platelet viability. Additional technical details are given in the footnotes to Table 1.

8. J. Gross, *Comp. Biochem.* **5**, 307 (1963).
9. R. L. Katzman, M. H. Halford, V. N. Reinhold, R. W. Jeanloz, *Biochemistry* **11**, 1161 (1972).
10. The structure of the homologous glycopeptide from rat skin collagen (α1-CB5) has been published [see W. T. Butler, *ibid.* **9**, 44 (1970)]. Both chick and rat skin α1-CB5 contain an amino-terminal Glc-Gal-Hyl and are identical in the next 14 residues. Six conservative substitutions occur in residues 16 to 37 (A. H. Kang and J. Gross, in preparation).
11. Inhibition experiments were carried out in the following manner. The potential inhibitor (50 μl of a 10 percent solution) was added to PRP (0.5 ml). None of the test substances by themselves induced aggregation within 10 minutes. The test substances were then preincubated with PRP for 3 minutes and challenged with α1 chain (12.5 μl of a 1 percent solution). Only *Metridium* Glc-Gal-Hyl oligopeptides (*9*), pure Glc-Gal-Hyl (*9*), and D-glucosamine · HCl blocked the α1 chain from inducing aggregation. Methyl-α-D-glucopyranoside, lactose, *p*-nitrophenyl-β-D-galactopyranoside, *N*-acetyl-D-glucosamine, D-galactosamine · HCl, L-fucose, D-mannose, and D-glucose did not block α1-induced aggregation.
12. To counter the argument that there might be a small amount of renaturation, we have run the reaction with chick skin α1 chain and its CB5 peptide at 37°C. There was no quantitative difference in the reaction at the higher temperature, except for a shortening of the latency period. The melting temperature (T_M) of chick skin α1-CB5 (A. H. Kang, unpublished observations) is very similar to the T_M of α1-CB2, approximately 13°C. Both are also similar in size and imino acid content [K. A. Piez and M. R. Sherman, *Biochemistry* **9**, 4134 (1970)].
13. Prepared as described by R. L. Katzman, E. Lisowska, R. W. Jeanloz [*Biochem. J.* **119**, 17 (1970)].
14. Extracted as described by R. L. Katzman and A. L. Oronsky [*J. Biol. Chem.* **246**, 5107 (1971)].
15. Preparation of R. L. Katzman and A. H. Kang [*ibid.* **247**, 5486 (1972)].
16. Diethylaminoethyl cellulose fraction 2 described by R. L. Katzman, A. K. Bhattacharyya, R. W. Jeanloz [*Biochim. Biophys. Acta* **184**, 523 (1969)].
17. By the method of J. Gross and D. Kirk [*J. Biol. Chem.* **233**, 355 (1958)]. Type II collagen was prepared by the method of R. L. Trelstad, A. H. Kang, S. Igarashi, J. Gross [*Biochemistry* **9**, 4993 (1970)].
18. Cyanogen bromide peptides were prepared as described by A. H. Kang, S. Igarashi, J. Gross [*Biochemistry* **8**, 3200 (1972)].
19. Prepared by the method of J. Gross, G. Maltosy, C. Cohen [*J. Biophys. Biochem. Cytol.* **1**, 215 (1955)].

20. This is paper No. 11 in the Invertebrate Connective Tissue series. The preceding paper in this series is by R. L. Katzman and A. H. Kang [*J. Biol. Chem.* **247**, 5486 (1972)]. Supported by grants from the Veterans Administration and NIH (AM-16506 and NS-09616).

* Present address: Biophysics Department, Weizmann Institute, Rehovot, Israel.

2 April 1973

Juvenile Hormone Analogs: A Possible Case of Mistaken Identity?

Abstract. *Many synthetic compounds that exhibit activity in juvenile hormone assays have limited structural resemblance to natural juvenile hormones. The observed morphogenic action of many of the compounds considered to be biologically analogous to juvenile hormones is probably synergistic rather than intrinsically hormonal.*

The possibility of controlling insects by interfering with the action of hormones that critically control their postembryonic development has received considerable attention (*1*). Particular interest has centered around the compound methyl-*trans,trans,cis*-10,11-epoxy-7-ethyl-3,11-dimethyltrideca-2,6-dienoate (*JH* in Fig. 1), which is one of two juvenile hormones (JH) occurring naturally in the silk moth *Hyalophora cecropia* and which inhibits metamorphosis in species representing several insect orders (*2*).

In attempts to develop new and potentially more effective materials with this type of activity, a large number of compounds have been synthesized and evaluated as mimics of the naturally occurring compound (*1, 3, 4*). Among the compounds that have been shown to possess various degrees of activity are piperonyl 6,7-epoxy-3-ethyl-7-methyl-2-nonenyl ether (**2**), 10,11-epoxy-*N*-ethyl-3,7,11-trimethyl-2,6-dodecadienamide (NIA 23509) (**3**), isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate (ZR-515) (**4**), and ethyl 3,7,11-trimethyldodeca-2,4-dienoate (ZR-512) (**5**) (*5, 6*). Several insecticide synergists such as piperonyl butoxide (**1**) and propyl 2-propynylphenylphosphonate (NIA 16388) (**6**) have also been found to exhibit appreciable activity in juvenile hormone assays (*7*).

Although these synthetic compounds are usually referred to as juvenile hormone analogs (JHA) (*1*), many have little or no structural resemblance to natural juvenile hormones and the apparent absence of any common structural feature has been difficult to rationalize in terms of their interaction with a specific hormone receptor. It now appears probable that the biological activity of many of these compounds is not due to their innate capacity to interact with the same target receptor as JH, but results mainly from their ability to interfere with the metabolic degradation of the natural hormone. Consequently the observed activity of many JHA is that of the insect's own unmetabolized JH.

The major pathways responsible for the metabolism of JH in the tobacco hornworm (*Manduca sexta*), the southern armyworm (*Prodenia eridania*), the vagrant grasshopper (*Schistocerca vaga*) and the flesh fly (*Sarcophaga bullata*), as well as in *H. cecropia* itself, have been clearly established (*8, 9*), and are shown in Fig. 1. Two major pathways are involved in which ester hydrolysis may either precede or succeed cleavage of the epoxide ring. Since each of the metabolites (an epoxy acid, **7**; a dihydroxy ester, **8**; and a dihydroxy acid, **9**) is considerably less active than JH in juvenile hormone assays, these are considered to constitute the natural deactivation pathways of JH in the insects studied. Except for the dipterous *S. bullata*, in which JH appears to be deactivated almost exclusively by epoxide ring cleavage, these pathways are common to all insect species examined. This suggests that the JH of each species is structurally similar to or identical with that of *H. cecropia* (*8*), a conclusion supported by chromatographic analyses of lipid extracts from a number of Lepidoptera including *P. eridania* and *H. cecropia* (*10*). In view of this, *P. eridania* was considered an excellent model for studying the effects of a variety of JHA on the metabolism of the natural JH of *H. cecropia*.

Studies in vitro with several insect species have established that the enzymatic pathways responsible for the metabolism of JH occur in a number of tissues including the midgut (*9*), and preliminary experiments indicated that this was also true in *P. eridania*. Midgut homogenates from early sixth-instar armyworm larvae were prepared by established procedures (*11*) and constituted the major enzyme source for this investigation. The enzyme preparation (0.5 ml of a homogenate of two guts per milliliter in 0.15*M* KCl) was incubated for 10 minutes at 30°C with JH (2.5 μg) labeled with ^{14}C in the 2-position (*12*) in 0.1*M* tris-HCl, *p*H 7.8, in the presence or absence of the candidate JHA at a concentration of $10^{-4}M$. The total volume of the reaction medium was 5.0 ml. Extraction of the incubation mixtures has been described (*8*), and subsequent thin-layer chromatographic analysis of the extracts revealed the well-defined radioactive areas associated with the major metabolites of JH (*8*). Elution of these from the plates and further analysis by scintillation counting yielded the quantitative data shown in Table 1.

It is clear from these data that JH

is indeed stabilized in the presence of each of the JHA evaluated, and with some compounds, particularly **1**, **2**, and **3**, the effect is substantial. Of additional interest is that the metabolite patterns produced in the presence of the different JHA studied show that they act primarily in one of two distinct ways. Thus, compounds **1**, **2**, and **3** appear to act predominantly on the pathways mediated by epoxide hydrase, whereas compounds **4**, **5**, and **6** have their major effect on the pathways mediated by esterase. However, the precise effect of any particular JHA is difficult to assess. The much larger accumulation of the epoxy acid **7** produced by compounds **1**, **2**, and **3** compared with the amount of dihydroxy ester **8** present with compounds **4**, **5**, and **6** could be a reflection of the fact that **1**, **2**, and **3** are better epoxide hydrase inhibitors than **4**, **5**, and **6** are esterase inhibitors. On the other hand, it could be a confirmation of an earlier indication (*8*) that ester hydrolysis is more rapid than epoxide cleavage. It is most likely, though, that the data in Table 1 result from a complex combination of both of these factors.

The different degrees of JH stabilization shown in Table 1 cannot be said with complete certainty to fully account for the observed in vivo biological activity of the various JHA used in this investigation since the latter have been evaluated by numerous testing procedures with several different insect species. In many insect species, however, compounds **1** and **2** have been reported to exhibit "significant" activity (*5*, *7*), and the activity of **3** has been shown to be "particularly pronounced" (*6*). The activities of **4** and **5** are sufficiently high to warrant field trials, but **6** is known to be considerably less active (*7*). In view of the many factors likely to be involved in determining the morphogenic action of the JHA, a directly proportional relation between their activities in vitro and their effects in vivo could not reasonably be expected. Thus factors such as differential rates of penetration and possible changes related to age and species in the rates of metabolism of both the natural JH (*8*) and the test compound will combine to present a very complex situation. Indeed it is likely that a great deal of the reported species specificity of various JHA results directly from factors of this type.

Our data confirms the view (*13*) that the activity of some JHA is synergistic rather than intrinsically hormonal and

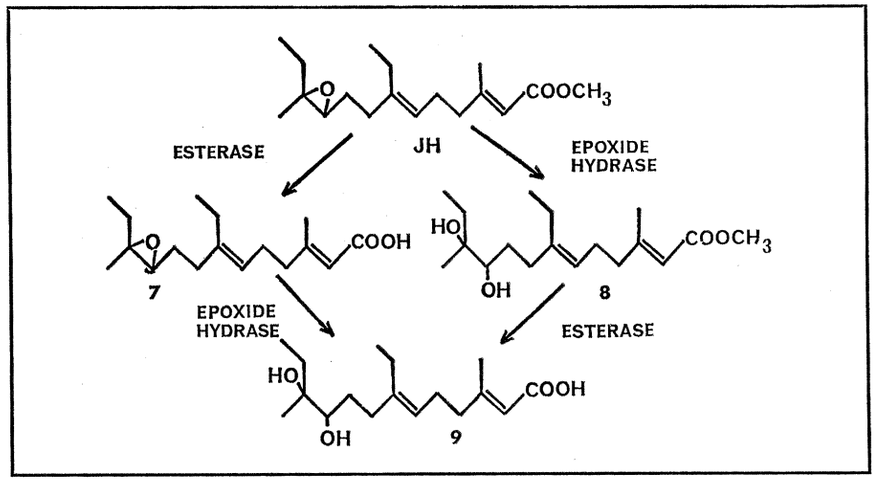
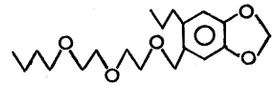
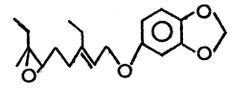
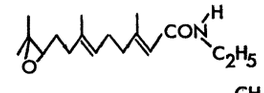
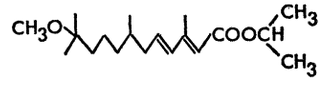
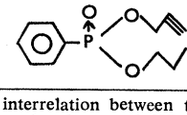


Fig. 1. Major pathways responsible for the deactivation of *Cecropia* juvenile hormone in insects.

supports the veracity of earlier suggestions concerning the possibility of exploiting this approach (*14*). Our results, however, do not entirely rule out the possibility that some JHA might have some intrinsic hormone activity. The fact that various compounds have been reported to produce juvenile hormone activity in allatectomized insects (*15*) has been put forward in support of this contention. This argument, though, is based on the assumption that the allatectomized insects are completely free of their own juvenile hormone at the time of testing. No direct evidence has yet been presented to support this claim. Indeed, on the basis of considerations of binding and metabolism it could be argued that low levels of endogenous juvenile hormone persist at the critical time of JHA application.

In addition to the JHA reported here. which appear to act by inhibiting either

Table 1. Effect of JHA on the metabolism in vitro of *Cecropia* JH by midgut preparations from *Prodenia eridania* larvae.

JHA		JH metabolites produced relative to control (1.0)			Stabilization of JH*
		Epoxy acid **7**	Dihydroxy ester **8**	Dihydroxy acid **9**	
1		3.8	0.5	0.3	14.6 ×
2		4.8	0.3	0.2	19.2 ×
3		3.3	0.2	0.2	12.7 ×
4		1.0	1.0	0.2	5.6 ×
5		0.7	1.1	0.3	3.8 ×
6		0.6	1.3	0.3	1.9 ×

* Because the interrelation between the two possible JH deactivation pathways is complex, no direct relation between the degree of JH stabilization and metabolite patterns is evident from these data. The relation is further complicated by the fact that the activities of the enzymes involved, and possibly also their susceptibility to inhibition, are dependent on the age of the insect. Since these data were obtained with different enzyme preparations, the different degrees of JH stabilization reported do not necessarily reflect the true relative potencies of the JHA.

the esterase or the epoxide hydrase responsible for JH metabolism, we have evidence (*9*) that other JHA may act through epoxide hydrase activation. The initial response of insects treated with each type of material is of course different though the end result, disruption of development, is apparently the same.

Our data may indeed provide the basis for a more rational approach to the design and development of a variety of compounds with potential JHA activity.

MICHAEL SLADE
C. F. WILKINSON
Department of Entomology,
Cornell University,
Ithaca, New York 14850

References and Notes

1. J. J. Menn and M. Beroza, Eds., *Insect Juvenile Hormones: Chemistry and Action* (Academic Press, New York, 1972).
2. H. Röller and K. H. Dahm, *Recent Progr. Hormone Res.* **24**, 651 (1968).
3. W. S. Bowers, *Naturally Occurring Insecticides* (Dekker, New York, 1971), pp. 307–332.
4. K. Slama, *Annu. Rev. Biochem.* **40**, 1079 (1971).
5. W. S. Bowers, *Science* **164**, 323 (1969).
6. P. A. Cruickshank, *Bull. WHO* **44**, 395 (1971).
7. W. S. Bowers, *Science* **161**, 895 (1968).
8. M. Slade and C. H. Zibitt, in "Chemical releasers in insects," *International IUPAC Congress 2nd, Pesticide Chemistry: Proceedings*, A. S. Tahori, Ed. (Gordon & Breach, New York, 1971), vol. 3, pp. 45–58.
9. M. Slade and C. F. Wilkinson, in preparation.
10. A. S. Meyer and H. A. Ax, *J. Insect Physiol.* **11**, 695 (1965).
11. R. I. Krieger and C. F. Wilkinson, *Biochem. Pharmacol.* **18**, 1403 (1969); R. S. H. Yang and C. F. Wilkinson, *Biochem. J.* **130**, 487 (1972).
12. W. Hafferl, R. Zurfluh, L. Dunham, *J. Labelled Comp.* **7**, 331 (1971).
13. C. E. Dyte, *Proc. Brit. Insec. Fungic. Conf. 5th* **1**, 393 (1969).
14. G. T. Brooks, in *Agricultural Research Council Annual Report for 1970–71* (Her Majesty's Stationery Office, London, 1972), p. 36.
15. T. Ohtaki, K. Kiguchi, H. Akai, K. Mori, *Appl. Entomol. Zool.* **7**, 161 (1972).
16. We thank H. K. Hetnarski for technical assistance, Dr. W. S. Bowers of the New York State Agricultural Experiment Station, Geneva, for compound **2**, and Dr. P. A. Cruickshank of FMC Corporation, Princeton, N.J., for compounds **3** and **6** (NIA 23509 and NIA 16388). Supported in part by PHS training grant ES 00098, PHS grant ES 00400, and Rockefeller Foundation grant RF 69073.

16 March 1973; revised 24 April 1973

Human Papovavirus (JC): Induction of Brain Tumors in Hamsters

Abstract. *Eighty-three percent of hamsters inoculated at birth with JC virus, a human papovavirus isolated from brain tissue of a case of progressive multifocal leukoencephalopathy, developed malignant gliomas within 6 months. Three brain tumors have been serially transplanted as subcutaneous tumors. JC virus was isolated from five of seven tumors tested. Cells from four tumors were cultivated in vitro. These cells contained an intranuclear antigen with the characteristics of a T antigen, and this antigen was antigenically related to SV40 T antigen. Although virus was not recovered from extracts of serially cultured tumor cells, JC virus was rescued when one tumor cell line was fused with permissive cells.*

A new human papovavirus, JC virus, was recently isolated (*1*) from brain tissue of a case of progressive multifocal leukoencephalopathy (PML). Although PML is a degenerative disease of the brain of man, the scattered giant astrocytes usually present in the lesions cannot be distinguished from the malignant astrocytes of pleomorphic glioblastomas (*2*). This observation in itself could raise a question concerning the oncogenic potential of this virus, but, in addition, the following points suggest that the tumor-inducing capacity of JC virus should be examined: (i) JC virus belongs in that subgroup of papovaviruses (polyoma, SV40) that have a strong capacity for transforming cells in vitro and for inducing tumors in laboratory animals; (ii) JC virus is found in deep tissues of man (in contrast to the human papilloma virus); and (iii) approximately 75 percent of adults have serum antibody against JC virus, which is evidence of infection by this virus (*3*). The data presented here are part of such an examination and indicate that JC virus is strongly oncogenic in the central nervous system of hamsters.

JC virus is distinct from polyoma, SV40, and other papovaviruses in several respects (*1*); however, we have unpublished evidence of a weak relationship between JC and SV40 virion antigens. Because of this relationship and because polyoma virus and SV40 are known to be highly oncogenic in newborn hamsters, care was taken to reduce the possibility that the observed effects could be due to adventitious SV40 or polyoma viruses.

Golden Syrian hamsters (*Mesocricetus auratus* from Lakeview Hamster Colony, Newfield, New Jersey) less than 24 hours old were inoculated both intracerebrally and subcutaneously with JC virus or with control material. Inoculation was into the right cerebral hemisphere and under the skin over the shoulders. The virus dose in each site was 10^6 $TCID_{50}$ (tissue culture infective dose, 50 percent effective) (2048 hemagglutinating units) in 0.02 ml.

The virus inoculum was from the third passage in primary human fetal glial (PHFG) cell cultures (*1*) and was concentrated and partially purified by differential centrifugation. The control inoculum was a concentrated and partially purified extract of uninfected PHFG cells. The inocula were cultured for mycoplasma (*4*) with negative results. That the virus inoculum had the characteristics typical of JC virus was checked by inoculation onto PHFG cells and two cells derived from African green monkey kidney cultures (Vero and CV-1). JC virus does not produce cytopathic effects in Vero or CV-1 cells, but SV40 virus does. During a 21-day observation period, cytopathic effects were seen only in the PHFG cultures, and only JC viral antigen was detected (in the PHFG cultures) when inoculated cultures of all three cell types were stained by the indirect immunofluorescent technique with anti-JC, anti-SV40, and anti-polyoma serums.

There was no evidence of acute disease caused by the inocula. Sixty-three of 100 hamsters inoculated with JC virus and 39 of 69 inoculated with control material survived the initial manipulations and were placed under long-term observation.

Three months after inoculation, four animals from each group were killed, and their tissues were examined for gross and microscopic lesions. Although no signs of disease had been seen in these animals, one of the four inoculated with JC virus had a definite microscopic tumor deep in one cerebral hemisphere. The other three had collections of abnormal cells in the brain that suggested early tumor foci. The control hamsters showed no evidence of disease.

Approximately 4 months (123 days) after inoculation overt signs of central nervous system disease were recognized in a JC virus–inoculated hamster. Additional animals with signs of central nervous system disease appeared at a fairly steady rate of five or six per week. Central nervous system disease usually was signaled by decreased activity, somnolence, unsteady gait, and circling. In order to obtain fresh tissues, most animals were killed within 5 days after onset of signs of disease; otherwise they usually died within 10 days.

Six months after inoculation the nine remaining JC virus–inoculated hamsters and the control animals were