Coptisine chloride (NSC-119754) exhibited ED_{50} 8.2×10^{-1} mcg./ml. and Alkaloid CM-1 exhibited $ED_{50} < 1.5 \times 10^{0}$ mcg./ml. against Eagle's 9 KB carcinoma.[4]

SUMMARY

An investigation of *Chelidonium majus* L. (*Papaveraceae*) rhizomes and roots has shown that they are devoid of activity against the L-1210 leukemia and the Walker 256 (intramuscular) carcinosarcoma. However, an identical extract displayed significant cytotoxicity against Eagle's 9 KB carcinoma of the nasopharynx in cell culture.

The alkaloids of the rhizomes and roots of *C. majus* were examined and coptisine was isolated as the chloride, in addition to a second alkaloid designated as CM-1. These alkaloids were shown to be two of the cytotoxic principles of *Chelidonium majus*.

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[4] An $ED_{50} \leq 1.0$ mcg./ml. is considered as active for pure compounds (32).

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Daily Susceptibility Rhythm to Morphine Analgesia

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Abstract □ Mice exhibit a daily susceptibility to morphine-induced analgesia, that is, maximum sensitivity (crest) at 2100–2400 hr. in the dark phase and a minimum (trough) at 1500 (light phase). Shifts in the crest and/or trough of from 3 to 6 hr. were evident on three experimental dates; however, the crests and troughs always remained within their respective light phases. A discussion is presented on the interrelationships among the rhythms for catecholamine metabolism, motor activity, and susceptibility to stimulant and depressant drugs, particularly morphine.

Keyphrases □ Morphine analgesia—daily response rhythm □ Susceptibility patterns, mice—morphine analgesia □ Light, dark phases—morphine analgesia □ Lunar phases—morphine analgesia

Several drugs have been demonstrated to possess persistent daily or circadian (about 24-hr.) susceptibility patterns in rodents standardized in an alternating light-dark regimen (1–11). The resultant susceptibility pattern of these drugs when tested at frequent intervals is usually characterized by the temporal placement within a 24-hr. period of a major peak (crest) followed by a minimum (trough) approximately 12 hr. later. The times for the crest and trough responses to a specific drug can be reproduced *reliably* providing all studies are performed under identical conditions. Until a recent preliminary report on morphine from this laboratory (11), no response pattern had been shown for an analgesic agent. The present study was designed to

characterize the daily susceptibility pattern to morphine-induced analgesia in mice whose biological functions had been standardized by a rigidly controlled environment (*i.e.*, light, temperature, humidity, noise, *etc.*) and then to determine the reproducibility of the crest and trough response times within the dark and light phases, respectively.

EXPERIMENTAL

Materials—Adult female albino mice[1], 26.7 ± 0.2 g. body weight, were housed 10 per cage with a floor area of 6.8 sq. in. per mouse, thereby eliminating the psychological variable of aggregation due to overcrowded housing. The mice were housed in a controlled environmental room maintained at a temperature of 23.3 ± 1.0° with a relative humidity of 65 ± 2.0%. The room was programmed so as to provide 12 hr. of incandescent lighting (four 40-w. bulbs) from 0605 to 1805 hr. and a totally darkened phase from 1805 to 0605 hr. Testing was conducted in an adjacent, controlled environmental room having identical conditions, except that in order to make observations during the dark phase a very low level of illumination was maintained in the test room by means of a 20-w. photographic safelight. All mice were provided a minimum of 14 days acclimation time in the controlled environment prior to testing. New mice were used for each of the three replicate experiments. Water and Purina mouse chow were freely available during the acclimation period.

Methods—On the basis of the preliminary experiments (11), an 8 mg./kg. dose of morphine sulfate, administered intraperitoneally, was selected as that dose of morphine which, over a 24-hr. period, should most nearly approximate an AD_{50} (analgesic dose in 50% of the mice tested) and which should induce peak analgesia within 20 min. of injection. Each mouse was pretested for a positive pain response by a modified Haffner test (12), that is, biting a pinch clamp placed dorsoventrally on the base of the tail. Mice which, after three tests, still did not react to the noxious stimulus within 7 sec. were replaced with new mice: the pretesting continuing until such time as the predetermined sample size of positive pain respondents had been obtained.

Beginning at 1500 hr. and at 3-hr. intervals over the succeeding 24-hr. period on each of three dates (I = June 30, 1965; II = August 8, 1967; III = October 17, 1967) groups of 20 mice (18 in Experiment I) were pretested, weighed, injected, and placed into separate, wide-mouth, gallon glass jars. Exactly 20 min. after the morphine injection, each mouse was retested. All mice that failed to display a positive pain response within 30 sec. were considered to be exhibiting analgesia (12). Responses were transformed into percent analgesia, relative to the total number of mice injected at that time period, and a mean 24-hr. morphine analgesia percent response was determined. The methods of Snedecor were used to perform all statistical analyses (13).

RESULTS AND DISCUSSION

Susceptibility Rhythm—An analysis of variance of the percentage data in Table I revealed that as previously reported (11) mice are more susceptible to the analgesic activity of morphine during the dark phase (65.5%) than during the light phase (48.4%) of a 24-hr. period ($p < 0.01$). In order to characterize more precisely the daily rhythm, chi-square analyses were performed on the pooled raw data for each of the eight test periods per day relative to the mean 24-hr. morphine response for pooled raw data (57.0%). A crest was found in the dark phase at 2100 and 2400 hr. ($p = 0.01$) and a trough in the light phase at 1500 hr. ($p = 0.02$). Similar analyses made on the data from each of the three separate experiments showed that there is a significant crest and trough for each of the three replicate experiments. Shifts in the crest and/or trough of from 3 to 6 hr. were evident on the three experimental dates (Table I); however, the crests and troughs always remained within their respective light phases.

General Stimulants—Drugs possessing central stimulant activity exhibit peak susceptibility in rodents from 2000 to 2400 hr. (dark phase) with a trough seen 12 to 15 hr. later in the light phase (Table II). Since rodents are nocturnal mammals in that peak motor and neural activity occur during the dark phase with the light phase being a quiet period (14), it could follow that increased susceptibility to central stimulants during the dark phase correlates with increased neural activity and increased concentrations of catecholamines. Norepinephrine content of the rat pineal gland varies threefold during each 24-hr. day, reaching the highest levels at the end of the dark period and falling during the light period (15). Significant daily rhythms were observed in the anterior and posterior hypothalamus of rats in that the norepinephrine contents of both regions were maximal at the middle of the daily dark period whereas catecholamine concentrations were lowest throughout the light period in the anterior hypothalamus and at the end of the dark period in the posterior hypothalamus (16). Tyrosine hydroxylase activity in the rat pineal gland varies over a 24-hr. period in the same manner as norepinephrine content (17). Increased tyrosine hydroxylase activity could therefore represent the mechanism by which norepinephrine concentrations are increased, since the enzyme is thought to control the rate of norepinephrine synthesis (18). A similar mechanism has been suggested as the basis for the inverse rhythm in pineal gland serotonin content (19). Serotonin content in the pineal gland is highest in the middle of the daylight period and falls with the onset

Table I—Morphine-Induced Analgesia, in Percent, in Mice

Clock Hour[a]	Expt. I 6-30-65 New Moon	Expt. II 8-8-67 New Moon	Expt. III 10-17-67 Full Moon	Pooled Data
		Light Phase		
900	56	45	40 T[b]	47.0
1200	39	48	65	50.7
1500	28 T	30 T	70	42.7 T
1800	50	50	60	53.3
Mean phase	43.25	43.25	58.75	48.43
		Dark Phase		
2100	67 C[c]	60	75	67.3 C
2400	44	85 C	70	66.3 C
300	50	50	89 C	63.0
600	61	65	70	65.3
Mean phase	55.50	65.00	76.00	65.48
Mean 24-hr. response	49.4 ± 4.4	54.1 ± 5.7	67.4 ± 4.9	57.0 ± 3.1

[a] Lighting schedule: lights on from 0605 to 1805; lights off from 1805 to 0605. [b] T = trough statistically significant from corresponding 24-hr. mean ($p < 0.02$). [c] C = crest statistically significant from corresponding 24-hr. mean ($p < 0.02$).

Table II—Drug Susceptibility in Mice in a 24-hr. Period

	Crest	Trough	Reference
Dark cycle[a]			
Morphine analgesia	2100	1500	11
Lidocaine seizures	2100	1500	9
Flurothyl seizures (2000–0800)	2200	1000	6
Acetylcholine lethal seizures	2000	0800	7
Ethanol lethal depression	2000	0800	3
Chlordiazepoxide lethal depression	2400	1200	4
Nialamide lethal seizures	dark	light	7
Pentobarbital lethal depression[b]	2200	1600	22
Tremorine lethal seizures[b]	2200–0400	1500	22
Tremorine tremors[b]	2200	1400	22
Light cycle[a]			
Ouabain cardiac arrest	1000	2400	2
Pentobarbital sleep (0800–2000)	1400	0200	5
Nikethamide lethal seizures (sunrise–sunset)	1400	0200	1
Methopyrapone lethal depression	1600	0800	10
Aurothioglucose lethal depression (0700–1900)	light	dark	8

[a] Dark cycle = 1800-0600 (unless otherwise indicated); light cycle = 0600-1800 (unless otherwise indicated). [b] Rats.

[1] Carworth Farms, CF-1.

of darkness (20), perhaps, because hydroxy-indole-*O*-methyl transferase, the pineal enzyme which converts *N*-acetylserotonin to melatonin, is lowest at the end of the light period and rises with darkness (21). It is also possible that the nocturnal increase in pineal norepinephrine levels results from enhanced generalized sympathetic nervous activity perhaps, in response to darkness rather than to specific effects on the pineal gland (15).

Depressants—In contrast to the stimulants, drugs whose terminal effect is to cause central or peripheral depression had crests in the light period and troughs in the dark period (2, 5, 10, 22) except for methapyropone which exhibited a trough at 0800 (8). Ethanol, which precipitates overt stimulant behavior brought on by initial depression of the reticular formation, has a pattern similar to the central stimulants (23).

One mechanism presented for the established sedative activity of reserpine is reserpine-induced central depletion of catecholamines, particularly norepinephrine and dopamine, thereby reducing sympathetic nerve impulses in the reticular activating centers (24). Reserpine interferes with the storage of serotonin and antagonizes morphine analgesia (25, 26). Morphine and related narcotic analgesics lose much of their pain-killing effect in animals treated with *p*-chlorophenylalanine, an established inhibitor of the synthesis of serotonin (24) and α-methyl-tyrosine (27). Intact serotonin stores in the thalamic-hypothalamic area of the brain may, in contradistinction to the pineal gland, therefore, be essential to morphine analgesic activity (24). Morphine, a descending cortical depressant with ascending spinal cord stimulation, displays a susceptibility pattern in mice (Table I) parallel to the pre-existing degree of gross motor and electrocerebral activity, that is, the greater the motor and neural activity the greater the degree of morphine-induced analgesia. Consequently morphine analgesia is at a peak when motor and neural activity as well as catecholamine levels are maximal. Studies are in progress to clarify the relationship between catecholamine-serotonin levels and morphine susceptibility in mice.

Lunar Phases—The susceptibility to morphine-induced analgesia was found to be statistically different on the three test dates ($p < 0.05$). Comparing the data on the basis of lunar phase (*i.e.*, new moon on June 30, 1965 and August 8, 1967; full moon on October 17, 1967) showed that there is a significant difference between the mean 24-hr. morphine responses during new (51.8%) and full (67.4%) moon phases ($p = 0.01$). Such factors as seasonal and annual changes as well as genetic variations among the mice may have a bearing on the shifts in the crests and troughs for each study. However lunar effects have been well established in the plant and animal kingdom (28), although not associated with mammalian drug susceptibility rhythms.

Human Implications—In the application of this study to man, whose social structure is the dominant synchronizer, diurnal man should be expected to display maximal susceptibility to morphine during the most active daylight hours, providing pain thresholds remain constant. Preliminary data from this laboratory indicate however, that the pain threshold in mice exhibits a 24-hr. rhythm: low pain threshold during the light period (quiet phase) and high pain threshold during the dark or activity phase (29). Some appreciation can now be obtained of the variables attendent with the unpredictable degrees of clinical analgesia resulting from the parenteral administration of the standard 10-mg. dose of morphine sulfate to chronically ill, pain-wrought patients. A clearer understanding of the pain cycle and morphine susceptibility rhythm in man should facilitate more adequate therapy in such patients.

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