relative distribution of fatty acids were noted. That singlet oxygen is responsible for the observed effect was established by substituting D_2O for water as the fluid phase. It has been established experimentally that the lifetime of singlet oxygen is markedly extended (10 times) in D_2O (*16*). We detected a further twofold decrease in unsaturated free fatty acids when D_2O was employed as the medium under otherwise identical conditions. The less dramatic effect of the solvent on the rate of attack observed in this case may be due to the fact that the reactions noted occur on or near the surface of the pollen grain rather than in a homogeneous system.

These results confirm that surface and near surface components of common viable particulate matter in the atmosphere may be subject to rapid oxidation by singlet oxygen, leading to products which are probably allylic hydroperoxides (*2*). We have alluded to the known lethal properties of such compounds mimicking the effects of ozone in leading to pulmonary edema and lung congestion in rats and to our interest in the effects on pollen viability. The recent increase of atmospheric pollutants which may serve as sensitizers enhances the probability that materials toxic to mammalian lung tissue (and potentially biologically damaging to pollens and spores) may be oxidatively produced on the surfaces of viable particulate matter. Adsorption of appropriate sensitizers and substrates (polynuclear aromatics) on nonviable atmospheric particles might lead to similar interactions yielding toxic oxidative products.

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Collagen-Induced Platelet Aggregation: Involvement of an Active Glycopeptide Fragment (α1-CB5)

Abstract. *It is widely held that the tertiary structure of collagen is essential for induction of platelet aggregation. However, we have found that the purified α1 chain prepared from denatured chick skin collagen aggregates platelets. This activity appears to be confined to a distinct region of the molecule representing less than 4 percent of the length of the α1 chain. Of all of the cyanogen bromide peptides of the α1 chain tested, only one (α1-CB5) was active. This glycopeptide, devoid of any ordered tertiary structure, contains only 36 amino acids and one residue of* O-α-D-*glucopyranosyl-(1 → 2)*-O-β-D-*galactopyranosyloxy-(1 → 5)-lysine (Glc-Gal-Hyl). Blocking experiments strongly suggest that the Glc-Gal-Hyl is one of the structural determinants involved in collagen-induced platelet aggregation.*

Collagen-induced platelet aggregation is a phenomenon that is presently attracting considerable attention (*1–4*). Jamieson *et al.* (*2*) and Bosmann (*4*) have suggested that collagen-induced platelet aggregation is mediated by an interaction between a glycosyltransferase on the surface of the platelet membrane and a carbohydrate moiety of collagen. Therefore, it was of interest to examine in a systematic manner those structural features of collagen that might be involved in this process. In this report we describe the results we obtained from studies of collagens from various phyla and tissues. We also report, contrary to previously published papers (*1*, *3*), that denatured collagen from chick skin has platelet aggregating activity. Furthermore, one of the cyanogen bromide peptides (α1-CB5) (*5*) from chick skin collagen is active; none of the other of these peptides from chick skin are active, including those from the α2 chain. Results from blocking experiments with pure Glc-Gal-Hyl (*6*) and other sugars strongly suggest that this group is one of the structural determinants of the α1-CB5 peptide.

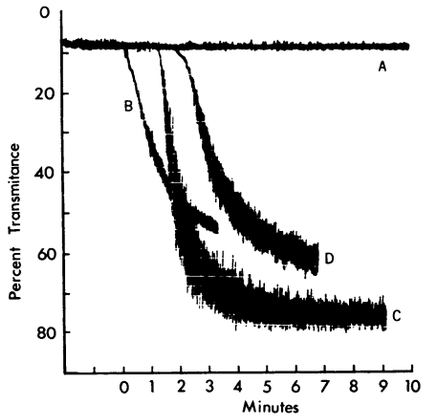

Fig. 1. Superimposed recordings of four typical results. (Curve A) *Metridium dianthus* insoluble collagen. This tracing was indistinguishable from all of the other negatively acting collagens listed in Table 1. (Curve B) Aggregation induced by adenosine diphosphate; (curve C) α1-CB5 chick skin peptide; and (curve D) α1 chain of chick skin collagen. Details, including amounts added, are given in (*7*) and in Table 1.

Platelet aggregation was measured in a Payton aggregometer, model 300A, with the use of a Goerz (Gelman) recorder, according to standard techniques (*7*). Typical recordings are shown in Fig. 1, and our results are summarized in Table 1. Under the test conditions employed, none of the

invertebrate collagen preparations were active. This was surprising since the collagen from these invertebrates has been shown to possess many of the ultrastructural characteristics (*8*) of mammalian collagen and to be relatively rich in carbohydrates specifically including Glc-Gal-Hyl (*9*). The dogfish was the lowest vertebrate we tested that gave a positive response (see Table 1). Both chick and human bone collagens were inactive, as was human dentin collagen, bovine corneal collagen, and bovine vitreous humor collagen. Chick cartilage collagen and bovine tendon collagen (a widely used standard in this assay) were active, as was bovine aortic collagen. Contrary to the reports of Chesney *et al.* (*3*) and Wilner *et al.* (*1*), who state that the tertiary or native structure of soluble collagen is essential for platelet aggregation, we find that the denatured $\alpha 2$ or $\alpha 1$ chain from chick skin collagen is an effective aggregating agent. This indicated to us that the primary structures of chick skin α chains were predominantly responsible for their aggregating activity. To test this hypothesis, we examined the cyanogen bromide peptides (see Table 1) of both α chains. All of the peptides were inactive with the exception of $\alpha 1$-CB5. This peptide contains only 36 amino acid residues and one amino-terminal residue of Glc-Gal-Hyl. Its sequence determination has not yet been completed (*10*).

Preincubation of platelets with either Glc-Gal-Hyl oligopeptides (*11*) or pure Glc-Gal-Hyl blocks platelet aggregation induced by chick skin $\alpha 1$, strongly suggesting that the Glc-Gal-Hyl of $\alpha 1$-CB5 is a structural determinant necessary for platelet aggregation. It is obviously not the structural determinant since pure Glc-Gal-Hyl is incapable of inducing aggregation. A number of other sugars (*11*) were incapable, with the exception of D-glucosamine (*2*), of blocking collagen-induced aggregation. Also, the nonreducing end of Glc-Gal-Hyl is structurally identical to D-glucosamine and both sugars bear a protonated amino group; possibly they act in the same manner.

We do not mean to imply that the tertiary structure of collagen is not a factor in platelet aggregation. There are probably other physical factors as well as structural (chemical) factors involved. Our data do indicate, however, that a small, well-defined segment of a collagen molecule, devoid of any ordered tertiary structure, is capable of inducing platelet aggregation (*12*). We are attempting to define all of the structural determinants of this glycopeptide in order to more fully understand its mode of interaction with the platelet membrane.

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Table 1. Collagen-induced platelet aggregation. Details of the assay are given in (*7*). Small pieces of insoluble collagens (approximately 0.5 mg) were placed in the cell at time zero. Soluble materials (50 μl) were added at time zero as 1 percent solutions in 0.145*M* NaCl with 0.02*M* Na_2HPO_4 (*p*H 7.4).

Animal	Type of collagen	Reaction
Sponge (*Hippiospongia gossypina*)	Spongin B, insoluble (*13*)	—
Sea anemone (*Metridium dianthus*)	Body wall, insoluble (*14*)	—
	Body wall, pepsin-solubilized α chain (*15*)	—
Sea cucumber (*Thyone briareus*)	Dermis, insoluble (*14*)	—
	Dermis, gelatin (*16*)	—
Dogfish (*Squalus acanthias*)	Skin, reconstituted native type fibril (*17*)	+
Chick (White Leghorn)	Cartilage, reconstituted native type fibril (*17*)	+
	Bone, insoluble chips*	—
	Skin, reconstituted native type fibril (*17*)	+
	Skin, lyophilized tropocollagen (amorphous)	—
	Skin, tropocollagen (in solution)	+
	Skin, isolated $\alpha 2$ chain (*18*)	+
	$\alpha 2$–CB1	—
	$\alpha 2$–CB3	—
	$\alpha 2$–CB4	—
	$\alpha 2$–CB5	—
	Skin, isolated $\alpha 1$ chain†	+
	$\alpha 1$–$CB1^{Ald}$	—
	$\alpha 1$–CB2	—
	$\alpha 1$–CB3	—
	$\alpha 1$–CB4	—
	$\alpha 1$–CB5	+
	$\alpha 1$–CB6A	—
	$\alpha 1$–CB7	—
	$\alpha 1$–CB8	—
Cow (Bovine)	Vitreous humor, insoluble (*19*)	—
	Cornea, insoluble‡	—
	Tendon, insoluble§	+
	Aorta, insoluble (*14*)	+
Human (*Homo sapiens*)	Dentin, insoluble‖	—
	Bone, insoluble‖	—

* Femur cleaned of one marrow and decalcified by 0.2*M* ethylenediaminetetraacetic acid. † Cyanogen bromide peptides prepared according to the method of A. H. Kang *et al.* (*5*). ‡ Unextracted lyophilized corneal fiber free of epithelial membrane. § Purchased from Sigma. ‖ Decalcified in 0.6*N* HCl at 2°C for 5 days and lyophilized. Gift from Dr. Alton A. Register.

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7. All operations were performed at room temperature. Platelet-rich plasma (PRP) was prepared from citrated blood (9 parts of blood and 1 part of 3.8 percent sodium citrate) obtained by venipuncture from two of the investigators (A.H.K. and E.H.B.). The citrated blood was centrifuged at 226*g* for 10 minutes. Samples of the supernatant PRP (0.5 ml), containing between 210,000 and 350,000 platelets per milliliter, were transferred to siliconized glass cuvettes (8 by 45 mm) containing a silicone-coated magnetic stirring bar. The recorder baseline with the PRP in the aggregometer cell (constantly stirring at 1100 rev/min) was adjusted to 10 percent transmission, and the baseline with water in the cell was adjusted to read 90 percent transmission. After establishing a stable baseline, test solutions were added in volumes of 50 μl. Insoluble substances were added in 0.5-mg amounts. Transmittance was recorded for 10 minutes. This technique is based on the turbidometric method originally described by G. V. R. Born [*Nature London* **194**, 927 (1962)]. If during this time the transmittance changed by less than 3 percent, the aggregating ability of the test substance was considered to be negative; in all positive responses the transmittance changed by more than 40 percent (that is, a final reading of 50 percent or greater). In tests in which negative results were obtained, a collagen preparation of known aggregating activity was added (after 10 minutes) to confirm the viability of the platelets. In cases where the test substance (such as Glc-Gal-Hyl) blocked the response of a known collagen inducer, 50 μl of adenosine diphosphate solution (40 μM) was added to confirm

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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)].
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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 its 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