was also abundant on Hevea flowers. Thrips were found to be so numerous during the course of these investigations that probably very few flowers escaped their visits. There are indications, however, that thrips are not as important pollinators of Hevea as are the midges. It is clear that thrips are not responsible for leaving the hairs on the stigmas. Although placed on the stigmas and roughly rubbed back and forth with a dissecting needle, several live thrips failed to leave any hairs on the sticky surfaces. Thrips also were observed to carry very little pollen on their bodies, and this was usually stuck in masses of latex with which the insect had come in contact. Pollen so held is extremely difficult to dislodge. Finally, this species of thrips appears to move largely by crawling and hopping and to have little or no capacity for sustained flight. This suggests that any pollination accomplished by thrips would likely be from pollen of the same inflorescence (self-pollination). In fact, the stigmas observed to have pollen grains but not hairs may be in part the result of thrips' activity. This viewpoint is strengthened by the observation that stigmas with pollen grains only are less adequately pollinated than those with both hairs and pollen (22 stigmas with pollen only had an average of 4 grains/stigma, as compared with more than 16 grains/stigma for 38 having both hairs and pollen).[5]

The various small flies caught in the adhesive on the cards and petals, though numerous, are not believed to be of importance in pollination. They were never seen to be carrying pollen on their bodies, and none was ever observed to enter a female flower. Any pollination accomplished by them would seem to be accidental rather than systematic.

Pollination in cacao (*Theobroma cacao*), prior to the recent work in Trinidad, had been about as much of a puzzle as that of Hevea, and it is interesting that eventually midges of the same family as those described in the present report were found to be the effective pollinating agents. Posnette (*5*) showed beyond any question that heleid midges (identified as *Forcipomyia quasi-ingrami* Macfie, *Lasiohelea nana* Macfie, and *L. stylifer* Lutz [*6*]) are the chief pollinators of cacao in Trinidad. Midges of this group are thus known to carry pollen and to be effective pollinating agents in another plant species.

The habits and life histories of the heleid midges are not well known. Some species have aquatic larvae, and others are thought to breed in damp soil or decaying organic matter. The breeding places of the midges found in the Hevea plantings at Mayaguez are not known; nor is it known whether these insects are of importance in Hevea pollination outside Puerto Rico. The family has a wide distribution, however, and it is possible that their habits and small size (about 1 mm in length) may have caused them to be overlooked in previous pollination studies. Actually, at no time during the present studies have these insects been observed in flight around Hevea inflorescences. If the hairs or bristles had not accidentally been found on the stigmas and the midges identified from this clue, their potential role in pollination might not have been discovered.

[5] That thrips are responsible for some pollination in Hevea seems certain after a recent observation. Female flowers tightly covered with soda straws immediately before anthesis sometimes were found to have pollen grains on their stigmas when carefully examined some days later. An occasional thrips was found inside such covered flowers, and it is highly unlikely that any other insect could have gained access to these stigmas.

References

1. MORRIS, L. E. *J. Rubber Research Inst. Malaya,* **1**, 41 (1929). (Repr. in *Trop. Agr. (Ceylon),* **72**, 135 (1929).
2. MUZIK, T. J. *Science,* **108**, 540 (1948).
3. MAAS, J. G. J. A. *Arch Rubbercult. Nederland.-Indië,* **3**, 288 (1919).
4. SEIBERT, R. J. *Ann. Missouri Botan. Garden,* **34**, 261 (1947).
5. POSNETTE, A. F. *Trop. Agr. (Trinidad),* **21**, 115 (1944).
6. MACFIE, J. W. S. *Bull. Entomol. Research,* **35**, 297 (1944).

Rate of Circulation of the Body Fluid in Adult *Tenebrio molitor* Linnaeus, *Anasa tristis* (de Geer), and *Murgantia histrionica* (Hahn)

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The rate of circulation of body fluid in insects and the time for an introduced material to become homogeneously mixed with it are important factors in certain studies in insect physiology and toxicology. Only brief, incidental references to the rate of circulation of insect body fluid have been found in the literature (*1–5*). Much more is known about the rate of movement and mixing of mammalian blood. The times for blood movement between comparable points in various mammals are: rabbit, 7.5 sec; dog, 16 sec; man, 23 sec; and horse, 28.8 sec (*6*). The time for complete mixing of the blood in man has been estimated to be between 2 and 4 min (*7–9*). The time for complete mixing of the blood in dogs is about 5 min (*10*).

Since insects have an open circulatory system, it may be more nearly correct to speak of the time for uniform mixing of insect blood rather than of the time for a complete circuit of any portion of it. One of the standard (and best) ways to determine the time of circulation of the blood of an animal is to introduce a substance whose concentration can be determined in a small portion of the blood. Elements such as phosphorus, which are normal constituents of insect blood, and which can be made radioactive, make it possible to use this method on even the smaller insects.

The usual path of the circulating blood in an insect is anteriorly through the dorsal vessel and posteriorly through the ventral portion of the body cavity (*11, 12*). A substance injected near the posterior end of the heart would be expected to reach first the wings, then the antenna, and first, second, and third pairs of legs in order. As the blood containing the injected substance reaches an appendage, e.g., the antenna, the amount present will increase to a maximum in each of the pair and then decrease as unadulterated blood fol-

lows. If we can find the time of this maximum we know the time for circulation. In the open circulatory system of an insect there is considerable mixing, and the maximum is not sharp, so that all one usually finds is a time beyond which no further increase in concentration of added substance occurs. This time for reaching maximum concentration can be found by removing one appendage of a pair and later, after mixing is complete, removing the other. If the concentrations are the same in the two appendages, the maximum occurred at or before the time of removal of the first appendage. If the first removed appendage has a lower concentration the time of maximum had not been reached.

The speed of mixing in the blood of an injected solution containing radiophosphorus was determined in the adults of three species of insects: the yellow meal worm, *Tenebrio molitor* Linnaeus; the squash bug, *Anasa tristis* (de Geer); and the harlequin cabbage bug, *Murgantia histrionica* (Hahn). Adults were injected with a 3% aqueous solution of Na_2HPO_4 containing radiophosphorus,[1] using a mounted microinjector. The solution used had a radioactivity of 100–200 μc/ml. About 0.001 μc was needed for a satisfactory test with the counter, and 0.1–0.6 μc was injected, in 1–3 μl of solution. All injections were made at room temperature. At various times after injection, an appendage (leg, antenna, or wing) was cut off, and the amount of radiophosphorus present was determined with a Geiger-Mueller counter. After a time long enough to ensure complete mixing of radiophosphorus in the blood, the corresponding appendage on the other side of the insect was removed and its content of radiophosphorus was determined. If the first appendage of a pair had less radiophosphorus than the second, the former was presumed to have been cut off before the injected solution was evenly distributed in the body fluid. If the same radioactivity was found in the two appendages, distribution of the radiophosphorus was considered to have been complete before the first was removed. It was shown that the radiophosphorus would reach corresponding appendages on each side of the insect at essentially the same time. In 10 experiments corresponding appendages from both sides were cut off simultaneously to check the assumption that the injected solution would reach them at the same time. The greatest percentage of deviation from the average radioactive content of a given pair of appendages was 30%; the least, 4%; the average, 12.5%.

Experiments showed that neither the presence of radiophosphorus nor the injection techniques caused any obvious departure from the normal in the insects used. Adult *T. molitor* that had been fed radiophosphorus as larvae reproduced. Seven adult *T. molitor* injected with 2 μl or more of the radiophosphor-solution survived on the average as long as 3 insects that were not injected. The rate of heart beat did not change significantly in at least 10 of 14 adult *T. molitor* injected with water, radiophosphorus solution, or a saline solution.

[1] Obtained through the courtesy of the Radiation Laboratory of the University of California in 1939.

TABLE 1

TIME FOR UNIFORM MIXING OF INJECTED RADIOPHOSPHORUS

Insect species and site of injection	Appendage									
	Wing*		Antenna*		First leg*		Second leg*		Third leg*	
Murgantia histrionica Posterior abdomen venter	—	14	15	15	16	17	23	22	21	22
Murgantia histrionica Anterior abdomen venter	10	14	18	15	19	23	24	25	23	25
Murgantia histrionica Head vertex	23	18	2	1.5	7.5	7.2	15	14	19	19
Anasa tristis Posterior abdomen venter	—	—	25	25	27	23	32	32	35	33
Tenebrio molitor Posterior abdomen venter	—	—	—	—	7	6.5	10	6.5	5	—
Tenebrio molitor Posterior abdomen dorsum	2	14	—	—	4.5	8	—	—	—	—

* First figure of each pair is maximum time in minutes at which mixing was not complete. Second figure is minimum time at which mixing was complete.

The results are shown in Table 1. It is to be expected that, since different individuals were used in each experiment, the minimum time in one insect may exceed the maximum time in another insect of the same species. The "average" time needed for complete mixing lies somewhere between the two values. The data showed that in *M. histrionica* and *A. tristis* radiophosphorus reached different appendages at different times. The injected solution usually reached the appendages in the following sequence: wings, antennae, and first, second, and third legs. The starting point of this sequence varies with the site of injection. Fluctuation in the radioactivity of appendages cut off before a maximum amount of radiophosphorus had reached them may occur, but in most experiments there were no significant fluctuations in radioactivity after the maximum had been attained. Apparently, therefore, the time for a complete circulation of the body fluid and the time for complete mixing of the injected solution were the same.

M. histrionica were injected in three places in different experiments, ventrally at the anterior and posterior ends of the abdomen, and at the vertex of the

head. The sequence in which appendages received the maximum radiophosphorus content was the same regardless of the point of injection; the wings were reached by the radiophosphorus first after injection in the abdomen and last after injection in the head. When the insect was injected in the head the first pair of legs consistently showed a decrease after an initial rise to a maximum radiophosphorus content. Apparently the injected solution was very poorly mixed with the body fluid in so short a distance. Complete mixing of the body fluid required about 25 min. In *A. tristis* the order in which the radiophosphorus reached the appendages was the same, with a time to a maximum of about 35 min. Not enough experiments were performed with *T. molitor* to show the sequence in which the radiophosphorus reached the appendages. The time for uniform mixing can be estimated at 8–10 min.

References

1. PAWLOWA, M. *Zool. Anz.*, **18**, 7 (1895).
2. BROCHER, F. *Arch. zool. exptl. et gén.*, **55**, 347 (1916).
3. SNODGRASS, R. E. *Anatomy and Physiology of the Honeybee.* New York: McGraw-Hill (1925).
4. FREUDENSTEIN, K. *Z. wiss. Zoöl.*, **A132**, 404 (1928).
5. GEROULD, J. H. *Acta Zoologica*, **19**, 297 (1938).
6. HOWELL, W. H. *A Text-Book of Physiology.* Philadelphia: Saunders (1940).
7. HALDANE, J., and SMITH, J. L. *J. Physiol. London*, **25**, 331 (1900).
8. PLESCH, J. *Z. exptl. Path. Therap.*, **6**, 380 (1909).
9. DOUGLAS, C. G. *J. Physiol., London*, **40**, 472 (1910).
10. MILLER, A. T., JR. *Am. J. Physiol.*, **151**, 234 (1947).
11. SNODGRASS, R. E. *Principles of Insect Morphology.* New York: McGraw-Hill (1935).
12. WIGGLESWORTH, V. B. *The Principles of Insect Physiology.* New York: Dutton (1938).

An Instance of the Occurrence of Carcinogenic Substances in Certain Barnacles[1]

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The chromatographic fractionation of some extracts obtained from a sample of the thatched barnacle (*Tetraclita squamosa rubescens*) showed on lime and alumina columns the presence of several zones that displayed intense blue fluorescence in ultraviolet light. Some of the fractions were crystallized and gave in hexane solution extinction curves that were typical for polycyclic aromatic hydrocarbons. The carbon and hydrogen content of one of these fractions (5 mg from 1 kg barnacles), as well as the observed molecular weight, corresponded to the values calculated for benzpyrenes. Furthermore, a spectroscopic examination, although also indicative of accompanying isomers or close analogs, demonstrated the presence of 3,4-benzpyrene in the crystalline mixture. Consequently, this particular sample was tested on mice for carcinogenic activity.

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The material was dissolved in tricaprylin, 5 mg/ml. Twelve male C_3H mice, 3 months old, received a single subcutaneous injection of 0.5 mg in 0.1 ml; 12 additional mice were injected with 0.25 mg in 0.05 ml. The mice were maintained on Purina dog chow and an unlimited supply of water. They were examined weekly for the presence of progressively growing tumor at the site of injection.

Four of 12 mice receiving 0.5 mg of the material developed subcutaneous tumors in 16, 17, 19, and 20 weeks following injection. Two of 12 mice receiving 0.25 mg developed tumors in 17 and 19 weeks. The remaining 18 mice were alive and free of tumor 36 weeks after injection.

The mice with tumors were sacrificed when the tumors reached 1–2 cm in diameter. On histologic examination, all 6 were seen to be spindle-cell sarcomas with local invasion of areolar and muscular tissue. Morphologically they were indistinguishable from tumors induced with 3,4-benzpyrene and other polycyclic carcinogenic hydrocarbons (*1*). The first tumor to be noted was transplanted into six C_3H mice and grew vigorously within 10 days, maintaining its sarcomatous appearance.

Previous data (*2, 3*) showed that 80–90% of C_3H male mice developed sarcomas within 20 weeks after the subcutaneous injection of 0.25–0.5 mg of 3,4-benzpyrene dissolved in tricaprylin. The incidence of approximately 25%, and the longer latent period of the tumors in this investigation, suggest that the material tested contained 10–40% of the active carcinogen, assuming that 3,4-benzpyrene was the only such compound present and that other substances in the sample did not alter the carcinogenic reaction. The presence of an active carcinogen was unquestionably demonstrated.

Comparative work on barnacles has shown that the polycyclic aromatic hydrocarbons do not constitute normal metabolic products but may reach these organisms accidentally. The possibility of tarry materials, from ships or submarine oil wells, being carried to the filter-feeding intertidal sedentary animals constitutes a potential external source for aromatic polycyclic hydrocarbons. We may also mention that the wooden pilings from which the material was collected at Corona del Mar, Calif., had been given a surface creosoting 10 years previously.

It has been observed that the goose barnacle (*Mitella polymerus*), collected from another habitat (on and among the mussels growing on the pier pilings at the Scripps Institution of Oceanography, La Jolla, Calif.), also yielded a fluorescent fraction whose extinction curve was indicative for the presence of 3,4-benzpyrene, although the quantity of this fraction was at least 10 times less than mentioned above. In contrast, our fluorescing fractions from *Tetraclita squamosa rubescens*, originating from rocks near La Jolla, were found to be of different nature and free of benzpyrene or similar compounds.