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6. For a general discussion of linear regression and associated statistical techniques, see N. R. Draper and H. Smith [*Applied Regression Analysis* (Wiley, New York, 1966)].
7. An MSE of 190.4 was obtained with a 26-variable model, which included two terms for trend, four pairs of terms for cyclic variation, terms for temperature through 6 days of lag, a three-way interaction term for temperature, and a carbon monoxide term. The coefficient for the logarithm of carbon monoxide was 22.8 ($P < .003$).
8. First-order autocorrelation coefficients were less than 0.05 ($P > .05$) for both models with temperature terms. For the model with trend and cyclic variation terms only, the autocorrelation coefficient was 0.155 ($P < .001$).
9. If the subsamples are regarded as statistically independent, and if the day-of-the-week effect, if any, is a simple displacement of the mean for total mortality, then each of the 28 subsamples provides an independent and unbiased estimate of the same set of regression coefficients. For the model shown in Table 1, the mean coefficient for the logarithm of carbon monoxide is 26.28, t (the ratio of the coefficient to its standard error) = 2.67 ($P < .01$).

10. We thank Dr. W. R. Gaffey and H. K. Ury for many helpful discussions and suggestions during the course of this study. Supported in part by contract PH 86-68-35 with the National Air Pollution Control Administration.

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Chemical Communication and "Propaganda" in Slave-Maker Ants

Abstract. *Slave-maker ants of the* Formica sanguinea *group direct their raids by means of odor trails. Artificial trails made from whole-body extracts and extracts of Dufour's glands and hindguts can be used to guide columns of workers to selected target colonies and to initiate raids. In workers of* F. pergandei *and* F. subintegra, *members of the* F. sanguinea *group, the Dufour's glands are hypertrophied and contain large quantities of three acetates (decyl, dodecyl, and tetradecyl), which are discharged at defending workers during the slave raids. The acetates produce very efficient, long-lasting alarm signals that attract the slave-makers but disperse the defenders; in effect, therefore, they are "propaganda substances."*

Slavery is practiced by species belonging to at least six ant genera: *Leptothorax, Strongylognathus,* and *Harpagoxenus* in the subfamily Myrmicinae; and *Formica, Polyergus,* and *Rossomyrmex* in the subfamily Formicinae. Although most or all of these cases represent independent phylogenetic developments, the basic pattern is the same. The slave-maker workers raid nests of another species in the same or a closely related genus, where they repel or kill defending workers, penetrate their nest, and capture their worker pupae. When adults later eclose from the pupae, they accept the slave-makers as nestmates and readily assist in the domestic work of the slave-maker nest. Workers of the most specialized of the slave-maker species are capable only of conducting raids and are wholly dependent on their slaves for their day-to-day existence.

Previous experimental work indicates that the raids of *Harpagoxenus americanus* (*1*) and *Polyergus lucidus* (*2*) are both initiated and guided by odor trails laid down by scout workers from the target nest back to the home nest of the slave-makers. Neither the glandular source nor the chemical identity of the trail pheromone has been identified in these species. We extended the result to the species of the *F. sanguinea* group in the following way. It was learned that raids could be initiated at the discretion of the investigator during late July and August by placing fragments of colonies belonging to slave species (*F. subsericea*) a short distance from the edge of the slave-maker nests. When slave-maker scouts encountered the colony fragments, they returned to their own nests, apparently laying odor trails. Columns of workers, indistinguishable from those seen during natural raids, immediately emerged and began attacking the colony fragments. They subdued the workers of the slave species and carried their pupae back to the slave-maker nests. We were able to initiate and guide raids in the following way. Whole-body extracts of ten slave-maker workers (*F. rubicunda*) were made in ether and land down with watercolor brushes over the soil, from the nest entrance to selected points about 1 m away. The *F. rubicunda* workers, accompanied by a few adult slaves, followed the trails to the end and milled in confusion in the area beyond. When fresh fragments of slave-species colonies (*F. subsericea*) were now placed at the end of the trail, a full-scale raid ensued. Similar results were obtained with workers from a *F. subintegra* nest. *Formica subsericea* slaves did not accompany their *F. subintegra* mistresses on these artificially induced raids. It was further discovered that *F. subintegra* workers can be easily diverted for distances of up to 1 m or more from raided colony fragments with the use of artificial trails consisting of synthetic acetates in the mixtures found naturally in the Dufour's gland (see further discussion of the chemistry of the secretions below). However, it was not demonstrated that the natural trail substances originate in the Dufour's gland. When a trail made from three combined Dufour's glands was laid in competition with one laid from three combined hindguts, the latter had far greater attracting power. Only a single worker followed the trail made from the Dufour's gland during 5 minutes, whereas over 30 followed the trail made from the hindguts. In view of the fact that the hindgut is the source of recruitment odor trails in other kinds of formicine ants (*3*), we conclude that this organ also produces a trail pheromone in *F. subintegra.* It remains to be determined whether substances from the Dufour's gland, and, in particular, the acetates, also serve as trail substances.

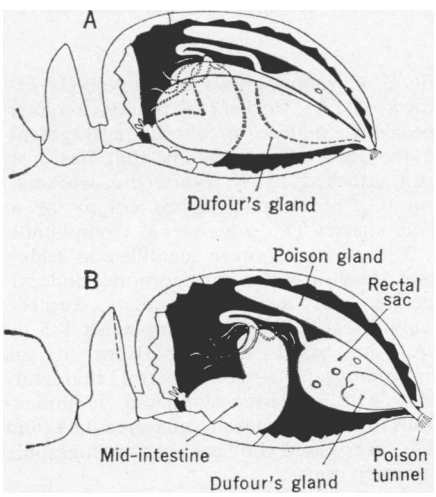

Fig. 1. The abdomens of workers of (A) a slave-maker ant species (*F. subintegra*) and (B) one of the ant species it utilizes as slaves (*F. subsericea*), showing the location of the gut and principal exocrine glands.

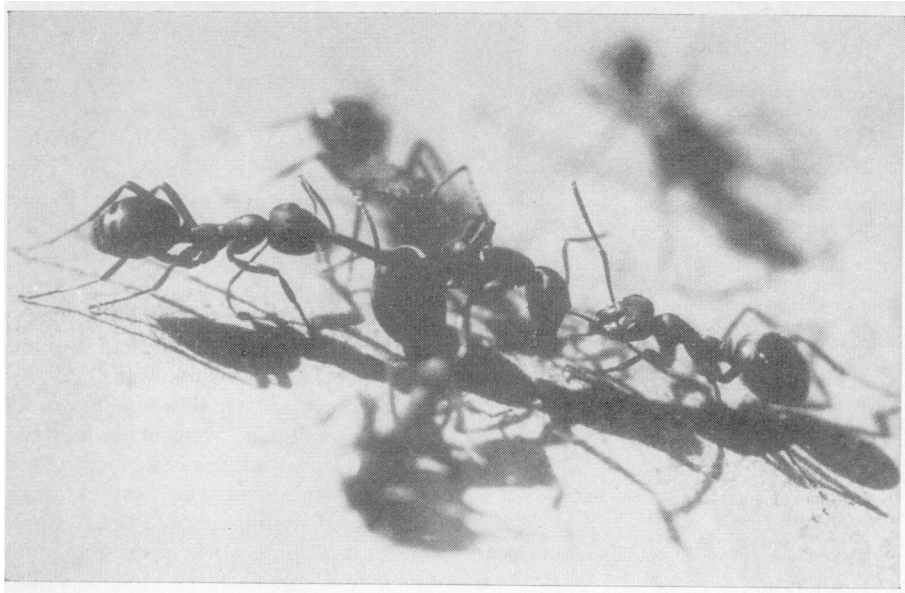

Fig. 2. A slave raid in ants. Workers of the slave-maker species *F. subintegra* are shown attacking a defending worker of the species *F. subsericea* during an artificially induced raid. [Photograph by Bert and Turid Hölldobler]

In two of the slave-maker species of the *F. sanguinea* group we studied, *F. pergandei* and *F. subintegra*, the Dufour's gland is hypertrophied (Fig. 1). Analysis of the contents revealed the presence of a series of esters: decyl acetate, dodecyl acetate, and tetradecyl acetate (*4*, *5*). These substances are present in astonishingly large quantities in *F. subintegra*. Each worker contains approximately 700 μg of these acetates, or 10 percent of its entire body weight. The percentages of these acetates are as follows: decyl, 57.1; dodecyl, 21.8; and tetradecyl, 21.1. The amounts of these esters in *F. pergandei* are much smaller but still substantial relative to that of other formicine species (*6*). The acetates in one worker total about 40 μg, in the following percentages: decyl, 20.5; dodecyl, 53.8; and tetradecyl, 25.7. These substances are discharged at intruders when the nest is disturbed and can serve as defensive substances against arthropod enemies. We broke open a nest of *F. subintegra* and allowed the workers to attack the tips of wooden applicator sticks thrust at them. The sticks were then washed with ether, and the material on the sticks was analyzed by gas chromatography. Substantial quantities of the acetates were detected, amounting to a large fraction of the contents of a single Dufour's gland. When 200 μg of a synthetic mixture of acetates corresponding to the natural mixtures were applied as droplets to the heads and other parts of the bodies of *F. subsericea* workers, these ants showed temporary discomfort and disorientation. However, no long-term ill effects were observed. Experiments with a colony of *F. pergandei* showed that these substances also operate as effective alarm substances for these ants. On two occasions, single Dufour's glands of *F. pergandei* workers were crushed onto the tips of wooden applicator sticks and then inserted into the edge of a laboratory nest containing a complete colony. Within minutes the entire colony became highly excited, and most of the workers were attracted to the odor source. Several attempted to attack the tip of the stick. The effect lasted for over 30 minutes. An untreated control stick elicited short-lived response from only a single worker present at the place where the tip intruded. The same extreme alarm response was obtained when 40 μg of decyl acetate, the equivalent of the content of a single Dufour's gland, was absorbed into a small square of filter paper and inserted into the nest.

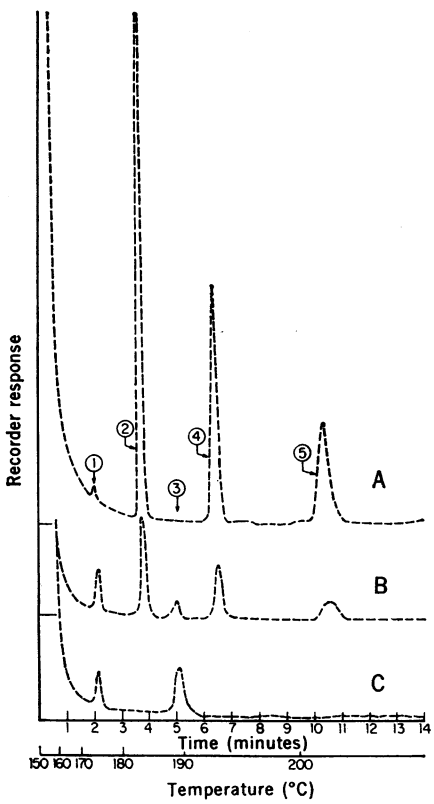

Fig. 3. Chromatograms of whole-body extracts of (A) workers of the slave-maker species *F. subintegra*, of (B) a fragment of the slave-species colony that has just been attacked by *F. subintegra* workers, and (C) of an undisturbed colony of a slave species (*F. subsericea*). Compounds 1, 2, 3, 4, and 5 were identified as tridecane, decyl acetate, 2-tridecanone, dodecyl acetate, and tetradecyl acetate, respectively. Identifications of compounds 1–5 in (A) are based on comparisons of gas chromatographic–mass spectral characteristics with authentic standards. Identifications of compounds in mixtures (B) and (C) are based on gas chromatographic retention times.

Workers of *F. subsericea* were also extremely alarmed by crushed Dufour's glands and single gland–equivalents of decyl, dodecyl, and tetradecyl acetates presented separately. But they were not attracted by these preparations, and they tended to scatter when exposed for more than a few seconds. This observation reminded us of the description given in 1810 by Pierre Huber, the discoverer of slavery in ants: "One of the principal features of the wars levied on the Ash-colored ants [*F. fusca*] seems to consist of exciting fear, and this effect is so strong that they never return to their besieged nest, even when the oppressors [*F. sanguinea*] have retired to their own nest; perhaps they realize that they could never remain in safety, being continually liable to new attacks by their unwelcome visitors" (*7*). Observations similar to those of Huber have been repeatedly made in the field by subsequent investigators, including ourselves. It occurred to us that the panic reaction of the colonies under attack might well be enhanced by the discharge of the acetates by the slave-makers. Accordingly, during three raids we collected workers of *F. subsericea* that had just been attacked by *F. subintegra* workers (see Fig. 2). The results, illustrated in Fig. 3, show conclusively that the defenders are sprayed by relatively

large quantities of the acetates. The amount of acetates individual *F. subsericea* workers received, on the average, was equal to or greater than the entire volatile contents of their own gland reservoirs, and more than enough to cause an alarm reaction. We feel certain that this accounts not only for the disorientation observed in many defenders during the raids but also, to some extent, for the panic and rapid retreat displayed by the slave-species colonies, and the relative ease with which their nests are breached by the slave-makers. The acetates, by virtue of the large quantities dispensed and their relatively low evaporation rate (as compared with that of other common alarm substances, such as undecane and citronellal), are, in effect, "superpheromones." They create penetrating and long-lasting alarm signals. They also serve as offensive "propaganda substances" against the colonies of the slave species, which cannot help but respond to them as alarm pheromones (*4, 6*).

It thus appears that the acetates of the Dufour's gland of *F. pergandei* and *F. subintegra* perform no less than three distinct functions in the life of the slave-maker colonies: as defensive and offensive chemical weapons, as alarm pheromones for communication within the colony, and as offensive "propaganda substances" directed at alien colonies during slave raids.

F. E. REGNIER
Department of Biochemistry, Purdue University, Lafayette, Indiana 47907
E. O. WILSON
Department of Biology, Harvard University, Cambridge, Massachusetts 02138

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Insulin and Microtubules in Rat Adipocytes

Abstract. *Insulin appears to promote microtubule assembly in rat adipocytes. Neither oxytocin nor high concentrations of glucose has this effect. Colchicine inhibits stimulation by insulin of lipid and glycogen synthesis without influencing stimulation by insulin of glucose oxidation. The anabolic effects of oxytocin or high concentrations of glucose are not inhibited by colchicine. The "directive effect" of insulin may involve microtubules.*

Attempts to correlate the diverse metabolic effects of insulin with ultrastructural changes in target cells have met with little or no success. The view, based upon a single study of fat cells (*1*), that insulin stimulates the formation of pinocytotic and cytoplasmic vesicles has not been supported by subsequent investigations (*2*). We now present evidence which suggests that in the fat cells of rats insulin stimulates the formation of microtubules—structures known to be associated with such diverse cellular processes as mitosis, axonal flow, and melanin granule movement (*3*). In addition, we show that characteristic anabolic responses of adipocytes to insulin are inhibited by colchicine, which disrupts microtubules by binding to their constitutent subunits (*4*). We suggest that the directive effects of insulin on anabolic processes (*5*) may depend upon microtubule assembly.

Fat cells were isolated from epididymal fat pads (*6*) from rats which were fasted for 2 to 3 days or which had free access to food. For electron microscopic studies, cells were incubated in Krebs-Ringer bicarbonate (KRB), containing 1 percent purified bovine serum albumin, but not glucose (unless otherwise stated), in the presence or absence of 10 microunits of insulin per milliliter (*7*), a dose that is nearly the maximum one for lipogenesis. The reactions were terminated by addition of glutaraldehyde, cells were postfixed in OsO_4; the cells were then embedded in Araldite (*8*).

Electron microscopic studies of fat cells incubated without or with added insulin suggested that hormone treatment promoted microtubule assembly.

Table 1. Effect of colchicine on basal and insulin-stimulated glucose oxidation and lipogenesis in adipocytes. The assay procedures have been described (*11*). Cells were incubated in KRB containing 1 percent bovine serum albumin and 1 m*M* [1-^{14}C]glucose. Values from a representative experiment are the mean values of three to four incubations ± standard error.

Colchicine concentration (*M*)	Glucose incorporation into CO_2*		Glucose incorporation into total lipid†	
	No insulin	Insulin‡	No insulin	Insulin‡
0	1.54 ± .07	3.40 ± .21	0.65 ± .03	2.25 ± .11
5×10^{-7}	1.47 ± .03	3.50 ± .16	0.74 ± .03	2.03 ± .12§
5×10^{-6}	1.56 ± .09	3.43 ± .14	0.64 ± .02	1.90 ± .17§
5×10^{-5}	1.53 ± .06	3.55 ± .22	0.72 ± .09	1.65 ± .04§

*Units are micromoles of glucose oxidized to CO_2 per gram of total lipid per hour. † Units are micromoles of glucose incorporated in the total lipid per gram of total lipid per hour. ‡ Insulin concentrates of 10 microunits per milliliter. § Effect of insulin (incremental difference between samples incubated with and without insulin) significantly reduced by colchicine ($P < .01$).

Table 2. Effect of colchicine (5×10^{-5} *M*) on adipocyte lipogenesis and glycogenogenesis stimulated by insulin, oxytocin, and high glucose. Results are mean values ± standard error from groups of three to four incubations from a typical experiment. Values are obtained as the difference between basal and treated incorporation rates and represent the effect of the treatment on the anabolic process.

Treatment	Glucose incorporation into total lipid*		Glucose incorporation into total glycogen†	
	No colchicine	Colchicine	No colchicine	Colchicine
Insulin (10 μunit/ml)	4.02 ± .15	3.00 ± .14‡	0.91 ± .08	0.65 ± .08§
Oxytocin (0.5 μg/ml)	1.37 ± .14	1.87 ± .35	<0.01	<0.01
Glucose 10 (*μM*/ml)	3.59 ± .19	3.50 ± .37	1.51 ± .01	1.50 ± .19

*Lipogenesis was measured as the incorporation of [1-^{14}C]glucose into the heptane-soluble fraction of cells (*11*). The basal rate of lipogenesis in this experiment was 1.23 ± 0.12 μmole of glucose incorporated per gram of total lipid per hour. † Glycogenogenesis was measured by incorporation of [1-^{14}C]glucose into KOH-soluble, ethanol-insoluble material left after removal of lipids with heptane, as measured by Gutman and Shafrir (*15*). Basal rates were <0.01 μmole of glucose incorporated per gram of total lipid per hour. ‡ Effect of colchicine significant, $P < .01$. § Effect of colchicine significant, $P < .05$.