Secretion of Benzaldehyde and Hydrogen Cyanide by the Millipede Pachydesmus crassicutis (Wood)

Abstract. The millipede *Pachydesmus crassicutis* (Wood) secretes a mixture of benzaldehyde and hydrogen cyanide when it is disturbed. These compounds are secreted through paired glands located on 11 of the notal projections of adult millipedes. Experiments with the imported fire ant demonstrate that these natural products serve a defensive function.

Pachydesmus crassicutis (Wood) is a large millipede whose known distribution is limited to Louisiana and southern Mississippi. When disturbed, this arthropod emits a highly persistent, pungent aromatic secretion. This secretory behavior is found frequently among many species of the order Diplopoda, and it is presumably defensive in function.

The secretion of *Pachydesmus* is ejected through small openings on the dorsal surface near the tips of some of the paired notal projections. These projections are present on all body segments. Secretory ducts are located on segments 5, 7, 9, 10, 12, 13, 15, 16, 17, 18, and 19 in both adult males and females. The immature stages of this millipede also discharge the odoriferous secretion. Associated with each secretory duct are paired glands which are similar in general make up to those which have been found in other millipedes (*1*).

The water-clear secretion of *Pachydesmus* was collected by touching the dorsal surface of the notal projections with small squares of filter-paper which rapidly absorbed the liquid discharge. The impregnated squares were immediately placed in methylene chloride for chemical analysis. By means of infrared spectra, gas chromatography, and the preparation of a chemical derivative, the major component of the liquid secretion of *Pachydesmus* has been identified as benzaldehyde. In addition, the secretion contains hydrogen cyanide and carbohydrates.

Infrared spectra of methylene chloride solutions of the secretion demonstrated the presence of a carbonyl compound. A strong carbonyl band at 5.92 μ along with two bands at 3.59 and 3.68 μ indicated that this substance was aldehydic (*2*). A strong band consistent with the hydrogen deformation of aldehydes was also present (12.15 μ) as well as medium to strong bands at 7.69, 8.36, and 8.61 μ which could correspond to C—C stretching in aromatic compounds (*2*).

Gas chromatographic analysis of the methylene chloride secretion at 200°C with a column (¼ inch diameter and 18 feet long) of 25 percent cross-linked diethyleneglycol adipate poylester which had been treated with 2 percent phosphoric acid on Chromosorb W (75 cm^3/min helium flow rate) gave a single peak. The characteristic odor associated with *Pachydesmus* was discernible at this first stage of analysis. The material from this peak was trapped with liquid nitrogen and identified by its orange 2,4-dinitrophenylhydrazone, which, recrystallized from aqueous (1 : 3) methanol, melted at 236° to 237°C. In admixture with the 2,4-dinitrophenylhydrazone of benzaldehyde (*3*) the melting point was not depressed. Both phenylhydrazones had identical infrared spectra. When analyzed by gas chromatography, the secreted compound had the same retention time as benzaldehyde.

The presence of a volatile acid in the secretion was demonstrated by the discoloration of congo red paper impregnated with alkali mercuric chloride (*4*). This acid was identified as hydrocyanic acid by its ability to form a colored cyanide complex with nickel and alkali palladium dimethylglyoxime (*4*).

Millipedes have been the subject of some of the earliest investigations on the natural products chemistry of arthropods. Hydrogen cyanide was one of the first compounds to be identified from millipedes, and it is now known to be a common secretory product (*5*). Several carbonyl compounds have been identified in millipede secretions, principally substituted *p*-benzoquinones, not aldehydes (*6*). However, salicylaldehyde has been identified in several coleopterous secretions (*7*). Hydrocyanic acid has been identified in all stages of zygaenid moths, and it is believed to have a defensive function against vertebrate predators (*8*).

Benzaldehyde is commonly found in the plant kingdom. It is one of the main odor constitutents in plum and chrysanthemum flowers (*9*). In some plants, benzaldehyde is found combined in cyanogenic glycosides such as amygdalin, lotusin, prulaurasin, prunasin, or sambunigrin (*10*). β-Glucosidases such as the emulsin complex are capable of hydrolyzing these cyanogenic compounds into benzaldehyde, hydrogen cyanide, and a carbohydrate component which is often glucose (*11*). Cyanogenic glucosides may also be the source of hydrogen cyanide and aromatic aldehydes in millipedes, and a cyanogenic glucoside which contained cuminaldehyde, hydrogen cyanide, and glucose was isolated from the millipede *Polydesmus vicinus* (L.) (*12*). Similarly, in addition to benzaldehyde and hydrogen cyanide, the secretion of *Pachydesmus* contains carbohydrates, of which one, by chromatography, has the same properties as glucose whereas the other behaves like a disaccharide.

It is worth while to note that the *p*-benzoquinones secreted by the roach *Diploptera punctata* (Eschscholtz) are produced by glands which contain a strong β-glucosidase which presumably hydrolyzes the glucoside which contains the benzoquinone (*13*). Similarly, the tanning of the ootheca by *o*-quinones which are present in the cockroach *Periplaneta americana* (L.) is initiated by the action of a β-glucosidase (*14*). The enzyme and glycoside of *P. americana* are present in separate glands, which could explain the function of the double gland structure associated with the secretory system in *Pachydesmus* and other millipedes.

The aldehydic secretion of *Pachydesmus* is not forcibly discharged but issues as an ooze on the notal projections. When these millipedes were placed on filter papers impregnated with 2,4-dinitrophenylhydrazine, the secretion, which was detected as orange spots, was only observed directly below the stimulated notal areas. The secretion of *Pachydesmus* is usually discharged only from the segments that are stimulated; it does not become general until the millipede is highly irritated.

The defensive function of the *Pachydesmus* secretion was demonstrated by experiments with imported worker fire ants (*Solenopsis saevissima* v. *richteri* Forel). The millipede discharged its secretion only after contact from the workers and only in the segmental areas involved, and thus caused the ant workers to withdraw from the millipede in a high state of excitation. Such ants frequently performed cleansing movements. The millipede was then immune to immediate further attack, possibly because of the residual benzaldehyde still on its body. On the other hand, millipedes whose secretion had been exhausted by previous stimulation were highly susceptible to attack by the fire ant.

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Marrow Chimerism in Marmosets

Abstract. In the femoral marrow of three adult marmosets, two male *Callithrix jacchus* and one female *Leontocebus rosalia,* a number of opposite-sexed metaphases were found. It is inferred that this chimeric state resulted from intrauterine placental anastomoses between heterosexual twins. The lack of the freemartin effect in connection with this chimerism is discussed, and the structural nature of the Y chromosome in the Callithricidae is described.

The mosaic constitution of circulating blood in twin cattle was first demonstrated by Owen (*1*). It is the result of placental anastomoses between fraternal twins through which hematopoietic precursor cells are exchanged in embryonic life. These immature elements settle in the fraternal twin partner and, by virtue of aquired tolerance, continue to propagate throughout life in the new host and are instrumental in producing reciprocal tissue tolerance (*2*). If the metaphases are opposite-sexed, the resulting population of red cells may be detected by blood typing.

Similar chimerism has been demonstrated on rare occasions in human fraternal twins (*3–5*), but placental anastomoses are extremely uncommon in human fraternal twins (*3*) if they occur at all—they have never been demonstrated unequivocally (*6*). Wislocki (*7*) examined the placentas in 19 marmoset pregnancies and reviewed previously recorded observations. Of 40 pregnancies, 87.5 percent were twin pregnancies. Wislocki's data indicate that fraternal twinning is the rule in marmosets and that early fusion of the blastocysts results in the establishment of placental anastomoses between the twins. Despite these vascular channels, he points out, in marmosets the female partner of heterosexual twins is never a freemartin, whereas in cattle it usually is (*8*).

Ryan *et al.* (*9*) have recently presented support for their hypothesis that the enzymic constitution of the primate placenta may explain the nonoccurrence of freemartins in marmosets and in the rare instances of blood chimerism in man. According to their view in the primate the placenta may be able to convert the male gonadal hormones passing through it to estrogens, a faculty that the cattle placenta has not been shown to possess.

Despite the gross appearance of anastomotic channels in the marmoset placenta (*7, 9*), actual mixing of fetal blood has not yet been demonstrated. This report describes the occurrence of chimerism in the bone marrow of three adult marmosets, which is held to be the result of prenatal exchange of circulating hematopoietic tissue (*10*).

Six marmosets were available for study, but only five of these could be utilized. The mature animals [four male *Callithrix* (or *Hapale*) *jacchus,* one male and one female *Leontocebus rosalia*] were injected intramuscularly with colchicine (0.07 mg per gram of body weight) 5 to 6 hours prior to sacrifice through intraperitoneal injection of Nembutal. The male *L. rosalia* died, before marrow studies could be made, from infection with *Prosthenorchis elegans* (*11*); however, chromosome preparations were made from tissue cultures of the kidney. In the other five animals both femora were removed and the marrow was aspirated and placed for 30 minutes in 7 ml of hypotonic solution (Earle's solution and water, 1:4). After centrifugation the cells were fixed in a mixture of acetic acid and methanol (1:30) and subsequently treated as leukocyte cultures according to the method of Moorhead *et al.* (*12*). The air-dried cells were stained with aceto-orcein, and random mitoses were photographed by an individual who had no concern with the outcome of the experiment, selection being made only to obtain well-spread, intact cells. The sex chromosomes were identified by karyotyping the males, and subsequently karyotypes were prepared for doubtful cells, as indicated in Table 1.

The results of chromosomal analysis in five animals are shown in Table 1. In two of the five marmosets (M_3, M_4) all the cells analyzed contained the XY sex chromosomes expected in male monkeys, while in the three other marmosets cells were chimeric with respect to the sex chromosomes. Numerous polyploid cells were seen in M_6, few in the other animals. Figures 1 and 2 are representative male karyotypes for the two genera. As may be seen (Fig. 1), the Y chromosome of *Callithrix jacchus* is exceedingly small and thus is readily recognizable in well-spread metaphases. Consequently, chimerism in this genus is easily established through analysis of metaphase plates. The Y chromosome of *Leontocebus rosalia* (Fig. 2) is a small metacentric element, and karyotype analysis was more important in this genus for recognizing the chimeric nature of the animal's marrow. It is of course possible that M_3 and M_4 as well as the other three marmosets of Table 1 were members of pairs of fraternal twins but that their chimeric constitution could not be recognized because of the possibly isosexual nature of the twins. Alternatively, these animals could have been singletons, although Schultz (*13*) finds that twin pregnancy is the rule in marmosets in the wild state, singletons having been born only in captivity.

These results indicate prenatal exchange of marrow elements among heterosexual twins in marmosets; however, it is impossible to assess adequately the quantitative aspects of admixture, whereas this has been possible in the human chimeras. While it is not likely that selection of the metaphases in this study was biased, the number of metaphases analyzed is probably too small to give representative results. Thus, the findings of an 18-percent admixture of cells from the fraternal twin in M_2 and of a 34-percent admixture in M_6 may not indicate the true state of chimerism in these animals. Likewise, the finding of occasional "drumsticks" in polymorphonuclear leukocytes of the male chimeric monkeys is inadequate as a basis for quantitative assessment of the degree of chimerism, whereas a finding of "drumsticks" did provide such a basis in the studies of human blood chimerism reported by Woodruff (*14*). In Woodruff's studies, in the members of one set of heterosexual twins with chimer-