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 E. S. McCluskey, unpublished.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).

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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.

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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.

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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-