melting range. The melt endotherms of wet collagen obtained in this way were more diffuse than those of pure water, with the onset of melting commencing at least 15°C below the melting point of pure water. This is consistent with the freezing-point lowering expected for a liquid trapped in small, somewhat irregular capillaries. For each of six samples (Table 1) I calculated the quantity of ice from the measured heat of melting, using the value 79.71 cal/g (1 cal = 4.1840 joules) for the heat of fusion. As shown in the second column of Table 1, the amount of water remaining unfrozen was approximately the same for the six samples. Replicate endotherms on the same sample agreed to within ± 2 percent. However, some errors are necessarily involved in the preparation and weighing of the samples, and it is not likely that significant differences exist in this set of numbers.

The NMR estimate of the mobile water in frozen collagen, expressed in terms of H_2O rather than D_2O, is about 0.54 g of H_2O per gram of collagen. This amount is very close to the quantity obtained by calorimetry and implies that the NMR splitting constant K depends only on the amount of mobile water in the collagen and is not affected by the presence or absence of ice. It is also apparent from the combined results that essentially all of the water that does not actually freeze remains in a state of high mobility. On the basis of an average amino acid molecular weight of 90 (*8*), the "unfreezable" water amounts to 2.6 molecules per residue.

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References and Notes

1. H. J. C. Berendsen and C. Migchelsen, *Fed. Proc.* **25**, 998 (1966).
2. R. E. Dehl and C. A. J. Hoeve, *J. Chem. Phys.* **50**, 3245 (1969).
3. H. J. C. Berendsen, *ibid.* **36**, 3297 (1962).
4. C. Migchelsen and H. J. C. Berendsen, in *Magnetic Resonance and Relaxation*, R. Blinc, Ed. (North-Holland, Amsterdam, 1968).
5. J. A. Jackson and S. W. Rabideau, *J. Chem. Phys.* **41**, 4008 (1964).
6. I. D. Kuntz, Jr., T. S. Brassfield, G. D. Law, G. V. Purcell, *Science* **163**, 1329 (1969).
7. See (*4*), p. 764. A similar plot was obtained in this laboratory, extending over a wider range of moisture contents, from 0.2 to 1.2 g of D_2O per gram of collagen.
8. See, for example, J. E. Eastoe, in *Treatise on Collagen*, G. N. Ramachandran, Ed. (Academic Press, New York, 1967), vol. 1, chap. 1.
9. Supported in part by the U.S. Army Medical Research and Development Command, MOD No. 9914.

29 July 1970

Pheromone Transport and Reception in an Amphipod

Abstract. *Sexual dimorphism in the second antennae of the amphipod* Gammarus duebeni *Lilljeborg is connected with the reception in the male of a female sex pheromone transported through the water. Investigations on tritium-labeled specimens were carried out with scintillator and autoradiographic techniques.*

Experiments were designed to test whether the sexual dimorphism in the supposed chemo-sensory antennal receptor mechanisms in the amphipod *Gammarus duebeni* Lilljeborg indicates that the calceoli, not present in the female, are receptors for a female sex pheromone.

Females of *G. duebeni* were fed liver from rainbow trout which had received an intraperitoneal injection of [^{3}H]-acetylglucosamine. This diet produced radioactive accumulation in the females, as shown by liquid scintillation tests on whole animals (7,000 to 30,000 count/min) (Packard Tri-Carb liquid scintillation spectrometer, model 3320).

Unlabeled males were put into one compartment of an aquarium partitioned by a wall of double nylon net which permitted water circulation but prevented direct contact between the two compartments. When the labeled females were put into the other compartment, the males became agitated, and most of them swam to the net wall and clung to it. After some time the males again dispersed, which might indicate that the original gradient in the stimulus concentration had been obliterated by the circulation maintained in the aquarium. Control tests with males instead of females produced no activity.

After the males were exposed to the female stimulus for 30 to 60 minutes, they were removed and one group was prepared for liquid scintillation tests. The remaining specimens were fixed and prepared for light microscope autoradiography. Liquid scintillation measurements on the males were conducted on whole animals, on the second antennae (which carry the calceoli), and on the body minus the antennae (Table 1). The average weight of both second antennae of three males was 0.07 mg; the average weight of three whole animals was 33.70 mg.

The scintillation measurements indicated that the radioactivity per unit weight was approximately 1000 times higher in the second antennae than in the remainder of the body. Considering, however, the minute quantities of tissue and the limited number of sexually active males which could be used, the readings, which in the antennae were only 8 to 52 count/min above the background, could not be statistically significant. Nevertheless they contributed to focus the interest on the second antennae. Experiments from both the May and the November reproduction periods gave similar results.

Light microscope autoradiography was carried out on males in transverse

Table 1. Incorporation of radioactivity in males of *Gammarus duebeni*, kept for either 30 or 60 minutes in the same aquarium with labeled females of the same species. By means of a double, fine-meshed nylon net, direct contact between males and females was prevented, but there was free circulation of water between the two compartments. The figures represent counts, recorded for extracts of the animals measured for 20 minutes in a liquid scintillation spectrometer, and have been corrected for controls.

Subjects	Radioactivity		
	Second antennae	Total body minus second antennae	Total body
	Males kept for 30 minutes		
A	8	0	8
B	13	23	36
C	18	32	50
D	*	*	41
E	*	*	151
	Males kept for 60 minutes		
F	32	0	32
G	52	4	56
H	0	80	80
I	*	*	28
J	*	*	76
K	*	*	100

* Not measured.

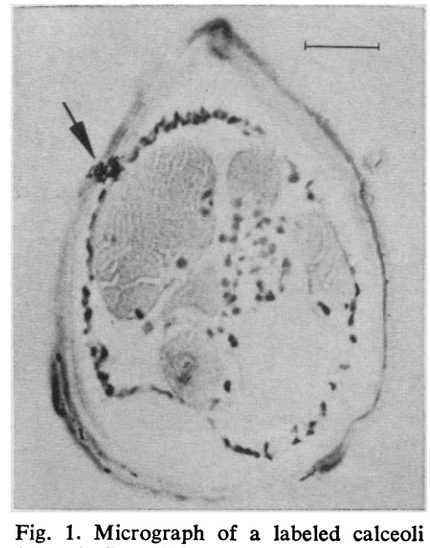

Fig. 1. Micrograph of a labeled calceoli (arrow). Bar, 50 μm.

and sagittal sections. The entire animals were sectioned and every section examined. Selective labeling was found exclusively in the calceoli (Fig. 1). The calceoli are extremely thin and fragile, and many of them were torn or completely lost in histological processing. The remaining ones, however, were very distinctly labeled. In several instances, labeling could be traced in different parts of the same calceolus in two or three consecutive sections.

It was concluded that the females produce a compound, apparently a sex pheromone, which acts over a distance in the water and agitates and attracts the males. The experiments prove that the calceoli on the male second antenna contain the receptors for this stimulus.

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Secondary Structure of Ribosomal RNA

Abstract. *Infrared spectra were obtained for 16S and for 23S ribosomal RNA's in D_2O solutions. The percentage of each base in the paired and unpaired regions of the RNA was determined from the spectra. The secondary structures of 16S and 23S ribosomal RNA's (from* Escherichia coli*) are significantly different from each other and are also different from those of yeast ribosomal RNA, formylmethionyl–transfer RNA, and the anticodon fragment of this transfer RNA.*

The secondary structure of ribosomal RNA (rRNA) in the ribosome is considered to be similar to that of protein-free rRNA (*1*), which consists of single-stranded regions (with no hydrogen bonds between bases) alternating with helical, double-stranded regions with hydrogen bonds between bases (*1*, *2*). These hydrogen bonds perturb vibrational modes and thus alter the infrared spectra of the bases. This effect has been used in a new method that gives the percentage of RNA bases in the double-stranded and single-stranded regions and the average base composition of these regions (*3*). This method has been applied to unfractionated rRNA from yeast (*3*) and from *Escherichia coli* (*4*), to crystallizable fragments of yeast rRNA (*2*), to a transfer RNA (tRNA) (*5*), and to anticodon fragment of a tRNA (*6*).

Table 1. Percentage of rRNA bases in paired and unpaired states at 26°C. The base composition (*1*) of 16*S* rRNA is (in percent) 24.5 A, 21.5 U, 31.5 G, and 22.5 C; that of 23*S* rRNA is 25.5 A, 20.7 U, 32.8 G, and 21.0 C.

Bases	16*S* rRNA	23*S* rRNA
A + U paired	24.0	25.0
G + C paired	36.0	33.0
A unpaired	12.5	13.0
U unpaired	9.5	8.2
G unpaired	13.5	16.3
C unpaired	4.5	4.5

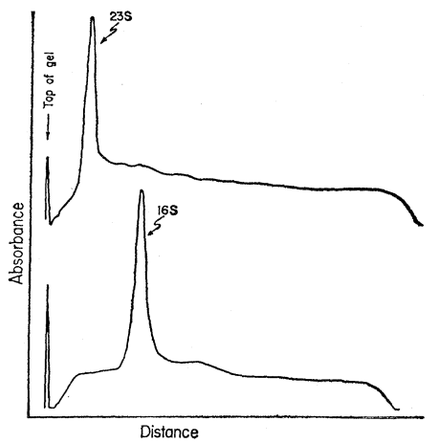

Fig. 1. Polyacrylamide-gel electrophoresis of 23*S* rRNA (upper curve) and 16*S* rRNA (lower curve) recovered from the infrared cell after spectra were recorded.

We measured the infrared spectra of purified 23*S* and 16*S* rRNA's from *E. coli*. Analysis shows that small but significant differences exist in the secondary structures of these two species.

The 16*S* and 23*S* rRNA's were extracted by the sodium dodecyl sulfate (SDS)–phenol method from 30*S* and 50*S* ribosomes isolated from *E. coli* D10 (*7*). After the ether treatment, the alcohol precipitation step was repeated three times to ensure that all phenol was removed from the RNA. After the final precipitation, the alcohol solution was removed from the pelleted RNA, which was dried with a flow of dry air to a hard transparent solid.

To obtain infrared spectra, dried RNA was dissolved to a concentration of 40 mg/ml in D_2O (99.8 percent; Diaprep Inc.) containing 0.1 percent SDS. No buffers were used because RNA is self-buffering at these concentrations (*2*, *3*). The *p*D of the solutions was 7.2 ± 0.4. Dust and air bubbles were removed from the RNA solutions by centrifugation at 6000*g*. The total concentration of RNA bases in the solution was determined by measuring the ultraviolet spectrum of a portion of the RNA solution which had been hydrolyzed with NaOH (*2*). Infrared spectra were obtained in a thermostated cell with path length of 75 μm. A reference cell was used to compensate for the absorbance of the D_2O (*2*).

The integrity of the RNA in the solutions recovered from the infrared cell was determined by electrophoresis on 3.75 percent polyacrylamide gels (*7*). The gels were scanned for absorbance at 2600 Å with a Gilford spectrophotometer and scanning attachment. The electrophoretograms of both 16*S* and 23*S* rRNA's recovered from the infrared cell showed one large band at the correct location with only slight amounts of lower molecular weight material and were quite similar to the electrophoretograms of the input samples (Fig. 1). We conclude that no significant amount of degradation occurred while the spectra were recorded.

To obtain quantitative estimates of the secondary structure of RNA, we

Table 2. Base composition (mole percent) of the paired and unpaired regions of rRNA's.

Base	16*S* rRNA	23*S* rRNA
A paired	21.5	20.0
U paired	21.5	20.0
G paired	28.5	30.0
C paired	28.5	30.0
A unpaired	31.0	31.2
U unpaired	19.5	23.8
G unpaired	38.8	33.8
C unpaired	10.7	11.2

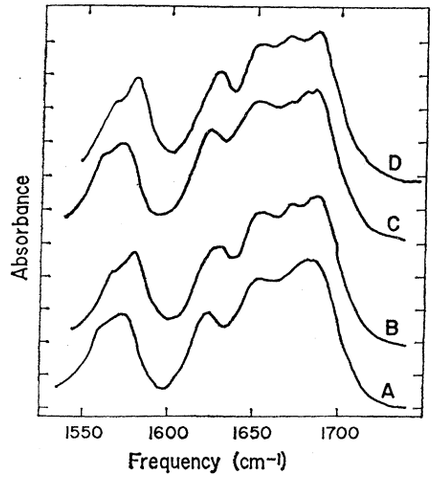

Fig. 2. Infrared spectra of 16*S* and 23*S* rRNA's. (A) Observed spectrum of 16*S* rRNA; (B) synthesized spectrum of 16*S* rRNA; (C) observed spectrum of 23*S* rRNA; and (D) synthesized spectrum of 23*S* rRNA.