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THE ALKALOIDS OF *PAPAVER SOMNIFERUM* L.—I.
EVIDENCE FOR A RAPID TURNOVER OF THE MAJOR ALKALOIDS

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Abstract—Previous work on *Conium maculatum* L. has shown that remarkably rapid changes in the pattern and concentration of alkaloids take place during development of the fruit.[1] Using special sampling techniques, similar work has been done on the content of morphine, codeine and thebaine in the latex of developing fruits of *Papaver somniferum* L. The results lead to two major conclusions which agree well with those based on the work on *Conium*. Marked variations in the content of all three alkaloids occur at intervals of 1 to 3 hr: this suggests that these alkaloids play an active part in metabolism rather than occurring as a slowly accumulating amount of waste matter. Secondly, though morphine has been shown to occur as the irreversible end-product of the sequence thebaine→codeine→morphine,[6] it has been found to decrease markedly at certain times during the day. This suggests that it is periodically converted into a non-alkaloidal molecule which may remain in the latex or pass out into other tissues of the capsule.

IN A PREVIOUS communication[1] it was shown that remarkably rapid changes in the alkaloidal picture took place in the fruits of hemlock (*Conium maculatum* L.) during development of the pericarp. If these results are typical of alkaloids in general then the widely held view that alkaloids are slowly accumulating waste products would seem unlikely. It was therefore decided to investigate the alkaloids of opium poppy (*Papaver somniferum* L.), especially as Pfeifer[2] has recently analysed poppy plants collected at 6-hr intervals and showed that marked variations in the content of all the alkaloids occurred. Pfeifer's sampling method involved collecting whole plants on each occasion; this clearly limits the number of plants in each sample with a consequent increase in sampling error. To eliminate this error as far as possible we decided to use the same plants for all the samples. For each sample minute quantities of latex were withdrawn from every plant of a series. This enabled succeeding samples to be collected from the same plants at intervals of 1 or 2 hr, without significant damage to the plants.

RESULTS

Two strains of poppy were used; seeds of the first (*Papaver somniferum* L. subsp. *anatolicum* M. Vesel, var. *albescens* M. Vesel) were obtained from Turkey in 1956[3] and plants were raised from their own seed in the experimental gardens at Enfield, Middlesex, each season afterwards. This strain is referred to as 'Turkey'. The second strain, (*P. somniferum* L. subsp. *eurasiaticum* var. *glaucum* Rothm.) was grown from seeds obtained from Berlin in 1960 and is referred to as 'Berlin'.

[1] J. W. FAIRBAIRN and P. N. SUWAL, *Phytochemistry* **1**, 38 (1961).
[2] S. PFEIFER and K. HEYDENREICH, *Sci. Pharm.* **30**, 164–73 (1962).
[3] J. W. FAIRBAIRN and L. D. KAPOOR, *Planta Medica* **8**, 49 (1960).

During 1961 preliminary work was carried out using two series of Turkey capsules sampled every 3 hr (series T_1 and T_2) and one series of Berlin capsules sampled every 2 hr (series B_1). Samples were collected from dawn to dusk on one day except in series T_2 where

TABLE 1. ANALYSES OF SAMPLES OF LATEX DRAWN FROM CAPSULES 2 WEEKS AFTER PETAL FALL. Results expressed as mg/100 mg fresh latex

Time of day	Series B_1 (26 July 1961)		Series T_1 (11 July 1961)	Series T_2 (13 July 1961)		(14 July 1961)	
	Morphine	Total solids	Morphine	Morphine	Total solids	Morphine	Total solids
4.00	12·4	52·0					
6.00	11·4	43·4	7·2	15·2	57·0	11·3	39·0
8.00	5·9	54·1					
9.00			5·4	14·6	63·0	8·3	41·0
10.00	13·4	52·5					
Noon	7·5	61·0	12·1	15·6	58·0	7·2	31·0
14.00	11·7	63·8					
15.00			10·0	14·4	77·0		
16.00	11·1	69·6					
18.00	10·3	57·8	11·4	8·4	42·0		
20.00	9·4	45·1					
21.00			7·1	7·8	40·0		
22.00	13·1	57·0					
Midnight	10·7	53·0					

sampling was continued the following morning. In all samples the morphine content only was determined. In 1962 Turkey plants only were used; two series with samples every 2 hr (T_3 and T_4) and two with samples every hour (T_5 and T_6) were collected. All samples were

TABLE 2. ANALYSES OF SAMPLES OF LATEX DRAWN FROM CAPSULES 2 WEEKS AFTER PETAL FALL. Results expressed as mg/100 mg fresh latex

Time of day	Series T_3 (25 July 1962)				Series T_4 (31 July 1962)			
	Morphine	Codeine	Thebaine	Total solids	Morphine	Codeine	Thebaine	Total solids
6.00	5·8	0·76	0·55	44·6	3·4	0·62	0·46	26·6
8.00	5·5	0·74	0·66	45·8	1·5	0·54	0·37	21·1
10.00	4·0	0·66	0·55	67·4	2·8	0·71	0·50	27·8
Noon	3·3	1·01	0·89	30·0	2·6	0·76	0·70	22·4
14.00	2·6	0·98	0·72	36·2	2·7	0·70	0·62	23·7
16.00	1·8	0·66	0·50	26·6	1·2	1·06	0·68	26·5
18.00	2·8	0·74	0·60	25·2	2·2	1·02	0·54	22·0
20.00	2·5	0·73	0·46	33·2	2·3	0·80	0·25	20·1

collected between dawn and dusk on a given day and the content of morphine, codeine and thebaine determined in each. The results are summarized in Tables 1–3. In Fig. 1 the results for T_5 are represented as percentage variation from the average alkaloidal content present

during the day. This enables the variation of the three alkaloids to be compared on a common basis, since the absolute amounts of codeine and thebaine are considerably lower than those of morphine. Figure 2 shows similar results but calculated on the basis of dry weight of latex.

TABLE 3. ANALYSES OF SAMPLES OF LATEX DRAWN FROM CAPSULES 2 WEEKS AFTER PETAL FALL. Results expressed as mg/100 mg fresh latex

Time of day	Series T_5 (10 July 1962)				Series T_6 (30 July 1962)			
	Morphine	Codeine	Thebaine	Total solids	Morphine	Codeine	Thebaine	Total solids
4.00	4·1	0·29	0·35	29·3	4·0	0·53	0·47	29·2
5.00	3·6	0·54	0.41	29·6	4·1	0·64	0·49	27·9
6.00	4·6	0·14	0·11	37·6	3·1	0·78	0·59	25·6
7.00	8·0	0·10	0·29	37·2	2·7	0·71	0·53	19·8
8.00	8·9	0·50	0·31	35·1	2·5	0·95	0·69	32·0
9.00	9·4	0·45	0·50	35·1	2·6	0·94	0·58	31·9
10.00	9·7	0·66	0·45	36·0	2·2	0·96	0·60	33·5
11.00	6·6	0·54	0·51	34·8	4·0	0·82	0·75	32·5
Noon	5·9	2·15	0·44	38·1	2·7	0·82	0·83	29·2
13.00	6·1	0·86	0·73	36·6	3·6	0·73	0·85	30·9
14.00	3·6	0·80	0·74	32·6	3·3	0·76	0·81	30·5
15.00	3·5	0·73	0·65	26·5	2·5	0·74	0·70	30·9
16.00	2·7	0·53	0·42	31·4	2·8	0·74	0·59	29·5
17.00	3·1	0·93	0·63	33·5	2·7	0·92	0·85	28·6
18.00	2·9	0·75	0·63	31·5	2·0	0·65	0·79	26·8
19.00	2·5	0·83	0·67	31·7	1·1	0·76	0·71	26·8
20.00	2·5	1·06	0·70	27·0	2·2	0·62	0·51	25·4
21.00	2·9	0·86	0·52	29·4	1·9	0·59	0·60	26·0
22.00	3·2	0·88	0·56	29·1	3·5	0·88	0·77	46·6

DISCUSSION

Rapid Variation in the Alkaloidal Picture

The results of all series of experiments show that marked variations in the amount of alkaloids in the latex takes place at short intervals. The extent of these variations is indicated in Fig. 1; for morphine −51 per cent to +96 per cent of the daily average; for codeine (omitting the very high value at noon) −90 per cent to +80 per cent; and for thebaine −84 per cent to +41 per cent. These variations far exceed those involved in the analytical techniques and are unlikely to be due to sampling errors in view of the precautions taken in sampling and the fact that the variations are exhibited in all seven series. Neither are the variations due to fluctuations in the water content of the latex, since Figs. 1 and 2 show that similar variations occur whether the alkaloidal contents are calculated on a fresh- or dry-weight basis. (For some of the other series, greater variation is shown when the results are calculated on a dry-weight basis than on a fresh-weight basis.) Pfeifer's work,[2] based on analyses of whole capsules sampled every 6 hr, show the following variations from the daily average; for morphine −30 per cent to +16 per cent; for codeine, −83 per cent to +46 per cent; for thebaine −84 per cent to +64 per cent. These variations agree well with ours but they are not so marked (probably because only 4 samples per day were involved as against 8–19 per day in our work) nor do they illustrate so well the rapidity of the changes. Both sets

of results support the conclusions derived from similar experiments on hemlock, that the alkaloids play a dynamic role in the metabolism of the plant. The laticifers therefore cannot be considered merely as traps or 'drain pipes' in which unwanted alkaloids are slowly accumulated as has been previously suggested,[3,4] but rather as actively metabolizing tissues.

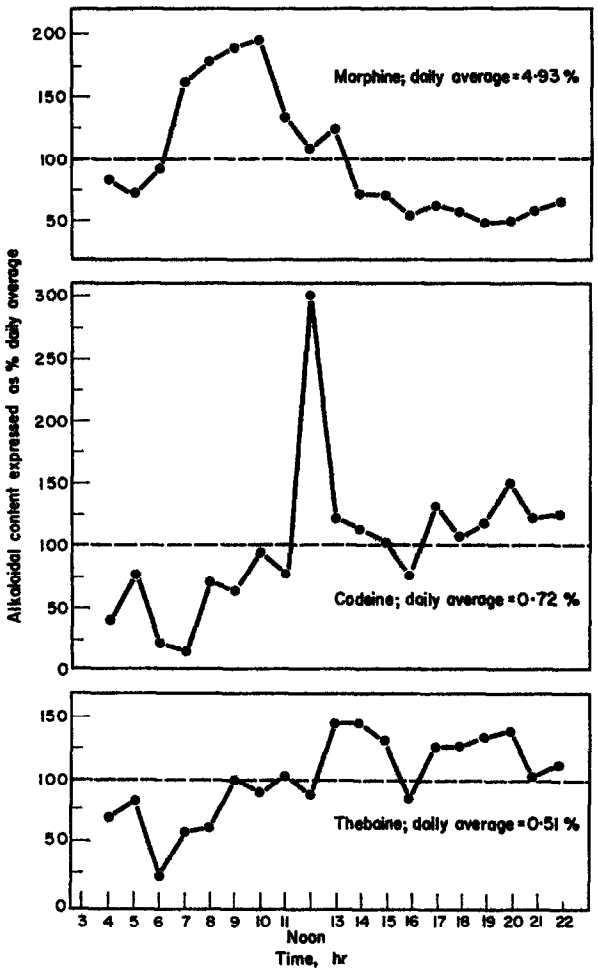

FIG. 1. ALKALOIDAL CHANGES IN THE LATEX DURING 1 DAY (SERIES T_5) EXPRESSED AS PERCENTAGE VARIATION OF THE DAILY AVERAGE.
Based on *fresh weight* of latex.

This is confirmed by the remarkable results reported by Kleinschmidt and Mothes[5] that isolated poppy latex is able to synthesize morphine from glucose or tyrosine.

Disappearance of Morphine

The periodic decrease in morphine content, shown in all series, is probably the most significant observation, as it is the reverse of what would be expected. It has been clearly

[4] A. A. ZAITSEVA, *Botan. Zhurnal* **44**, 1567–77 (1959).
[5] G. KLEINSCHMIDT and K. MOTHES, *Arch. Pharm.* **10**, 293, 948 (1960).

demonstrated that, in the plant, the sequence thebaine→codeine→morphine is irreversible.[6] Our results show, however, that when thebaine and codeine accumulation and disappearance are proceeding actively there is no accumulation of morphine; in fact, a rising content of thebaine and codeine sometimes corresponds with a marked fall in morphine content (Fig. 1).

It is very unlikely that this fall is due to transfer of the morphine to other parts of the plant as a developing fruit acts as a 'receiving' organ. Furthermore, Pfeifer's work[2] indicates

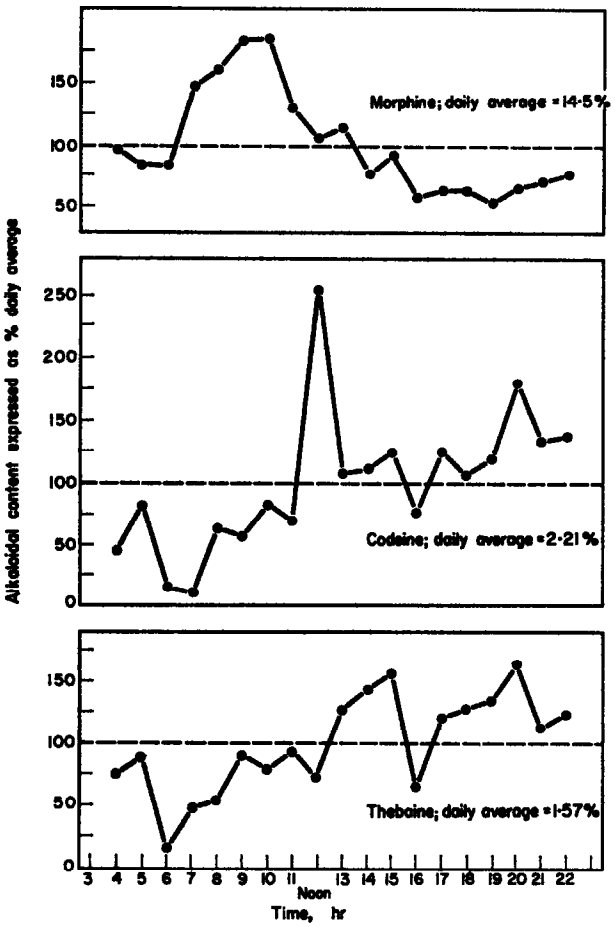

FIG. 2. ALKALOIDAL CHANGES IN THE LATEX DURING 1 DAY (SERIES T_5) EXPRESSED AS PERCENTAGE VARIATION OF THE DAILY AVERAGE.
Based on *dry weight* of latex.

that morphine is synthesized in the root overnight and transferred to the aerial parts, but never in the reverse direction (our results for T_2 series (Table 1) are consistent with his suggestion since the morphine content of the capsule latex increased from 7·8 per cent to 11·3 per cent overnight). His work on decapitated plants[7] also indicates that the developing capsule acts as a receiver of thebaine, produced in the vegetative parts, and transformed into codeine and morphine in the capsule.

[6] F. R. STERMITZ and H. RAPOPORT, *Nature* **189**, 310–11 (1961).
[7] K. HEYDENREICH and S. PFEIFER, *Sci. Pharm.* **30**, 17–25 (1962).

A tentative outline of the situation would therefore be that thebaine, codeine and morphine are formed in varying amounts during the day and, at certain times, the morphine is converted into a non-alkaloidal molecule which remains in the latex or passes into some other tissue of the capsule. It is interesting that a similar conclusion can be drawn from our work on hemlock,[1] where the decrease in coniine content at certain periods far exceeds the corresponding increase in γ-coniceine.

EXPERIMENTAL

Sampling Techniques

For each series of experiments 20–30 flowers were selected immediately after petal fall and labelled 'Week 0'.[3] Two weeks later (week 2), when the laticifer density in the capsules is at a maximum,[3] samples were drawn at 1- or 2-hr intervals on a given day. Each capsule was gently pierced with a needle about four times (the position of the pricks was varied throughout the 20 or 30 capsules in the series) and the exuding latex was scraped into a special cup as soon as possible. (The cup was made of aluminium or stainless steel, about 2 cm dia., with one side dented inwards so as to fit the convex shape of the capsule. Before use it was transferred to a squat-shaped weighing bottle and the whole weighed.) After collecting the latex droplets the cup and weighing bottle were reweighed. Immediately afterwards about 5 ml of acid methanol (hydrochloric acid 1 ml, water 30 ml, methanol to 100 ml) was stirred into the latex and filtered through a tared filter paper into a 10-ml volumetric flask. The cup and filter were washed with more acid methanol and the filtrate made up to volume. The filter paper was dried and reweighed to give the dry weight of the methanol insoluble solids (which was mainly caoutchouc, whose presence at subsequent stages in the assay would be inconvenient). The filtrate was used for determination of alkaloids and methanol soluble solids. The results of alkaloidal assay could then be expressed in terms of fresh or dry weight of latex.

Determination of Morphine, Codeine and Thebaine

The methods of Pfeifer[8] and Milos[9] were used initially but gave variable results on replication and were tedious to use with large numbers of samples. Accordingly, a quantitative paper chromatographic method was devised[10] which gave the following limits of error ($P = 0{\cdot}95$) with replicate determinations; morphine and codeine ± 5 per cent; thebaine ± 7 per cent. For each determination a low and high 'dose' of standard solution of alkaloid and a low and high 'dose' of a suitable extract of the latex are applied to the paper. The chromatogram is run in *n*-butanol–acetic acid–water (5:1:2) at 18° (± 1) for 18 hr and the spots revealed by spraying with Dragendorff's reagent. The areas of the spots are measured planimetrically and the amount of alkaloid present calculated on the basis that area of spot is proportional to the logarithm of the quantity of alkaloid originally applied.

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[8] S. PFEIFER, *Bull. Narcotics* **10**, 18–33 (1958).
[9] C. MILOS, *J. Pharm. Sci.* **50**, 837–9 (1961).
[10] J. W. FAIRBAIRN and G. WASSEL, *J. Pharm. Pharmacol.* **15**, Suppl. (1963).