as photosensitizers if longer exposure conditions were employed. This is so because rotenone itself is highly unstable to sunlight, its half-life on foliage under the conditions of study being no longer than 30 minutes (*1*). It is known that the majority of rotenone photodecomposition products are less active than rotenone or are inactive as photosensitizers (*1*); thus the effective sensitizing period resulting from rotenone applications is expected to be a short one.

The fact that rotenone is a natural plant product suggests that other naturally occurring sensitizers might be used for pesticide chemical photodecomposition. Except for earlier reports that pyrethrins are more susceptible to photodecomposition when plant pigments are present in crude pyrethrum extracts (*11*), there are apparently no indications that such sensitizers exist. However, their existence is confirmed by the results of our preliminary studies which show that aqueous suspensions of spinach chloroplasts are potent sensitizers for the decomposition of certain ^{14}C-labeled carbamate and pyrethroid insecticides in sunlight but not in darkness; this is probably the result of the chlorophyll content of these chloroplasts. Whether or not chloroplasts sensitize the photodecomposition of other pesticide compounds is, as yet, unknown. Although an interesting speculation, it may be difficult to assess the possible *in situ* significance of chloroplast pigments in the alteration of pesticide residues in plants.

The present studies are not definitely applicable to the field use of pesticides, although they show that very low concentrations of a photosensitizing compound can greatly alter the persistence pattern of a pesticide chemical. Further, they show that rotenone, which is currently being used for insect control on food crops and which degrades quickly by photodecomposition to nontoxic products, is quite effective as a photosensitizer for certain chlorinated cyclodienes. It is likely that other interesting pesticide-photosensitizer mixtures exist in addition to the combinations reported here. This means that one pesticide chemical can greatly affect the residual persistence of a second pesticide chemical on a crop, not only by altering its rate of biotransformation (*12*) but also by sensitizing its photoalteration. It is important to determine such interactions because pest control frequently involves the application of more than one type of pesticide chemical to the plants. Perhaps the most intriguing possibility is that of "managing" residues or pesticide persistence by applying an appropriate photosensitizer to cause, at will, an increased rate of residue alteration, including dissipation, when this is desirable from the standpoint of efficient pest control procedure, good agricultural practice, or minimum environmental contamination.

GLEN WAYNE IVIE
JOHN E. CASIDA
Division of Entomology, University of California, Berkeley 94720

References and Notes

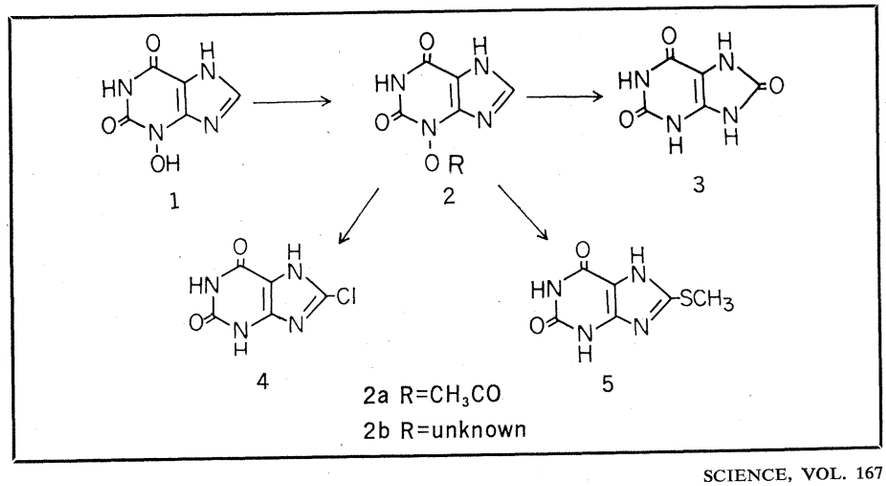
1. G. W. Ivie and J. E. Casida, in preparation.
2. L. C. Mitchell, *J. Ass. Offic. Agr. Chem.* **40**, 999 (1957); M. F. Kovacs, Jr., *ibid.* **46**, 884 (1963); J. J. Faucheux, Jr., *ibid.* **48**, 955 (1965).
3. Chemicals from the Photosensitizer and Quencher Kit, Commodity No. Z901, J. T. Baker Chemical Company, Phillipsburg, N.J.
4. J. D. Rosen and W. F. Carey, *J. Agr. Food Chem.* **16**, 536 (1968).
5. Compounds were provided by: L. Crombie, University College of South Wales and Monmouthshire, Cardiff, United Kingdom; M. Matsui, University of Tokyo, Japan; and M. Nakajima, University of Kyoto, Japan.
6. L. Crombie, *Fortschr. Chem. Org. Naturst.* **21**, 275 (1963).
7. J. D. Rosen, D. J. Sutherland, G. R. Lipton, *Bull. Environ. Contamination Toxicol.* **1**, 133 (1966).
8. J. D. Rosen and D. J. Sutherland, *ibid.* **2**, 1 (1967).
9. ———, M. A. Q. Khan, *J. Agr. Food Chem.* **17**, 404 (1969).
10. D. D. Phillips, G. E. Pollard, S. B. Soloway, *ibid.* **10**, 217 (1962).
11. N. C. Brown and R. F. Phipers, *Pyrethrum Post* **3** (4), 23 (1955); G. D. Glynne Jones, *Ann. Appl. Biol.* **48**, 352 (1960).
12. J. E. Casida and L. Lykken, *Annu. Rev. Plant Physiol.* **20**, 607 (1969).
13. Aided by PHS grants ES 00049 and GM 12248 and AEC contract No. AT(11-1)-34, project agreement No. 113. For advice and assistance we thank L. Lykken and J. L. Engel of this laboratory; R. W. Risebrough, Department of Nutritional Sciences, and B. B. Buchanan, Department of Cell Physiology, University of California, Berkeley. We especially thank J. D. Rosen, Department of Agricultural Chemistry, Rutgers University, and S. B. Soloway, Biological Sciences Research Center, Shell Development Company, Modesto, California, for supplying authentic standards of the photoisomers used.

3 November 1969

Oncogenic Purine Derivatives: Evidence for a Possible Proximate Oncogen

Abstract. *Two additional urinary metabolites of the chemical oncogen 3-hydroxyxanthine are now identified as 8-chloroxanthine and 8-methylmercaptoxanthine. Such products are thought to be derived from a reactive intermediate which can be tentatively considered to be a proximate oncogen. Since each of these 8-substituted xanthines has also been obtained in vitro by reactions of 3-acetoxyxanthine with chloride ion or methionine, their production in vivo can be explained as resulting through the metabolic formation of an activated ester with a reactivity similar to that of the chemical model.*

Guanine 3-*N*-oxide and 3-hydroxyxanthine are chemical oncogens (*1*) with potencies comparable to those of the most oncogenic arylamines and many oncogenic hydrocarbons (*2*). We have now identified two additional urinary metabolites of 3-[8-^{14}C]hydroxyxanthine as 8-chloroxanthine and 8-methylmercaptoxanthine. Each has been separated and characterized by ion-exchange and subsequent paper chromatography (discussed below). Samples of each so purified were reduced to xanthine with no loss of specific activity. The identity of 8-chloroxanthine was confirmed by the isolation of 8-[^{36}Cl]chloroxanthine when Na^{36}Cl was administered with unlabeled 3-hydroxyxanthine. The results in Table



1 include measurements of the previously identified urinary metabolites of 3-hydroxyxanthine: allantoin, 3-hydroxyuric acid, uric acid, and xanthine, which result from the reducing or oxidizing actions of xanthine oxidase (*3*, *4*), and which, with unaltered 3-hydroxyxanthine, account for more than 90 percent of the radioactivity in the urine. An analogous series of metabolites formed by xanthine oxidase has been shown to result from guanine 3-*N*-oxide (*5*).

The guanine 3-*N*-oxide and 3-hydroxyxanthine are stable compounds under many conditions, but it has been found (*6*, *7*) that they can undergo a remarkable reaction involving an apparent rearrangement of the oxygen from the 3-nitrogen to the 8-carbon. For example, 3-hydroxyxanthine (**1**) is converted to uric acid (**3**) upon acetylation, and similarly guanine 3-*N*-oxide is converted to 8-hydroxyguanine. The intermediate, 3-acetoxyxanthine (**2a**), has been prepared (*8*) and shown to react readily with nucleophilic agents. In water it yields uric acid (**3**); in dilute NaCl it also yields 8-chloroxanthine (**4**); with methionine it yields 8-methylmercaptoxanthine (**5**), all at room temperature and *p*H 5 to 6 (*8*).

The metabolic formation of the 8-substituted chloro- and methylmercapto- derivatives, **4** and **5**, is logically explained if it is postulated that **1** is converted in vivo to an ester, **2b**, with reactivities similar to those of **2a**. 3-Hydroxyxanthine would thus be an additional example of an oncogen that is converted in vivo to a metabolite having a chemical reactivity that could permit it to react with cell components to initiate the process of chemical oncogenesis (*9*). This behavior of 3-hydroxyxanthine in vivo and the reactions of 3-acetoxyxanthine in vitro are reminiscent of the findings of Miller and coworkers on the metabolism of the oncogenic arylhydroxylamines, the reactivity in vitro of their *O*-acetyl derivatives, especially of *N*-acetoxyacetamidofluorene (*9*, *10*), and the possible formation in vivo of sulfate (*11*) or phosphate esters (*12*). The postulated reactive intermediate (**2b**) which is formed in vivo from 3-hydroxyxanthine, and for which 3-acetoxyxanthine (**2a**) is a chemical model, may well have a similar origin. No reaction of esters of *N*-hydroxyacetamidofluorene with such weak inorganic nucleophiles as chloride has been reported, and it is probable that the active metabolites from the two types of compounds will show different reactivities with different nucleophiles.

Table 1. Metabolites of 3-hydroxyxanthine as percentage of total ^{14}C excreted* by five rats: four male and one (rat E) female; four of the Wistar strain (Carworth) and one (rat D) of Sprague-Dawley strain (Charles River Breeding Laboratories). Rat A (350 g) received 0.4 μmole, 11 mc per mmole for each experiment; B (220 g), 1 μmole, 11 mc per mmole; C (190 g), 1 μmole, 25 mc per mmole; D (260 g), 0.7 μmole, 25 mc per mmole for each experiment; E (160 g), 0.4 μmole, 25 mc per mmole.

Metabolites	Percentage ^{14}C excreted by rats				
	A	B	C	D	E
Allantoin fraction†	80.				
Uric acid	0.33				
3-Hydroxyuric acid	0.13				
8-Chloroxanthine	0.88			0.35‡	0.24
3-Hydroxyanthine	13.0 13.7§	8.8	24.5		21.5
Xanthine‖					5.5
8-Methylmercaptoxanthine	1.0 2.0§	1.0	1.0	1.4 2.1‡	1.0
Unidentified metabolite accompanying 8-methylmercaptoxanthine	0.4 0.9§	0.4		0.55 0.86‡	0.37

* Urines were collected for 8 hours, at which time more than 75 percent of the radioactivity is excreted (*4*, *5*). † As described (*4*). With the smaller dose administered here the excretion of allantoin is greater, and that of uric acid, 3-hydroxyuric acid, xanthine, or **1** is less than the amount that resulted with a much larger dose (*4*). ‡ Prior treatment with ~30 mg of **1**, 0.1 mg/ml in water, in 1 week, followed by a week on regular diet and then the second experiment. § Prior treatment with ~100 mg of **1**, 2.2 mg per gram of chow, 12 g per day for 1 week, followed by a week on regular diet and then the second experiment. Four weeks later this rat was also used for the $Na^{36}Cl$ experiment. ‖ This fraction contains two additional labeled products.

Because male rats are more susceptible to both these types of oncogens (*11*, *13*), both sexes, as well as the two strains that have been used in our oncogenicity assays, were included in this study. The female (rat E, Table 1) excreted slightly more 8-methylmercaptoxanthine than the four males of either strain. The experiments in which the rats were given a preliminary feeding of 3-hydroxyxanthine (rats A and D) resulted in some increase in excretion of the two new metabolites and of the unidentified metabolite accompanying 8-methylmercaptoxanthine. Since it is quite possible that 8-methylmercaptoxanthine is itself subject to further metabolism, the significance of these differences cannot yet be interpreted.

Upon column chromatography (Table 2) the radioactivity of 8-chloroxanthine is a poorly resolved peak on the trail of the radioactivity eluted with allantoin and 3-hydroxyuric acid, but the 8-chloroxanthine can be well separated from these metabolites by paper chromatography (Table 2). The radioactivity of 8-methylmercaptoxanthine coincides symmetrically with the added marker in the eluate from column chromatography. Upon subsequent paper chromatography in NH_4Cl, however, it is consistently found to be accompanied by 28 to 30 percent of an unknown metabolite (Table 2). That is not an artifact arising from 8-methylmercaptoxanthine, which is stable under the conditions of handling. Confirmation of the identity of 8-methylmercaptoxanthine was obtained by reducing about 3 μmole of a purified sample of this metabolite and its carrier to xanthine by the use

Table 2. Separation of urinary metabolites by ion-exchange and paper chromatography. For column chromatography, a Dowex 50 (H^+) 200- to 400-mesh column (50 cm by 1.29 cm^2) was eluted with 50 ml of H_2O, 60 ml of 1.1*N* HCl and 2.2*N* HCl. Elution volumes in milliliters indicate volumes of eluate which contain about 95 percent of the compound. For separation of 8-[^{36}Cl]chloroxanthine a column 75 cm by 1.29 cm^2 was used. For ascending paper chromatography, Schleicher and Schuell paper 597 was used and the substances were applied in 6*N* NH_4OH.

Metabolite	Chromatograms		
	Column	Paper (R_F values)	
		Glacial CH_3COOH	3% NH_4Cl
None (NaCl alone)	11–16		0.77*
Allantoin	15–35	0.65	0.82
3-Hydroxyuric acid	15–28	0.42	0.55
Uric acid	20–33		0.42
8-Chloroxanthine	35–45	0.79	0.24 0.32†
3-Hydroxyxanthine	135–155	0.50	0.62
Xanthine	180–200	0.67	0.47
8-Methylmercaptoxanthine	215–255	0.85	0.31
Unknown compound	215–255	0.80*	0.80*

* Located by its radioactivity. Strips were scanned (*7*) or cut for measurement by scintillation. † Substance applied in HCl. Such a difference is not found with the other compounds.

of Raney nickel, followed by H_2S treatment of the nickel to release the small amount of xanthine. 8-Methylmercaptoxanthine of 34,900 count min^{-1} $\mu mole^{-1}$ (*14*) was thus reduced to xanthine, which, after chromatography on 10 ml of Dowex 50, had a specific activity of 35,500 count min^{-1} $\mu mole^{-1}$ (*15*). Similarly, 8-chloroxanthine of 99,000 count min^{-1} $\mu mole^{-1}$ (*16*) could be catalytically hydrogenated over Pd-C to yield xanthine of 79,000 count min^{-1} $\mu mole^{-1}$. Additional evidence for the identity of the 8-chloroxanthine was obtained in an experiment with $^{36}Cl^-$ (*17*). An injection of 2.3 mmole of $Na^{36}Cl$ and 20.3 mmole of 3-hydroxyxanthine was administered to rat A. By 6.5 hours the urine contained 5.7 percent of the $^{36}Cl^-$ at a dilution of only 1 : 5.5, which corresponds with the fact that most of the radioactive chloride was still extracellular during that period (*18*). From a longer column (see Table 2), the $H^{36}Cl$ was eluted as an initial sharp peak, the trail of which obscured the 8-chloroxanthine radioactivity peak. When 660 count min^{-1} of radioactivity from the 8-chloroxanthine peak was rechromatographed on paper with NH_4Cl, 40 percent clearly accompanied the carrier 8-chloroxanthine and somewhat more accompanied the sodium chloride (Table 2). The low activity of the 8-chloroxanthine suggests that it was formed from intracellular chloride, as would be expected.

GERHARD STÖHRER
GEORGE BOSWORTH BROWN
Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, New York 10021

References and Notes

1. The more common "carcinogenic" should be restricted to agents that induce carcinomas. Oncogenic is an inclusive term for agents that induce any form of neoplasia. H. Martin, *Arch. Surg.* **7**, 534 (1963); P. Rous, *Cancer Res.* **27**, 1919 (1967).
2. K. Sugiura, M. N. Teller, J. C. Parham, G. B. Brown, *Cancer Res.* **30**, 184 (1970).
3. G. Stöhrer and G. B. Brown, *J. Biol. Chem.* **244**, 2498 (1969).
4. A. Myles and G. B. Brown, *ibid.*, p. 4072.
5. G. Stöhrer and G. B. Brown, *ibid.*, p. 2494.
6. U. Wölcke and G. B. Brown, *J. Org. Chem.* **34**, 978 (1969).
7. U. Wölcke, W. Pfleiderer, T. J. Delia, G. B. Brown, *ibid.*, p. 981.
8. U. Wölcke, N. J. M. Birdsall, G. B. Brown, *Tetrahedron Lett.* **10**, 785 (1969).
9. J. A. Miller and E. C. Miller, *Progr. Exp. Tumor Res.* **11**, 273 (1969).
10. P. D. Lotlikar, J. D. Scribner, J. A. Miller, E. C. Miller, *Life Sci.* **5**, 1263 (1966).
11. J. R. DeBaun, J. Y. Rowley, E. C. Miller, J. A. Miller, *Proc. Soc. Exp. Biol. Med.* **129**, 268 (1969).
12. C. M. King and B. Phillips, *Science* **159**, 1351 (1968).
13. M. N. Teller, G. Stohr, H. Dienst, *Cancer Res.* **30**, 179 (1970).
14. ε (283 nm; 2*N* HCl) = 15.8×10^3; determined on reference samples.
15. ε (260 nm; 1*N* HCl) = 9.2×10^3; determined on reference samples.
16. ε (278 nm; 0.1*N* HCl) = 11.9×10^3; R. K. Robins, *J. Org. Chem.* **26**, 447 (1961); J. B. Lloyd, *Chem. Ind.* **1963**, 953 (1963).
17. Obtained from the Oak Ridge National Laboratory, 11.7 μc per mole. For determination of ^{36}Cl, the channel ratio technique was used.
18. K. Nagao, *Osaka Shiritsu Daigaku Igaku Zasshi* **8**, 221 (1959).
19. We thank E. Corbin for assistance, I. Wempen, P. H. Stahl, N. J. M. Birdsall for suggestions on the chemical reductions, and U. Wölcke for a sample of 8-methylmercaptoxanthine. Supported in part by the American Cancer Society grant P-295, PHS grant CA 08748, and AEC contract AT(30-1)-910.

30 October 1969; revised 15 December 1969

Neuroendocrine Control of Ecdysis in Silkmoths

Abstract. *An adult moth sheds its pupal skin only during a specific period of the day. The brain is necessary for the synchronization of this behavior with the environmental photoperiod. This function is fully preserved when all the brain's nervous connections are severed or when a "loose" brain is transplanted into the tip of the abdomen. By appropriate experiments it was possible to show that the entire mechanism is brain-centered. The components include a photoreceptor mechanism, a clock, and a neuroendocrine output. The clock-controlled release of the hormone acts on the central nervous system to trigger a species-specific behavior pattern which culminates in ecdysis.*

Lepidopterists have long been aware that the escape of butterflies and moths from their old pupal cuticle occurs at a certain time of the day which is characteristic of the species (*1*). The three silkworms used in this study, *Hyalophora cecropia, Antheraea polyphemus,* and *A. pernyi,* proved to be no exception. When developing moths were placed in a photoperiod, the subsequent ecdyses, which were recorded by means of a 48-hour kymograph (*2*), were confined to a specific "gate" (*3*), the time of which depended on the particular photoperiod regimen (*4*). Figure 1 (left) shows the ecdyses of moths that were exposed to a regimen consisting of 17 hours of light per day (17L : 7D). The gates of the three species differed in respect to both time and width. The "clock" which determines the time of ecdysis also has a circadian component. Thus, when developing Pernyi were transferred into constant darkness from a 17L : 7D regimen, the subsequent ecdyses continued to occur synchronously with gates at 22-hour intervals after lights-off (*4*). In addition, an examination of the kymograph records (Fig. 1, right) showed that ecdysis is immediately preceded by a period of hyperactivity (the emergence behavior), the pattern of which was also species-specific. The present report describes a brain-centered neuroendocrine mechanism by which ecdysis is synchronized with a light-dark regimen.

The preliminary stage of this study involved an examination of the role of various cephalic nerves, ganglia, and endocrine organs in the ecdysis of silkmoths (*5*). The operations were performed primarily on diapausing pupae of the Cecropia moth (*6*); the animals were then allowed to begin adult development, and about 3 weeks later the effect of the surgery on the ecdysis of the resulting moths was ascertained (*7*). Sectioning of the optic nerves and circumesophageal connectives or extirpation of the compound eye anlagen, the subesophageal ganglion, the frontal ganglion, or the corpora allata and corpora cardiaca was inconsequential. All the operated moths emerged within the normal Cecropia gate, that is, between 1 and 9 hours after lights-on in the 17L : 7D regimen. By contrast, when the brain was removed, the moths were no longer influenced by light. Both brainless Cecropia and brainless Pernyi emerged randomly throughout the day and night (Fig. 2B). The removal of the brain somehow interrupted the chain of events leading from the reception of light to ecdysis.

It is important to note that the brain functioned correctly even after its connections to the eyes (*8*) and the ventral nerve cord were severed. That being so, the implantation of a brain into a brainless animal should restore the competence to synchronize emergence with photoperiod. To test this hypothesis, "loose-brain" moths were prepared by removing the brain from the head of each pupa and immediately reimplanting it into the tip of the abdomen. These animals proved to be fully able to respond to the photoperiod regimen. The emergence of "loose-brain" moths was gated and occurred at about the same time as that of unoperated moths (Fig. 2C). Also, as with unoperated animals, the ecdysis rhythm of "loose-brain" moths was free-running in continuous darkness (*9*).

In order to synchronize the emergence behavior with photoperiod, a minimum of three components are nec-