of the unsubstituted and α-hydroxy acids. Divalonic acid (a six-carbon, growth-promoting β-hydroxy acid), for example, exhibits a *p*H ½ value of 4.3, according to Wolf *et al.* [*J. Am. Chem. Soc.* 78, 4499 (1956)].
7. M. N. Camien and M. S. Dunn, *J. Biol. Chem.* **211**, 593 (1954).
8. Neither the isolated barium salt (2 percent in water) nor the free acid (2 percent in either water or chloroform) yielded a significant optical rotation with sodium light.
9. C. G. Baker and A. Meister, *J. Am. Chem. Soc.* 73, 1336 (1951).
10. A paper describing the details of their characteristics is in preparation.

11 April 1958

Daily Rhythms in Male Harvester and Argentine Ants

The nuptial flights of various species of ants occur at characteristic times—for example, in a species of *Myrmica,* early morning; of *Lasius,* late afternoon (*1*); of *Eciton,* night (*2*). To gain a better understanding of such field observations, male and female harvester ants [*Veromessor andrei* (Mayr)] and male Argentine ants [*Iridomyrmex humilis* (Mayr)] (*3*) were studied in a constant-temperature room in alternating light and dark (*4*). Under these conditions, as is shown below, the males of both species exhibited a sharp daily activity peak, but at opposite ends of the light period.

The *Veromessor* females were obtained at a nest entrance on 26 July and kept until September with workers under room conditions (about 19° to 26°C). The males were collected at the entrance of the same nest on 13 September and kept by themselves.

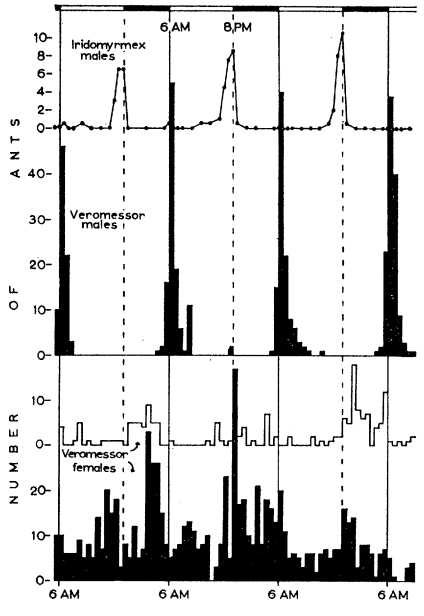

Fig. 1. Comparative activity patterns under alternating light and dark. *Iridomyrmex*: number out at time observed (mean of two counts 1 minute apart); *Veromessor*: half-time tunnel count per hour.

To obtain automatic activity samples, a circular "race track" was used. Two lots of ten males and two lots of ten females were placed in tiny chambers of clear plastic. Cotton-plugged test tubes of water and of sugar water projected down through the roof of each chamber; no other food or moisture was supplied. A transparent tube circled from one exit from the chamber back to the other, narrowing midway between to form the lining of a polystyrene tunnel. An ant passing through the tunnel, and thus between "sensitive" and "ground" screws, triggered a capacity-operated relay. In another room the total number of passes was recorded on a digital counter which was photographed each hour.

Since there were only two such relays available, a motor-driven cam device was used with each to alternate the contacts between two tunnels, so that a group of males were counted for half a minute, then females, and so forth. Thus each machine made a half-time count for each sex (*5*).

To simulate the darkness of the normal nest, the entire chamber was covered by an orange Plexiglas box (*6*). The circular "track" was left fully exposed to the light.

The counts of *Iridomyrmex* males, on the other hand, were made on a complete colony, consisting of nest queens, numerous workers and young, and about 14 males. The nest had been under laboratory conditions for over a year. Two adjacent plaster units opened onto a common board, which had legs set in DDT (*7*). The microscope-slide roof of each unit was overlapped with orange Plexiglas (*6*). Water and sugar water were continuously available in tubes suspended from a post set on the board (*7*). Solid food was provided, and the plaster was moistened, at irregular (though recorded) times; this had no observable effect on the rhythm. The males came out and wandered over the units or near them. Their wings made them easy to spot, and the total out at any given time was counted by eye.

Both species were kept on the same table in a darkroom at 25.4° ± 0.4°C. A 40-watt fluorescent light, automatically turned on at 6 A.M. and off at 8 P.M., provided an intensity of about 50 ft-ca. Two 40-watt clear ruby darkroom lights (two feet from the ants) were on continuously. Mechanical disturbance was minimized by sponge-rubber pads.

Figure 1 shows the simultaneously recorded activity patterns from 30 September to 3 October 1957, after the ants had been under the experimental conditions for several days. The replicate pattern of *Veromessor* males was similar to the one shown. Both female counts are given because of their greater variability. In fact, the activity patterns of the females are of interest, under these particular conditions, principally because of their lack of well-marked peaks, by contrast with the males.

The activity peak of *Veromessor* males occurred the first hour of each light period and was preceded consistently by a rise the last hour of the dark, with relative quiet the rest of the time (*8*). The *Iridomyrmex* males, on the other hand, were out of the nest during the last 2 hours of the light period only. The increases in activity *before* the changes in lighting suggest endogenous control of the rhythms. Further evidences for such an internal "clock" (*9*) in both species studied here were (*10*): (i) persistence of rhythm in constant darkness (that is, red light), though the *Veromessor* peak averaged 0.5 to 1 hour later each day, and (ii) a shift of phase following a single 5 P.M.–5 A.M. light period.

Veromessor mating flights occur locally early in the morning (*10*), thus suggesting the significance of the laboratory activity peaks. A species-characteristic light-phase relationship would seem valuable in synchronizing the nuptial flights of colonies of the same species. However, simple environmental response to the dawn might be insufficient, unless the ants were near enough to the surface at the right time. Endogenous control could bring them to the entry in time for the dawn. Their clocks could be "set" when the winged ants emerge on nonflight days, as do *Veromessor* (and other ants—for example, see *1, 11*). Such inherent control was postulated for worker leaf-cutting ants which were in the nest entry 1 hour before dawn, though even artificial light would not bring them up earlier (*12*). Similarly, bees trained to forage at a certain time may remain in a remote part of the hive until shortly before that time (*13*).

The value of the clocklike emergence of *Iridomyrmex* males is more difficult to explain, since mating takes place within the nest (*7, 14*). If a general male-and-female flight ever occurs, the results reported here would suggest the end of the day as the time. Perhaps the exit rhythm is a vestige of such a flight. The males sometimes fly, and they are found at artificial lights (*7, 14*). If the males normally fly before mating, a clock with the phase relationship noted could bring them out when late afternoon and night remain for encountering another nest or column of workers, before it again becomes too hot or dry for survival. In certain army ant species, where the virgin queens are wingless, some of the males, after their night flights, find their way into other colonies (*2*).

ELWOOD S. MCCLUSKEY*
Department of Biological Sciences,
Stanford University,
Stanford, California

References and Notes

1. M. Talbot, *Am. Midland Naturalist* 34, 504 (1945).
2. T. C. Schneirla, *Zoologica* 33, 89 (1948).
3. Determined by M. R. Smith, U.S. Department of Agriculture.
4. I am indebted to A. C. Giese, under whose direction this work was done, and to C. S. Pittendrigh for helpful discussions.
5. The contact was damped each minute by flexible contact arms. The male rhythm was checked later after some days in constant darkness (that is, red light) by turning off the alternators, and it was found to persist as before.
6. The Plexiglas permitted good visibility, but, to judge from the color choices made by *Iridomyrmex* workers with queens and brood, it was nearly as "dark" as a black cover.
7. S. H. Skaife, *Trans. Roy. Soc. S. Africa* 34, 355 (1955).
8. This was true also for earlier eye counts made under conditions somewhat similar to those of the *Iridomyrmex* counts.
9. C. S. Pittendrigh and V. G. Bruce, in *Rhythmic and Synthetic Processes in Growth* (Princeton Univ. Press, Princeton, N.J., 1957), pp. 75–109.
10. E. S. McCluskey, unpublished.
11. W. M. Wheeler, *Ants* (Columbia Univ. Press, New York, 1910), p. 288.
12. E. S. Hodgson, *Ecology* 36, 293 (1955).
13. K. von Frisch, *The Dancing Bees* (Harcourt Brace, New York, 1955), p. 146.
14. W. Newell and T. C. Barber, *U.S. Dept. Agr. Bur. Entomol. Bull. No. 122* (1913).

* On leave from the College of Medical Evangelists, Loma Linda, Calif.

31 March 1958

New Metabolites of Serotonin in Carcinoid Urine

5-Hydroxyindoleacetic acid, the only metabolite of serotonin so far identified, represents less than 20 percent of an exogenous dose of serotonin (*1*). The reported presence of other unidentified indole derivatives in carcinoid urines (*2*) and rat liver perfusates (*3*), however, is indicative that metabolic reactions other than deamination occur. Suggestive evidence has also been presented that free serotonin is present in normal urine (*4*). Now it has been found that carcinoid urine is much more oxytocic than normal urine (personal observation) and it was thought that, if in this syndrome there was an increase in the excretion of the known metabolite, there would also be an increase in the unknown metabolites.

By an adaptation of the method described by Bumpus and Page (*4*), the indoles were extracted from 3 gal of carcinoid urine from one patient which contained 350 mg of 5-hydroxyindoleacetic acid as assayed by the method of Udenfriend, Titus, and Weissbach (*5*). Removal of excess urea and a partial fractionation of indoles was accomplished by using a cellulose column and a single phase solvent of *n*-propanol/ammonia.

Paper chromatography of the concentrated extracts and fractions revealed the presence of six indole derivatives. Five of these were identified by means of paper chromatography in three solvents, oxytocic activity, and fluorescent spectra (see Table 1). One of these proved to be the normally occurring urinary indican, but the other four—5-hydroxyindoleacetic acid, 5-hydroxyindoleaceturic acid, 5-hydroxytryptamine and N-acetyl 5-hydroxytryptamine—were evidently metabolites by serotonin. The 5-hydroxyindoleaceturic acid was further characterized by enzymic hydrolysis of an eluate with chymotrypsin, to yield 5-hydroxyindoleacetic acid and glycine.

Table 1. R_F values, oxytocic activity, and fluorescent spectra of metabolites of serotonin and the normally occurring urinary indican.

Metabolite	R_F in solvent*			Oxytocic† activity	Fluorescent spectra (mμ)‡		
	A	B	C		Activation (max.)	Fluorescent (max.)	*p*H
Serotonin creatinine sulphate	0.48	0.64	0.86	+++	295	540	2
5-Hydroxyindoleacetic acid	0.15	0.80	0.03		300	355	7
5-Hydroxyindoleaceturic acid	0.23	0.84					
N-acetyl serotonin	0.75	0.81	0.86	±	310	370	7
Indican	0.40	0.43	0.56		300	400	7

* Blue spots were obtained when sprayed with *p*-dimethylaminobenzaldehyde in 1.5*N* HCl. Solvent systems used: A, propan-1-ol saturated with ammonia; B, *n* butanol–acetic acid–water (4:1:5); C, ethyl methyl ketone–2*N* ammonia (2:1).
† Oxytocic activity was determined on an eoestrus rat uterus. Activity was antagonized by brom-lysergic acid diethylamide.
‡ Fluorescent spectra were determined with an Aminco Bowman spectrophotofluorometer.

The metabolism of endogenous serotonin in carcinoid patients therefore appears to be very similar to that of exogenous serotonin in experimental animals which we have studied (*6*). Autoradiographs obtained from urinary extracts of rats and rabbits given radioactive serotonin have shown the presence of the same four metabolites with the addition of two other minor metabolites. One of these has been identified as the glucuronide of serotonin since it gave a positive indole test but did not give a blue color with 2:6 dichloroquinonechloroimide, indicating that the hydroxyl group was not free. An eluate of this compound gave a positive naphthoresorcinol reaction, confirming that it was an ether glucuronide. Quantitative estimations of glucuronic acid and ethereal sulfate excretion after administration of serotonin have also shown that some conjugation does take place.

Oxidation of serotonin in vivo is a theoretical possibility, and it was thought that the other minor metabolite might represent the product of such a reaction, though so far no definite experimental confirmation has been obtained since it is present in such small quantities.

No evidence has been found in these experiments to suggest that methylation of serotonin might occur.

The normal metabolic fate of serotonin therefore appears to be (i) deamination to 5-hydroxyindoleacetic acid with (ii) some subsequent glycine conjugation to form the aceturic acid, (iii) N-acetylation, (iv) conjugation with glucuronic acid, (v) excretion unchanged and (vi) possible oxidation.

Preliminary studies have shown that, although great amounts of serotonin are metabolized by carcinoid patients, there appears to be no qualitative difference from the normal mode of metabolism.

WILLIAM M. MCISAAC
IRVINE H. PAGE
Research Division, Cleveland Clinic Foundation, and *Frank E. Bunts, Educational Institute, Cleveland, Ohio*

References

1. V. Erspamer, *J. Physiol. (London)* 127, 118 (1955); S. Udenfriend, E. Titus, H. Weissbach, R. E. Peterson, *J. Biol. Chem.* 219, 335 (1956).
2. J. B. Jepson, *Lancet* 1955, 2, 1009 (1955).
3. C. E. Dalgliesh and R. W. Dutton, *Biochem. J.* 65, 21p (1957).
4. F. M. Bumpus and I. H. Page, *J. Biol. Chem.* 212, 111 (1955).
5. S. Udenfriend, E. Titus, H. Weissbach, *ibid.* 216, 499(1955).
6. W. M. McIsaac and I. H. Page, *ibid.*, in press.

14 April 1958

Spontaneous Changes in Corn Endosperm Tissue Cultures

Spontaneous changes in the characteristics of plant tissue cultures are known to occur from time to time. The best documented change is that which occurs in connection with the isolation of habituated tissues (*1*). Habituated tissues are independent of exogenous auxin, in contrast to the normal tissues from which they are derived. The latter tissues require external supplies of auxin for growth in vitro. Another change which has been observed to arise is the appearance of a purely parenchymatous tissue from woody tissue cultures (*2*). Reinert (*3*) and Torrey (*4*) have described irreversible changes from compact calli to cultures of very loose masses of cells from *Picea* and pea root callus, respectively. The latter changes, however, were as-