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THE ALKALOIDS OF *PAPAVER SOMNIFERUM* L.—III.
BIOSYNTHESIS IN THE ISOLATED LATEX

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Abstract—Solid L-tyrosine-U-^{14}C hydrochloride was mixed with poppy latex isolated from the base of the capsule and samples of the mixture were withdrawn at short intervals and analysed. The results showed that after 10 min a significant incorporation of radioactivity into the alkaloids had taken place, the isoquinoline alkaloids having the highest activity. It was shown that, there is a rapid variation of the alkaloidal pattern with time, the morphine "disappears" from the latex instead of accumulating and a fall in the radioactivity of the morphine, codeine and thebaine is sometimes accompanied by an increase in the amount of the alkaloids present. These results are in agreement with previous work on whole plants[3,4] but, since isolated latex was used it is possible to conclude more definitely both that the morphine is transformed into a non-alkaloidal molecule in the latex itself and that there is an additional "non-tyrosine" route to the alkaloids.

KLEINSCHMIDT and Mothes[1,2] fed radioactive tyrosine and radioactive glucose to isolated poppy latex and showed that radioactive alkaloids could be recovered 24 hr later. We decided to repeat their experiments but to analyse samples of the isolated latex at short intervals in an attempt to gain a clearer insight into the rapid changes in the concentration and specific activity of alkaloids we have already reported in intact plants.[3,4]

RESULTS

Fresh latex was mixed with solid L-tyrosine U-^{14}C hydrochloride and samples of the mixture were withdrawn at intervals and analysed. The changes in the amounts and specific activities [counts/sec(cps)/μM] of morphine, codeine and thebaine are given in Table 1.

TABLE 1. ANALYSES OF SAMPLES TAKEN AT INTERVALS FROM ISOLATED LATEX WHICH HAD BEEN ADDED TO SOLID L-TYROSINE HYDROCHLORIDE U-^{14}C ($3{\cdot}7 \times 10^6$ cps)

Amounts of alkaloid expressed as mg/100 mg fresh latex; specific activities as cps/μM

Time after addition	Morphine		Codeine		Thebaine	
	Amount (mg)	Sp. act.	Amount (mg)	Sp. act.	Amount (mg)	Sp. act.
10 min	2·64	14·16	0·34	3·10	0·09	75·08
2 hr	2·94	3·25	0·28	0·63	0·16	30·09
4 hr	1·50	5·91	traces	traces	traces	traces
6 hr	1·53	2·25	0·11	13·60	0·06	65·96

[1] G. KLEINSCHMIDT and K. MOTHES, *Z. Naturforsch.* **14b**, 52 (1959).
[2] G. KLEINSCHMIDT and K. MOTHES, *Arch. Pharm.* **293**, 948 (1960).
[3] J. W. FAIRBAIRN and G. WASSEL, *Phytochem.* **3**, 253 (1964).
[4] J. W. FAIRBAIRN, A. PATTERSON and G. WASSEL, *Phytochem.* **3**, 577 (1964).

The total radioactivity of the minor alkaloids (phenolic and non-phenolic separately) per known weight of latex (cps/100 mg latex) was also determined and the results compared with the corresponding figures for morphine, codeine and thebaine (Table 2). The morphine from some of the samples was crystallized by the method of Leete,[5] authenticated by reference to pure morphine, and its specific activity determined. The results confirmed the figures quoted in the tables.

In another experiment fresh latex was mixed with an aqueous solution of the residue left

TABLE 2. RESULTS OF ANALYSES OF THE SAME SAMPLES AS IN TABLE 1, BUT EXPRESSED AS RADIOACTIVITY (cps/100 mg FRESH LATEX) CONTRIBUTED BY EACH ALKALOID OR GROUP OF ALKALOIDS

Time after addition	Morphine	Codeine	Thebaine	Minor (phenolic)	Minor (non-phenolic)	Total radioactivity of the sample
10 min	130	3·5	22	574	683	95,060
2 hr	34	0·5	16	63	53	83,560
4 hr	31	traces	traces	not done	32	90,895
6 hr	12	5·1	12	80	103	—

from the previous experiment. This residue had been freed from alkaloids but was still highly radioactive. After 48 hr the mixture was analysed and the morphine present found to have a specific activity of 172·5 cps/μM.

DISCUSSION

Rapid Variation in Alkaloidal Pattern

The rapid synthesis of alkaloids from tyrosine is the most striking observation; in 10 min a significant incorporation of ^{14}C through the isoquinoline alkaloids to thebaine, codeine and morphine, in that order, has taken place. During the next 4 hr this synthetic activity slows down but increases again 2 hr later, when the experiment was terminated. The second experiment, lasting 48 hr, indicates that this increase continues since morphine of high specific activity was recovered. The results therefore confirm our earlier work,[3,4] based on whole plant studies, that the amounts of alkaloid being synthesized fluctuates markedly at short intervals. The initial surge of activity may be related to aeration of the latex during isolation, since Meissner and Mothes[6] found that isolated poppy latex showed the highest gaseous interchange during the first 20 min. On the other hand they found that glycine incorporation into protein showed only a steady increase during the first 2 hr.

Periodic Disappearance of the Morphine

Since this occurs equally in isolated latex (Tables 1 and 2) as in whole plants[3,4] the morphine must "disappear" by transformation into some other molecule rather than by physical removal out of the latex.

Specific Activities of the Alkaloids

In the first 2 hr the total radioactivity due to the morphine, codeine and thebaine fell from 156 to 50 cps/100 mg latex but, at the same time, the quantities present increased from 3·07

[5] E. LEETE, *J. Amer. Chem. Soc.* **81**, 3948 (1960).
[6] L. MEISSNER and K. MOTHES, *Phytochem.* **3**, 1 (1964).

to 3·38 mg/100 mg latex, thus leading to a fall in specific activities. As this occurs in isolated latex the possibility that it is due to a fluctuating inflow of ^{14}C tyrosine is ruled out. The results therefore support our earlier suggestion[4] based on whole plant studies that there is an additional, non-tyrosine, route to the alkaloids.

Site of Biosynthesis

We hope to confirm the radioactivity of the codeine, thebaine and the minor alkaloids in later experiments as has been done for morphine. There is sufficient evidence, however, in our one experiment to confirm the conclusions of Kleinschmidt and Mothes[1,2] that the isolated latex is capable of synthesizing alkaloids. The fact that this biosynthesis occurs even after the distortion and agitation of the latex involved in isolating it suggests that the major part of the biosynthetic process is associated with the same cellular organelle. We propose to investigate this interesting possibility further.

EXPERIMENTAL

Plants of *Papaver somniferum* L. subsp. *anatolicum* M. Vesel, var. *albescens*, M. Vesel were used as on previous occasions.[3,4] Two weeks after petal-fall ten capsules were cut off at the region of the thalamus. The exuding latex from the severed end of the pedicel and the capsule was drawn into a syringe, until about 0·7 to 1·0 ml had been collected, and transferred to a small flask containing about 1·3 mg solid L-tyrosine-U-^{14}C hydrochloride ($3·7 \times 10^6$ cps). The flask was gently rotated for 10 min and a sample of about 100 mg withdrawn. The flask was then allowed to stand and further samples withdrawn at 2,- 4- and 6-hr intervals. All operations were carried out at room temperature in subdued daylight; the analyses[7] and radioactive counting[4] were carried out as in previous work.

The final sample contained the bulk of the radioactivity, presumably unchanged ^{14}C tyrosine. After removal of the alkaloids from this sample the solution was evaporated to about 2 ml and added to a similar quantity of latex which was kept for 48 hr then analysed.

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[7] J. W. Fairbairn and G. Wassel, *J. Pharm. Pharmacol.* **15**, 216T (1963).