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THE ALKALOIDS OF *PAPAVER SOMNIFERUM* L.—II.
^{14}C ISOTOPIC STUDIES OF THE RAPID CHANGES IN THE MAJOR ALKALOIDS

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Abstract—L-tyrosine-U-^{14}C in solution was fed to the phloem region of the pedicels of poppy plants and the latex from the corresponding capsules was sampled at various intervals afterwards. Analyses of the samples confirmed the results previously reported on the rapid turnover of the alkaloids and the periodic disappearance of morphine. Practically no variation was found between samples taken simultaneously thus showing that the variation with time was not due to sampling error. Incorporation of tyrosine into the alkaloids was at least 4% and, in view of the rapid turnover, probably considerably exceeded this figure. The synthesis of these alkaloids from tyrosine cannot therefore be considered aberrant, but the results also indicate that there may be an additional precursor for the alkaloids.

OUR EARLIER paper[1] showed that the quantities of morphine, codeine and thebaine in the latex of developing capsules of *Papaver somniferum* varied considerably over short intervals and that morphine disappeared from the system from time to time. While these results are consistent with those found for the fruits of *Conium maculatum*[2], they are sufficiently remarkable to warrant confirmation by the use of radioactive isotopes. It was also hoped that this technique would provide a more detailed picture of the changes taking place and enable us to check our sampling methods. Clearly, if the latter were faulty the changes observed might be largely accounted for by sampling errors.

RESULTS

Sampling Error

Twenty-eight poppy capsules, each exactly at week 1 stage (i.e. 1 week after petal fall), were fed with L-tyrosine-U-^{14}C solution ($10{\cdot}6 \times 10^4$ counts/sec (cps) each) by the method described below. One week later three workers collected simultaneously one sample of latex each by the method described in our earlier paper.[1] These samples were analysed and the results recorded in Table 1.

Radioactive Changes in the Alkaloidal Pattern

Two series of experiments were carried out. In the first series the ^{14}C tyrosine was fed at week 1 and samples were drawn at various intervals up to week 2. In the second ^{14}C tyrosine was fed at week 0 stage (petal fall) and 2 weeks later the latex was sampled every 2 hr for a day. The results are recorded in Tables 2 and 3.

[1] J. W. FAIRBAIRN and G. WASSEL, *Phytochem.* 3, 253 (1964).
[2] J. W. FAIRBAIRN and P. N. SUWAL, *Phytochem.* 1, 38 (1961).

TABLE 1. ANALYSES OF THREE REPLICATE SAMPLES OF LATEX

	Sample		
	1	2	3
Wt. of latex in sample (mg)	124	271	246
Total solids (%)	29·4	30·3	29·3
Morphine (%)	3·49	3·30	3·23
Sp. act. of the morphine (cps/μM)	69·3	69·9	60·0

L-Tyrosine-U-^{14}C hydrochloride ($10{\cdot}6 \times 10^4$ cps) fed to each capsule on 9 July 1963, one week after petal fall. The three samples were collected simultaneously from 28 capsules at 10 a.m. on 17 July 1963.

DISCUSSION

Sampling Error

The remarkable agreement between the three sets of figures in Table 1 justifies the confidence we expressed earlier[1] in our sampling methods. The error involved is obviously too small to account for the large variations in alkaloidal composition which was found to occur with time. The uniform radioactivity of the morphine also indicates that the radioactive tyrosine applied to the pedicel is uniformly distributed throughout the capsules.

Periodic Disappearance of the Morphine

In Fig. 1 is shown the variation during 12 hr in total activities of the alkaloids per 100 mg. fresh latex. The values are calculated by combining the specific activities with the amounts of each alkaloid/100 mg latex (Table 2). The values for thebaine and codeine have been com-

TABLE 2. SPECIFIC ACTIVITIES (cps/μM) AND AMOUNTS (mg/100 mg FRESH LATEX) OF ALKALOIDS AT VARIOUS STAGES OF CAPSULE DEVELOPMENT (FIRST EXPERIMENT)

July 1962		Morphine		Codeine		Thebaine	
Day	Time	Sp. act	Amount	Sp. act.	Amount	Sp. act.	Quantities
24	12 noon	0		0		0	
24	2 p.m.	0·11	6·8	1·67	0·51	6·83	0·48
24	4 p.m.	1·24	4·8	11·0	0·47	43·7	0·34
24	8 p.m.	7·62	3·8	41·1	0·89	142·4	0·63
25	12 noon	33·0	4·7	224·2	0·69	67·2	0·29
26	10 a.m.	45·7	3·5	201·7	1·16	245·0	0·65
30	12 noon	156·6	3·9	479·2	1·00	1952·0	0·25
31	6 a.m.	107·2	3·4	590	0·62	704	0·46
	8 a.m.	264·9	1·5	979	0·54	1545	0·37
	10 a.m.	204·8	2·8	554	0·71	656	0·50
	12 noon	74·5	2·6	835	0·76	696	0·70
	2 p.m.	129·3	2·7	474	0·70	423	0·62
	4 p.m.	257·3	1·2	342	1·06	461	0·68
	6 p.m.	181·7	2·2	425	1·02	284	0·54
	8 p.m.	145·4	2·3	562	0·80	682	0·25

L-Tyrosine-U-^{14}C ($18{\cdot}5 \times 10^4$ cps) fed to each of 15 capsules (one week after petal fall), at noon on 24 July.

TABLE 3. SPECIFIC ACTIVITIES (cps/μM) AND AMOUNTS (mg/100 mg FRESH LATEX) OF ALKALOIDS AT VARIOUS STAGES OF CAPSULE DEVELOPMENT (SECOND EXPERIMENT)

July 1962 Day	Time	Morphine Sp. act.	Morphine Amount (mg)	Codeine Sp. act.	Codeine Amount (mg)	Thebaine Sp. act.	Thebaine Amount (mg)
11		0		0		0	
18	12 noon	26·7	4·4	49·7	0·25	34·7	0·52
25	6 a.m.	39·1	5·8	50	0·76	83	0·55
	8 a.m.	39·2	5·5	41	0·74	76	0·66
	10 a.m.	49·3	4·0	139	0·66	108	0·55
	12 noon	66·1	3·3	68	1·01	74	0·89
	2 p.m.	57·6	2·6	88	0·98	84	0·72
	4 p.m.	58·6	1·8	116	0·66	83	0·50
	6 p.m.	53·5	2·8	76	0·74	93	0·60
	8 p.m.	43·5	2·5	83	0·73	65	0·46

L-Tyrosine-U-^{14}C ($18{\cdot}5 \times 10^4$ cps) fed to each of 16 capsules (immediately after petal fall) at noon on 11 July.

bined in Fig. 1, so as to cancel out increases in codeine corresponding to decreases in thebaine, since the sequence thebaine→codeine→morphine has been shown to be irreversible.[3] It can be seen that there is a marked decrease in "hot" morphine between 10 a.m. and noon and smaller decreases at other intervals of time. These decreases are even more remarkable if the behavior of the thebaine and codeine is considered, since the *net* fall in their activity should

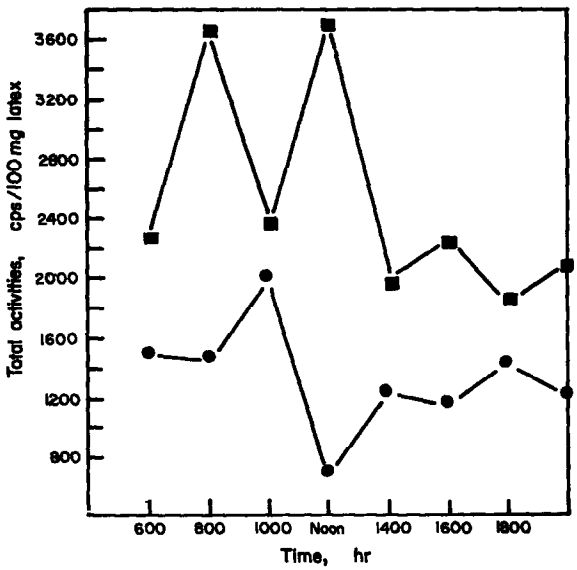

FIG. 1. VARIATION IN TOTAL ACTIVITIES OF THE ALKALOIDS DURING A PERIOD OF 14 hr. First experiment; radioactive tyrosine fed to plants 7 days before samples were drawn on 31 July. Thebaine + codeine —■—■—, morphine —●—●—.

[3] F. R. STERMITZ and H. RAPOPORT, *J. Amer. Chem. Soc.* **83**, 4045 (1961).

correspond to an equivalent rise in morphine. Between 8 a.m. and 2 p.m. two falls in thebaine/codeine occur, corresponding to a loss of 3027 cps. But instead of the expected increase in "hot" morphine molecules there is actually a decrease of 230 cps.

This periodic disappearance of morphine explains the otherwise puzzling results reported by Stermitz and Rapoport.[3] After feeding large quantities of thebaine to poppy plants, subsequent analyses showed a substantial increase in thebaine and codeine but none in morphine: in fact a slight decrease occurred. The rapid turnover we find also explains the fact they reported that when radioactive thebaine or codeine in comparatively large quantities were fed to the plant the specific activities of these alkaloids, extracted 24–40 hr later, were considerably lower than would be expected by dilution alone.

The Specific Activities of the Alkaloids

The results in Table 2 shows that initially the specific activity of the thebaine rises more rapidly than that of the codeine which in turn is higher than that of the morphine consistent with the biosynthetic sequence referred to. The rapid rise in specific activity of the morphine (Table 2) during the first 2 days is also consistent with the continued addition of newly formed radioactive molecules to a pool of non-radioactive ones. Since morphine is continually being removed from the pool, however, the specific activity of the morphine should gradually approximate those of the parent thebaine and codeine. Ultimately, as the supply of "hot" parent molecules falls off, the morphine should have a specific activity higher than that of thebaine and codeine. In the first experiment, even after 7 days, the specific activity of the morphine (average 145) is significantly lower than that of the codeine (average 600) and the thebaine (average 680); Table 2. In the second experiment (14 days after feeding) it is obvious that the supply of "hot" thebaine and codeine is falling off (average specific activities 85 and 83 respectively) yet the morphine is still not as active (average 51); Table 3.

These results suggest that the morphine pool is being continuously supplied from a non-radioactive source, and indicates that the tyrosine pathway is not the only route to morphine synthesis. Similar doubts have been expressed by Stermitz and Rapoport[3] and Kleinschmidt and Mothes.[4] The latters' work is particularly relevant to our own; when ^{14}C tyrosine was fed to isolated latex the relative incorporation of radioactivity into the morphine was significantly less than when ^{14}C glucose was fed. Mothes interprets this as indicating that half of the morphine molecule is derived from a non-tyrosine source.[5]

This suggestion of an alternative route for morphine synthesis is supported by a more detailed consideration of our quantitative results. In the first experiment (Fig. 1) there is a marked decrease in "hot" thebaine and codeine molecules between 8 and 10 a.m. but at the same time there is an actual increase (of 30%) in the amount of these alkaloids during this period (Table 2). A similar situation is shown for morphine between 10 a.m. and noon, where a marked fall in "hot" molecules does not correspond to a similar decrease in the amount of morphine in the latex. In fact every decrease in *specific activity* (Table 2) seems best explained by the influx of substantial amounts of alkaloids from a non-tyrosine source. (Another possibility is that the proportion of radioactive to non-radioactive tyrosine entering the latex fluctuates considerably. This would contradict the generally accepted view that radioactive molecules are rapidly diluted to a constant proportion by the normal molecules; an assumption which seems confirmed by the uniformity of labelling shown in our sampling experiment, Table 1). It is possible that the tyrosine route is used mainly when the demand for

[4] G. KLEINSCHMIDT and K. MOTHES, *Arch. Pharm.* **293**, 948 (1960).
[5] K. MOTHES, *Deut. Apotheker Ztg.* No. 49, 1424 (1960).

morphine is high (e.g. between 8 a.m. and 2 p.m., Fig. 1); this would explain Kleinschmidt's observation that in some experiments ^{14}C tyrosine was rapidly removed to form alkaloids, yet analyses of latex shows that free tyrosine is a normal constituent of the latex.[6]

Amount of incorporation

Stermitz and Rapoport (1961) criticized the tyrosine precursor hypothesis on the basis of low incorporation of ^{14}C tyrosine[3] (e.g. 0·017%[7]) though Battersby *et al.*[8] subsequently reported higher values of about 0·8% for morphine, codeine and thebaine combined and 1·6% for morphine alone on one occasion. Our results at 8 a.m. on the 31st (Table 2) show a total of 5090 cps/100 mg. latex for morphine, codeine and thebaine combined. We have roughly estimated that each capsule contains at least 150 mg latex and on this basis the incorporation is of the order of 4·1%. In view of the rapid changes in the alkaloidal pattern however this figure, like those quoted by other workers, represents the situation at one moment only. A more realistic appraisal is possible by considering the successive increases in activity of codeine and thebaine shown in Fig. 1; these correspond to an incorporation of about 3·0% in 14 hr. While such a rate would almost certainly not have been maintained during the 7 days of the experiment the order of incorporation will obviously be considerably higher than 3 or 4%. This indicates that, while tyrosine may not be the only route to the alkaloids it does represent a significant source and cannot be considered as an "aberrant synthesis".[3]

EXPERIMENTAL METHODS

Materials

Papaver somniferum L. ssp. *anatolicum* M. Vesel, var. *albescens* M. Vesel was used throughout and L-tyrosine-^{14}C hydrochloride, generally labelled, was used as the ^{14}C source. It had a specific activity of about 0·1 mc/mg.

Feeding the ^{14}C Tyrosine

It was decided to apply the tyrosine in solution to the phloem region of the pedicel immediately below the developing capsule. The latter would then act as a "sink" sucking up the solution via the vascular bundles. The soft outer tissues from about 3 cm of pedicel was scraped away till the hard sclerenchymatous band just outside the phloem[9] was reached. A piece of polyvinylchloride tubing about 5 cm long and 0·8 cm dia. was slit longitudinally and slipped over the scraped area. The longitudinal slit was sealed with waterproof adhesive tape and the lower end of the cylinder sealed securely to the pedicel with an adhesive wax made up by heating together, white beeswax 20 g, liquid paraffin 4 ml and mastich (*Pistacia lentiscus* resin) 1 g. Before use, the cylinder was filled with distilled water to ensure all the sealing was water-tight (Fig. 2). The tyrosine solution was placed in the cylinders when needed and as the plant absorbed the solution it was replaced with distilled water to wash in all traces of radioactive substances.

Sampling of Latex and Estimation of Alkaloids

Sampling was carried out exactly as described in the previous paper,[1] and the alkaloids estimated by quantitative paper chromatography.[10]

[6] G. KLEINSCHMIDT, *Planta Medica* **8**, 114 (1960).
[7] E. LEETE, *J. Amer. Chem. Soc.* **81**, 3948 (1960).
[8] A. R. BATTERSBY, R. BINKS and B. J. T. HARPER, *J. Chem. Soc.* 3534 (1962).
[9] J. W. FAIRBAIRN and L. D. KAPOOR, *Planta Medica* **8**, 49 (1960).
[10] J. W. FAIRBAIRN and G. WASSEL, *J. Pharm. Pharmacol.* **15**, 216T (1963).

Counting the ^{14}C Labelled Alkaloids

The use of dimpled planchets and a low background Geiger Muller Counter (Panax Equipment Limited) was found to be more convenient than a scintillation counter. Experiments with an artificial mixture of radioactive tyrosine and morphine showed that consistent results could be obtained if (*a*) the substance was applied in 70% methanol, as the water present prevented the solution from creeping over the edge of the planchet during evaporation;

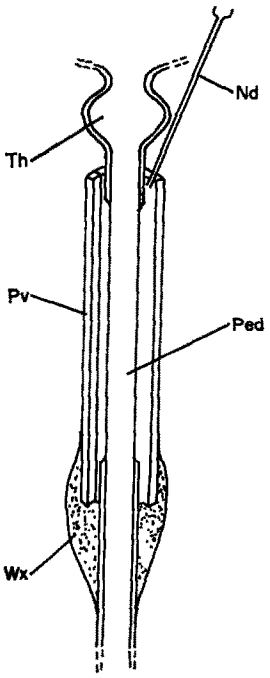

FIG. 2. METHOD USED FOR FEEDING RADIOACTIVE PRECURSORS.
Nd., needle; *Ped*, pedicel; *Pv*, polyvinyl tube; *Th.*, thalamus; *Wx.*, special wax used.

and (*b*) not more than 0·5 mg of total solid was applied (equivalent to 0·4 mg cm^2). With 10 replicated planchets, each counted till 1000 disintegrations were recorded, the coefficient of variation was 3·59%; when 10,000 disintegrations each were recorded the coefficient of variation was 3·82%. The machine was calibrated with a standard ^{14}C source and the results obtained with the alkaloids corrected accordingly.

The final solutions of the alkaloids obtained by the assay process were used for the radioactive counts. Band chromatograms of suitable volumes were prepared and the morphine, codeine and thebaine eluted by extraction with methanol.

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