young latex metabolism is quite rapid with about 40% of the morphine being converted into non-morphine in 5 min.

This detailed study of several latex fractions therefore confirms earlier work that, in the isolated latex, only the 1000 *g* pellet shows biosynthetic activity; none is found in the lighter fractions. However, the amount of alkaloid synthesized is extremely small even in the whole latex. In Table 1, 2nd experiment, the 65.7 mg of morphine had a sp. act. of 22800 dpm/mM corresponding to a total of 5256 dpm in the morphine. This was derived from 11 × 10^6 dpm of DOPA (corresponding to 117 μg) so that only 0.05% of the fed DOPA (0.06 μg) was converted into morphine. This quantity is very small when compared with the 65.7 mg of morphine in the approximately 2.6 g of latex used, and with the quantities involved in earlier *in vivo* work where net gains of morphine of 10, 34, 9 and 5 mg/g latex per hour occurred during the morning [ref. 11, series T_5]. It is important to remember that all work on latex is on expelled latex and it may be that the main site of biosynthesis is in the layers lining the latex vessel which would not be removed during tapping. Electron microscopy shows that, after tapping the capsule, the latex vessels still contain many vesicles. Since the vesicles arise from the endoplasmic reticulum lining the vessel wall [13] these residual vesicles will be younger than those expelled and almost certainly more active.

In marked contrast, however, morphine metabolism takes place in both the 1000 *g* pellet and its supernatant with proportionately more in the lighter 100000 *g* pellet and its supernatant. The values found are in agreement with the 17–40% morphine metabolism previously found in isolated stem latex and 1000 *g* pellets [5] although lower values ranging from 8–21% were found in another series [7]. In contrast again to the biosynthetic studies, our values for morphine metabolism agree with the rapid changes in morphine content both *in vitro* (isolated latex) when 43% of the morphine was 'lost' after 4 hr incubation [12] and *in vivo* [11] when the hourly decreases in morphine content after 11 00 hr were frequently of the order of 5–30 mg/g of latex, corresponding to percentage losses ranging from about 15–45% of the morphine content. These losses, however, are net, representing the excess of metabolism over biosynthesis (earlier in the day biosynthesis exceeds metabolism so there is a net gain in morphine). It would be tempting to explain the absence of evidence for biosynthesis in the supernatant by the fact that (*a*) only small amounts of alkaloid are present in this fraction as 95–99% are stored in the 1000 *g* pellet [5] and (*b*) the small amounts produced from radioactive DOPA would mix with this very small pool and be rapidly metabolized. We did indeed find that the pool sizes in the lighter fractions referred to in Table 1 were small; only 0.94 mg total alkaloids in the 10000 *g* pellet, 0.35 mg in the 100000 *g* pellet and 4.83 mg in the 100000 *g* supernatant (figures not given in Table 1) but as none of them was radioactive they were obviously not in transition between radioactive DOPA and alkaloid metabolites.

Electron microscopy shows clearly that vesicles and other organelles are present in the 100000 *g* and 10000 *g* fractions which would explain their metabolic and enzymic activities. However few of the larger capped vesicles are present. These are mainly associated with the 1000 *g* fraction and this is consistent with the suggestion previously made [9] that they are concerned with biosynthesis of the alkaloids.

EXPERIMENTAL

Plant material. Healthy outdoor plants of *Papaver somniferum* L. Halle 4/2 [14] and Soma SV 74204 [15] varieties were used. At stated times after petal opening capsules were cut off from the stem, the latex transferred to mannitol buffer and centrifuged as already described [6]. Incubation with L-3,4-dihydroxyphenyl-[3-^{14}C]-alanine and [1-*n*-^{3}H]-morphine hydrochloride (Radiochemical Centre, Amersham) was carried out at room temp., in the dark, for the periods described in the text. Controls were heated in a boiling water bath for 30 min before adding the radioactive precursors.

Extraction and purification of the alkaloids. After incubation the mannitol-latex mixture was transferred to 5% HOAc, shaken and filtered. The latter was made alkaline with 25% NH_4OH soln and the alkaloids extracted by shaking with successive portions of $CHCl_3$–*i*-PrOH (3:1). The combined organic solvent layers were back washed with H_2O, dried and concd to a suitable vol. A suitable aliquot was analysed by HPLC and individual alkaloids separated and purified by repeated TLC [4].

Electron microscopy. Portions of the latex pellet, obtained as described above, were fixed in 5% gluteraldehyde and post-fixed in osmium tetroxide; they were then double-stained in uranyl acetate and lead citrate and embedded in Araldite.

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REFERENCES

1. Miller, R. J., Jolles, C. and Rapoport, H. (1973) *Phytochemistry* **12**, 597.
2. Fairbairn, J. W. and El-Masry, S. (1967) *Phytochemistry* **6**, 499.
3. Wold, J. K. (1978) *Phytochemistry* **17**, 832.
4. Fairbairn, J. W. and Steele, M. J. (1980) *Phytochemistry* **19**, 2317.
5. Fairbairn, J. W., Hakim, F. and El Kheir, Y. (1974) *Phytochemistry* **13**, 1133.
6. Fairbairn, J. W. and Djoté, M. (1970) *Phytochemistry* **9**, 739.
7. Fairbairn, J. W., Handa, S. S., Gurkan, E. and Phillipson, J. D. (1978) *Phytochemistry* **17**, 261.
8. Böhm, H., Olesch, B. and Schulze, C. H. (1972) *Biochem. Physiol. Pflanzen* **163**, 126.
9. Dickenson, P. B. and Fairbairn, J. W. (1975) *Ann. Bot.* **39**, 707.
10. Roberts, M. F. and Antoun, M. D. (1978) *Phytochemistry* **17**, 1083.
11. Fairbairn, J. W. and Wassel, G. (1964) *Phytochemistry* **3**, 253.
12. Fairbairn, J. W. and Wassel, G. (1964) *Phytochemistry* **3**, 583.
13. Nessler, C. L. and Mahlberg, R. G. (1977) *Amer. J. Bot.* **64**, 541.
14. Fairbairn, J. W. and El-Masry, S. (1968) *Phytochemistry* **7**, 181.
15. Nyman, U. and Hall, O. (1976) *Heriditas* **84**, 69.