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Prehistoric Polymers: Rubber Processing in Ancient Mesoamerica

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Ancient Mesoamerican peoples harvested latex from *Castilla elastica*, processed it using liquid extracted from *Ipomoea alba* (a species of morning glory vine), and fashioned rubber balls, hollow rubber figurines, and other rubber artifacts from the resulting material. Chemical and mechanical analyses of the latex and of the processed rubber indicate that the enhanced elastic behavior of the rubber relative to the unprocessed latex is due to purification of the polymer component and to an increase in the strength and number of interchain interactions that are induced by organic compounds present in *I. alba*. These ancient peoples' control over the properties of latex and processed rubber gave rise to the Mesoamerican ball game, a central ritual element in all ancient Mesoamerican societies.

Ancient Mesoamerican peoples were processing rubber by 1600 B.C. (1), which predated development of the vulcanization process by 3500 years. They made solid rubber balls, solid and hollow rubber human figurines, wide rubber bands to haft stone ax heads to wooden handles, and other items (2). They used liquid rubber for medicines, painted with it, and spattered it on paper that was then burned in ritual. The ball game, played on a ball court with a solid rubber ball, was a key event in ancient Mesoamerican societies (3). The *Popol Vuh*, the Maya origin story, captures the game's religious and sacred function by pitting the ball playing skills of the Hero Twins against those of evil lords of the underworld, using complex imagery of human sacrifice, fertility, and regeneration (4). Sixteenth-century Spanish invaders reported that apart from its religious significance the ball game also was a sporting event in

which contenders gambled for land, slaves, and other valuables.

The raw material for most Mesoamerican rubber balls and for other Mesoamerican rubber artifacts is a latex acquired from the *Castilla elastica* tree (5). The tree is indigenous to tropical lowland Mexico and Central America. *Castilla* latex is a sticky white liquid that when dried is too brittle to retain its shape. Sixteenth-century Spaniards relate that ancient Mesoamerican peoples processed the raw material by mixing *C. elastica* latex with juice from *Ipomoea alba* (6) (a species of morning glory vine), one chronicler noting that "ulli is the resin from a tree that grows in the hot lands . . . when they mix it with another, the resin coagulates" (7). Pedro Martyr, the Spanish royal chronicler, commented that "they make these balls from the juice of a certain vine . . . once transformed into a mass they give it the form they desire" (8). In the present study, we investigated this processing technology, the extent to which it improves the mechanical properties of latex for balls, rubber bands, and hollow figurines, and the chemical changes responsible for property development.

Rubber artifacts are poorly preserved, but

archaeologists have recovered a few hundred ancient Mesoamerican examples. The oldest archaeological specimens are 12 solid rubber balls recovered at the Manatí site in Veracruz, Mexico. The six we examined range from 13 to 30 cm in diameter and weigh from 0.5 to 7 kg. The two oldest Manatí balls date to 1600 B.C., on the basis of radiocarbon dates (1). Manatí (1600 to 1200 B.C.) is an Olmec ritual burial site located in a swampy region along the lower Coatzacoalcos River. Other rubber artifacts were dredged from the Cenote Sagrado de Sacrificios, a water-filled limestone sinkhole at the site of Chichén Itzá on the Yucatán Peninsula. Cenote rubber artifacts, dated to between A.D. 850 and 1550 on the basis of stylistic attributes of associated artifacts, include small nodules, small solid balls, wooden tool handles wrapped in rubber, human figurines, human hands, a hollow human head, and a stone tool hafted with a rubber band (9).

The earliest dated ball court is the 1400 B.C. (uncalibrated) earthen court at Paso de la Amada, located in the Soconusco region on the coastal plain of Chiapas (10). Enclosed masonry ball courts proliferated in the Middle Classic Period (A.D. 400 to 700). By the time of the Spanish invasion of the Americas, versions of a ball game were played in the region from Arizona to northern South America. Several types existed, including one in which players hit a small rubber ball with a bat or stick. The more common hip-ball version of the game was still played in northwestern Mesoamerica until around 1970 (11).

We analyzed the Manatí rubber balls (12), and in Escuintla, Chiapas, we investigated traditional rubber processing methods. In Escuintla, the huleros (rubber-workers) incised the bark of the *C. elastica* trees and collected the sticky, raw latex in cups placed at the base of the trees. They cut a 5-m length of *I. alba* vine, stripped the leaves and flowers, and wrapped the vine into a coil. After beating and crushing the coiled vine on a rock, they squeezed the juice into a bucket containing latex; about 50 ml of the *I. alba* liquid extract was mixed with about 750 ml of the latex.

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After approximately 15 min of stirring, the liquid latex solidified into a white mass, which was then removed from the bucket and formed by hand into a solid ball 9.5 cm in diameter. When bounced on the ground, the ball exhibited typical rubbery behavior, rebounding into the air to a height of about 2 m.

We investigated the differences in the mechanical and chemical properties of the unprocessed (dried) (13) and processed Chiapas *C. elastica* latex to identify the mechanisms through which the processing technique alters and enhances the rubber's mechanical performance. The rubber samples from the ancient Manatí balls were too highly oxidized and delaminated for mechanical analysis. We quantified differences in the elastic behavior of the dried latex and the processed rubber by oscillating parallel plate rheometry, which measures the real (G') and imaginary (G'') components of the complex modulus of a material. The dried latex and processed rubber samples have similar G'' values (25.8 to 30 kPa; strain sweep), whereas the G' value, which relates to elasticity, of the processed rubber (285 kPa) is about double that of the dried latex (157 kPa) (14). Processing *C. elastica* latex with *I. alba* extract improves the elastic properties of the rubber. The increase in G' is indicative of increased stiffness and a higher degree of interchain interactions in the processed rubber, which are introduced by covalent cross-linking or by non-covalent interactions such as those involved in the packing of crystalline domains.

In addition to elasticity, the rubber used for Mesoamerican balls, figures, and bindings required initial formability (capacity to be deformed plastically and retain its shape) and toughness (resistance to fracture). In a simple replication exercise, we compared the performance of *C. elastica* latex and processed rubber when fashioned into thin-walled, hollow cylinders and wide, thin, circular bands. Our field observations in Chiapas indicated that thin sheets (0.1 cm thick) of raw latex and of rubber processed with *I. alba* dry and become brittle within minutes. Historical sources indicate that ancient Mesoamerican peoples heated the processed rubber (15) when forming it. In our replications, we heated two rectangular samples (4 cm by 2 cm by 0.2 cm), one of dried, unprocessed natural latex and the other of the rubber processed with *I. alba*, in a laboratory oven at 200°C for 1 hour. The rubber was easily rolled while warm and adhered to itself, producing a closed cylinder, which suggests that the material is not a fully cross-linked network akin to vulcanized rubber. In contrast, the unprocessed latex was brittle and developed long, longitudinal cracks upon rolling. Latex, like the rubber processed with *I. alba*, is flexible and compliant when warm, but latex lacks the elastic behavior that the processed rubber exhibits.

The increase in the shear modulus of the processed rubber measured by rheometry and

the differences in pliability and toughness evident from the replication tests may reflect an increase in cross-linking, chain entanglement, or the degree of crystallinity in the polymer structure. The molecular structures of the dried *C. elastica* latex, rubber prepared from latex and *I. alba*, and a sample from one of the Manatí rubber balls were examined by solid-state ^{13}C magic-angle spinning (MAS) nuclear magnetic resonance spectroscopy (^{13}C NMR) (Fig. 1). All three samples showed the five resonances characteristic of *cis*-1,4-polyisoprene (16), which is the polymer component of natural latexes and natural rubbers. The ^1H - ^{13}C cross-polarization (CP) MAS NMR technique (^{13}C CP NMR) was used to detect additional organic species that may be present in low abundance, such as cross-linking molecules. For the ancient Manatí rubber sample, a small amount of *trans*-1,4-polyisoprene was detected (17). The ^{13}C CP NMR spectrum of the processed rubber sample is unchanged relative to the ^{13}C NMR spectrum. The ^{13}C CP NMR spectrum of the unprocessed latex contains three resonances that are not present in the ^{13}C NMR spectrum, which suggests the presence of carbon-containing components, including proteins (18). However, these components are not

present after the latex has been transformed into rubber by treatment with *I. alba*. Thus, the processing of latex into rubber using *I. alba* is, in part, a concentration and purification process.

From NMR spectroscopy, there is no direct evidence of sulfur- or carbon-containing cross-linking groups in the processed rubber (19), although the observed change in mechanical behavior could arise from a number density of cross-links (about 1/1000) that is below the NMR detection threshold (about 1 weight %) (20). To identify molecules that are active in processing natural latex into rubber, we extracted *I. alba* juice with ether, purified it, and analyzed the resulting oil, which had previously been reported to induce latex coagulation but had not been characterized (21). In our experiments, use of the purified oil alone did not produce the same coagulation behavior as when the whole vine extract was used (22). The ^1H and ^{13}C NMR spectra and gas chromatography-mass spectrometric (GC-MS) analyses of the oil showed multiple, closely related species, which made definitive spectroscopic assignments difficult but indicated the presence of methylene and methyl groups within aliphatic chains (23). Fourier-transform infrared (FTIR) spectroscopy confirmed the presence of aliphatic

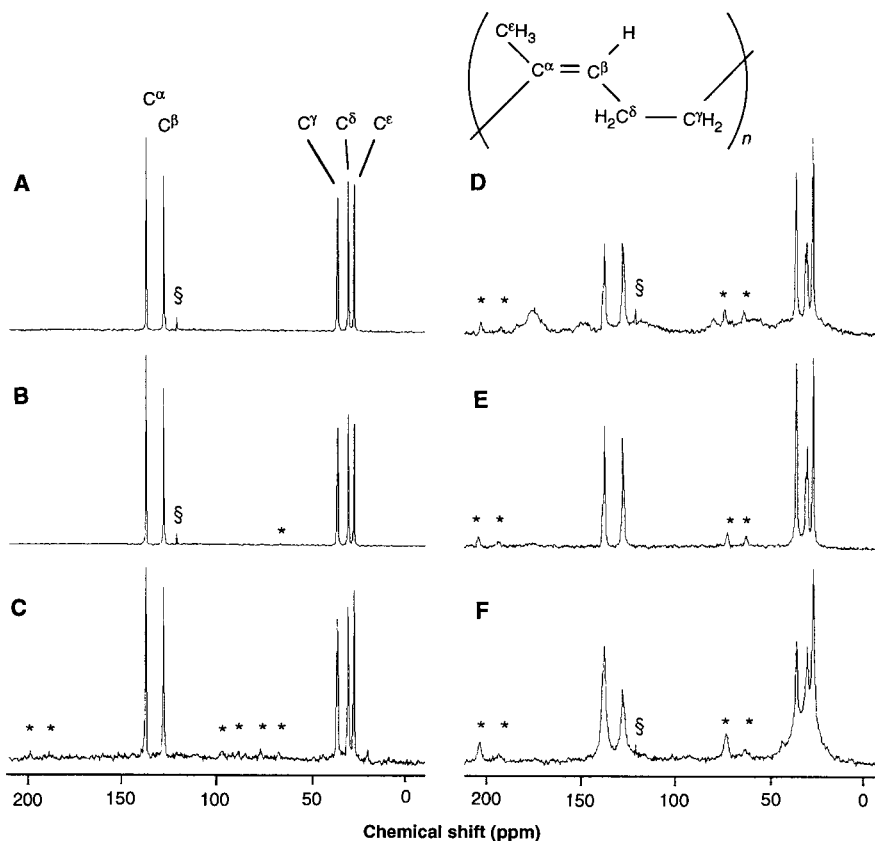


Fig. 1. Solid-state ^{13}C MAS (A to C) and ^1H - ^{13}C CP MAS (D to F) NMR spectra of unprocessed latex (A and D), *I. alba*-processed rubber (B and E), and ancient Manatí rubber (C and F). The ^{13}C NMR spectra were obtained at 68.055 MHz by using a single-pulse experiment with a 7-ms ^{13}C pulse length, a 15-s recycle time, and ^1H decoupling. The ^{13}C CP NMR spectra were obtained by using a 7-ms ^1H pulse length, a 2-ms contact time, a 3-s recycle time, and ^1H decoupling (Spectral Data Services, Champaign, Illinois). Spinning side bands (*) and a spectrometer artifact (S) are indicated.

ic methyl and methylene groups and indicated the presence of sulfonyl chloride and sulfonic acid moieties (24). These sulfur-containing organic groups are capable of reacting with alkenes such as those in the polymer chain, and a bifunctional molecule could act as a cross-link. Sulfonyl chlorides and sulfonic acids are also known to induce cyclization of 1,4-polyisoprene (25), which introduces rigid segments into the polymer chain. A small number of these segments would have the same effect on elastic behavior as would cross-links.

Natural latex is an emulsion composed of a *cis*-1,4-polyisoprene phase, an aqueous phase, and insoluble components (26). Our ^{13}C NMR data are consistent with the presence of soluble proteins and other organic species in the polymer and aqueous phases. Treatment of the fluid latex suspension with the *I. alba* vine extract destabilizes the emulsion, which separates the polymer and aqueous phases. In contrast, simple drying of the latex produces a brittle material that does not exhibit elastic behavior, consistent with its being a dried colloidal aggregate. This observation suggests that phase separation is not the sole process that occurs during coagulation of latex, and that processing increases entanglement and interactions among the polymer chains. We propose that organic species in the polymer phase of the unprocessed latex serve as plasticizers, which reduce its viscosity by disrupting interactions between the polymer chains. The *I. alba* extract solubilizes the plasticizing agents, thus allowing chain entanglement and interchain interactions, which give rise to the rubbery behavior of the processed rubber (27). The coagulated solid material is essentially amorphous, although powder x-ray diffraction provides evidence of some crystalline material. Crystalline domains may act as noncovalent cross-links, which inhibit movement of polymer chains and lead to rubbery behavior. Thus, the role of *I. alba* extract in the processing of natural latex into rubber involves purification of the *cis*-1,4-polyisoprene rubber by phase separation and by removal of plasticizing agents that inhibit interactions between the polymer chains.

In summary, our data show that *I. alba*-induced coagulation of *C. elastica* latex into a tough, compliant, elastic rubber arises from a combination of two phenomena: (i) purification of *cis*-1,4-polyisoprene by means of separation of the aqueous phase and dissolution of plasticizing organic species, which leads to solidification and chain entanglement; and (ii) an increase in noncovalent and covalent (cross-linking) interchain interactions due to reactions with organic sulfonyl chloride or sulfonic acid species present in *I. alba*. Mesoamerican people's successful experiments with this plant, which altered the mechanical properties of latex, led to the emergence of the ball game, a generative and integrating element in ancient Mesoamerican religious, ritual, and political life.

References and Notes

- This date is given for two solid rubber balls excavated at the Manatí site in Veracruz, Mexico [C. Rodriguez and P. Ortiz, *El Manatí, un Espacio Sagrado de los Olmeca* (Universidad Veracruzana, Jalapa, Veracruz, Mexico, 1994)].
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- Castilla elastica* is native to Mexico south of 21°N through northern South America. *Castilla elastica* grows chiefly in the state of Tabasco, Yucatán, and Chiapas at altitudes of 700 m or lower [P. C. Standley, *Trees and Shrubs of Mexico* (Government Printing Office, Washington, DC, 1920)].
- Ipomoea alba* is also known as *Calonyction aculeatum* and *Ipomoea bona nox* [S. J. van Ooststroom, *Blumea* **3**, 547 (1940)].
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- These artifacts, in the collections of the Peabody Museum of Archaeology and Ethnology, Harvard University (2), were extremely oxidized, and our pilot study of one produced no useful chemical data.
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- T. Leyenaar, *Ulama: The Perpetuation in Mexico of the Pre-Spanish Ball Game Ullamalitzli* (E. J. Brill, Leiden, Netherlands, 1978).
- The samples were taken from two ancient Manatí balls (MIT 3 and MIT 4) in the form of cones, each with a base radius of 2.3 cm and a height of 7 cm.
- The latex was dried for 4 to 5 days in air in dishes 10 cm in diameter filled to a height of 3 mm.
- Oscillating parallel plate rheometry was performed at room temperature on disks of sample material (25 mm in diameter and 3 mm thick). At room temperature, for a strain sweep of 0 to 1%, $G'_L = 157$ kPa, $G'_R = 30.0$ kPa, $G''_L = 285$ kPa, and $G''_R = 25.8$ kPa (subscripts L and R refer to latex and rubber). For a frequency sweep of 100 rad s $^{-1}$ to 0.01 rad s $^{-1}$ at 0.6% strain, $G'_L = 257$ kPa to $G'_L = 110$ kPa, $G''_L = 50.3$ kPa to $G''_L = 30.9$ kPa, $G'_R = 335$ kPa to $G'_R = 212$ kPa, and $G''_R = 48.5$ kPa to $G''_R = 17.4$ kPa. These values may be compared with the shear modulus of modern vulcanized rubber ($G' = 3.34 \times 10^3$ kPa at 30°C) [C. D. Hodgman, R. C. Weast, S. M. Selby, Eds., *Handbook of Chemistry and Physics* (Chemical Rubber, Cleveland, OH, 1958), p. 1553]. Polymer glass transition temperatures (T_g) determined by differential scanning calorimetry (DSC) are indicative of the mechanical behavior of polymers, and a rubbery material typically has a T_g significantly lower than its working temperature. DSC was performed on dried latex and on processed rubber (from -100°C to 300°C at a heating rate of 10°C min $^{-1}$). The T_g values of the unprocessed and processed Chiapas latex are similar [T_g (dried latex) = -63°C; T_g (rubber) = -62°C], and they do not account for the macroscopically observed difference in mechanical properties.
- I. Kelly, in *Notes on Middle American Archaeology and Ethnology* (Division of Historical Research, Carnegie Institution of Washington, Cambridge, MA, 1943), vol. 1, no. 26, p. 164.
- The principal resonances in the ^{13}C NMR and ^{13}C CP NMR spectra appear at 135 ppm (C $^\alpha$), 126 ppm (C $^\beta$), 33 ppm (C $^\gamma$), 27 ppm (C $^\delta$), and 24 ppm (C $^\epsilon$); carbon designations refer to *cis*-1,4-polyisoprene (Fig. 1).
- The appearance of a new resonance at 40 ppm and broadening at the base of the peaks in the 15- to 35-ppm region are consistent with the presence of *trans*-1,4-polyisoprene [134 ppm (C $^\alpha$), 124 ppm (C $^\beta$), 40 ppm (C $^\gamma$), 30 ppm (C $^\delta$), and 16 ppm (C $^\epsilon$)]; isomer-

ization from the *cis* to the *trans* form is slow but thermodynamically favorable.

- The ^{13}C CP NMR spectrum of the unprocessed latex contains resonances (77 ppm, 117 ppm, and 173 ppm) that are not present in the ^{13}C NMR spectrum. The resonance at 173 ppm is characteristic of amide carbons in a polypeptide chain.
- If inorganic sulfur were present as a cross-linking species, the resonances of the corresponding sulfur-bound carbon atoms should appear at 56 to 58 ppm, 35 to 45 ppm, and 13 to 16 ppm, but no such peaks were observed. Similarly, the sulfur content was not significant in the energy-dispersive x-ray analysis of the latex and processed rubber samples.
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- In our laboratory, 20 ml of the juice from the pressed morning glory vine was extracted three times with 20 ml of diethyl ether. The ether was removed in vacuo from the combined organic phases. The product was redissolved into toluene, purified with activated carbon, and dried with magnesium sulfate. The toluene solution was evaporated to dryness, yielding 5 mg of yellow oil. The oil was redissolved in a small amount of acetone and dispersed in 30 ml of water, and the acetone was removed in vacuo [S. G. Wildman, A. V. McMullan, R. Griggs, *Science* **97**, 471 (1943)].
- To evaluate the coagulative properties of the oil, we treated 10 ml of latex with the aqueous dispersion of the oil (0.7 mg ml $^{-1}$) but did not observe coagulation, and the latex appeared unchanged. In the work of Wildman *et al.* (21), the acetone used to prepare the aqueous dispersion of the oil was ostensibly removed by mild heating before use of the dispersion for latex coagulation. We observed that the addition of acetone induces latex coagulation. Because we found that our aqueous dispersion of morning glory oil did not coagulate latex, residual acetone likely was present in the aqueous dispersion used by Wildman *et al.* to induce coagulation; that is, the behavior they observed was due in part to the presence of acetone. At this time, the reason for the apparent inertness of our morning glory oil is not clear, but it is possible that the oil does not contain the same purported active species or that the coagulation phenomenon observed by Wildman *et al.* was due to the presence of acetone.
- The most intense peaks in the ^1H NMR spectrum in CDCl $_3$ were a sharp singlet at 1.25 ppm and a broad singlet at 1.29 ppm. The GC-MS data showed fragmentation of a (CH $_2$) $_6$ chain, and the isotopic ratios of coupled peaks were consistent with the presence of sulfur in these fragments. The presence of multiple, closely related species made definitive peak assignments difficult, but the spectra clearly indicated the presence of methylene and methyl groups within aliphatic chains. These methods and thin-layer chromatography (TLC) indicated that at least three different, closely related species were present in the oil. However, these species appeared to react with the chromatographic silica and could not be purified by TLC.
- The FTIR spectrum of the neat oil confirmed the presence of aliphatic methylene groups (2930 cm $^{-1}$, 2925 cm $^{-1}$, 2857 cm $^{-1}$) and methyl groups (1460 cm $^{-1}$). A carbonyl group (1733 cm $^{-1}$, 1641 cm $^{-1}$), a sulfonyl chloride group (1387 cm $^{-1}$, 1189 cm $^{-1}$), and a sulfonic acid group (1221 cm $^{-1}$, 1097 cm $^{-1}$) were also apparent.
- Cyclization of 1,4-polyisoprene can be initiated by sulfonic acid or hydrolyzed sulfonyl chloride. Protonation of an alkene group in the polymer chain produces a carbonium ion, which can undergo electrophilic addition to the next alkene group in the polymer chain (intramolecular reaction), leading to cyclization. The analogous intermolecular electrophilic addition reaction of the carbonium ion to an alkene group on an adjacent polymer chain would lead to formation of a cross-link [G. Odian, *Principles of Polymerization* (Wiley, New York, ed. 3, 1991)]. Reactions of sulfonyl chlorides with olefin groups to form β -halo sulfones is also known [J. March, *Advanced Organic Chemistry* (Wiley, New York, 1992)]. No spectroscopic evidence of other common known cross-linking agents for 1,4-polyisoprene, such as or-

ganic peroxides, nitro compounds, azo compounds, or inorganic sulfur, was detected.

26. J. I. Kroschwitz Ed., *Encyclopedia of Polymer Science and Engineering* (Wiley, New York, 1985).
27. This behavior is consistent with our observation that the addition of polar organic solvents such as acetone or ethanol to the latex emulsion induces latex coagulation, but addition of water alone does not produce this effect (26).

28. We thank Mexico's Consejo de Arqueología at the Instituto Nacional de Antropología e Historia (INAH) and J. García-Bárcena, C. Rodríguez, and P. Ortiz of INAH for permissions to perform this study; in Chiapas, J. Gasco, F. Guillen, L. Guillen, and A. Castañeda;

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Requirement for Croquemort in Phagocytosis of Apoptotic Cells in *Drosophila*

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Macrophages in the *Drosophila* embryo are responsible for the phagocytosis of apoptotic cells and are competent to engulf bacteria. Croquemort (CRQ) is a CD36-related receptor expressed exclusively on these macrophages. Genetic evidence showed that *crq* was essential for efficient phagocytosis of apoptotic corpses but was not required for the engulfment of bacteria. The expression of CRQ was regulated by the amount of apoptosis. These data define distinct pathways for the phagocytosis of corpses and bacteria in *Drosophila*.

Phagocytosis is the terminal event of the apoptotic process (1, 2) and is also critical for the engulfment of microorganisms (3). It has been proposed that the recognition of both nonself (microorganisms) and effete self (corpses) may share common receptors (4). Blocking experiments have implicated a

number of receptors as important for target recognition (2–4). Genetic studies indicate that some of these receptors participate in phagocytosis of pathogens in vivo (5, 6). However, the multiplicity and redundancy of recognition mechanisms in mammalian systems have made it difficult to evaluate the

relative roles of these receptors in the phagocytosis of corpses. Although several genes of *Caenorhabditis elegans* are involved in the phagocytosis of corpses (7–9), none of these molecules seem to act directly as a receptor in the recognition of the corpse.

In *Drosophila* embryos, like in mammals and in contrast to worms, the clearance of apoptotic cells is primarily mediated by macrophages, hemocytes that become phagocytic at the initiation of developmentally regulated apoptosis (10). Croquemort (CRQ), a *Drosophila* CD36-related receptor, is specifically expressed on all embryonic macrophages (11). Human CD36 acts as a scavenger receptor (12–14) and also binds apoptotic cells in combination with the macrophage vitronectin receptor and thrombospondin (15, 16). CD36 has the ability to confer phagocytic activity on nonphagocytic cells on transfection (17, 18). CRQ expression in nonphagocytic Cos7 cells allows these cells to recognize and engulf apoptotic thymocytes (11). Thus, CRQ may participate in the removal of apoptotic cells during *Drosophila* embryogenesis. We genetically evaluated the relative

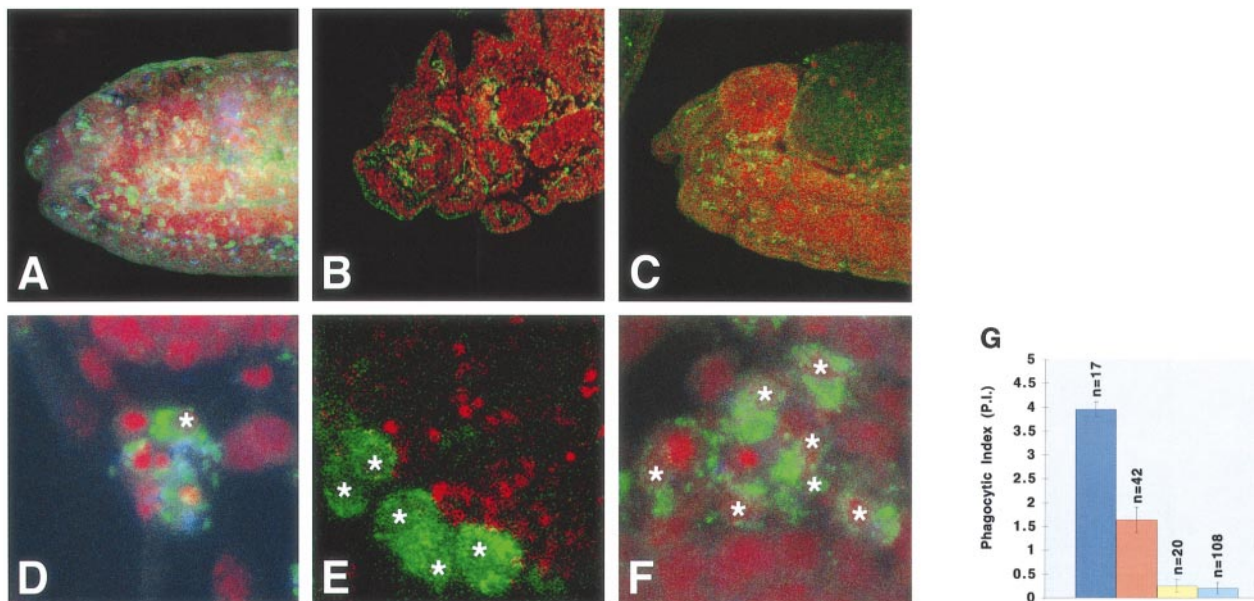


Fig. 1. Macrophages in *crq*-deficient embryos have very poor phagocytic activity for apoptotic cells. (A to F) In confocal micrographs, peroxidase-stained hemocytes appear green, CRQ staining appears blue, 7-AAD-stained apoptotic corpses appear as bright red round particles, and the nuclei of viable cells appear as large red diffused components. All images are the sum of eight focal planes. (A) to (C) show a $\times 40$ magnified lateral view of the head region of (A) a *In(2L)Cy* homozygous embryo, (B) a *Df(2L)al* homozygous embryo, and (C) a *W88* homozygous embryo. (D) to (F) show high-magnification views ($\times 400$) of their respective macrophages. As compared with the wild-type distribution (A) and phagocytic

activity (D) of macrophages within *In(2L)Cy* homozygous embryos, macrophages in *Df(2L)al* (B) and *W88* (C) homozygous embryos accumulate in the head and around the amnioserosa and show very poor phagocytic activity despite their recruitment at sites of abundant apoptosis (E and F). Asterisks indicate the nucleus of each macrophage seen in these fields. (G) A chart summarizes the efficiency of phagocytosis of apoptotic corpses observed within each genotyped embryos assayed. Results shown are the mean P.I. \pm SE; n is the total number of macrophages scored for each genotype. Dark blue, w; *In(2L)Cy/In(2L)Cy*; red, w; *In(2L)Cy/Df(2L)al*; yellow, w; *Df(2L)al/Df(2L)al*; and light blue, w; *W88/W88*.