effect of this milking action might be to decrease effective papillary plasma flow which in turn would decrease solute washout by the ascending vasa recta. In fact, Chuang et al.[9] have shown that removal of the upper ureter is accompanied by an increased papillary blood flow.

1 A portion of this work was conducted at The Mount Desert Island Biological Laboratory, Salsbury Cove, Maine and was supported by NIH grant 2 RO1-AM15972-10 awarded to Dr B. Schmidt-Nielsen. Additional support came from the Maine and Pennsylvania Affiliates of the American Heart Association.

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Epidermal sensitivity to hypoxia in the lugworm[1]

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Summary. Physiological and anatomical data support the idea that in the caudal epidermis of the lugworm, *Arenicola marina* (L.), there are chemoreceptors detecting variations of oxygen partial pressure in the ambient water.

Numerous aquatic organisms live in tubes or in more or less consolidated burrows through which they actively pump ambient water. Usually the main purpose of this irrigation is to provide the animal with oxygen. It thus corresponds to a true ventilation, and has been extensively analyzed in several species of polychaete annelids[2-4]. The lugworm, *Arenicola marina* (L.), forces sea water through its L-shaped burrow in tail-to-head waves running along its dorsal surface. This ventilatory behavior is part of a recurrent sequence of activities lasting 30–40 min and controlled by pacemakers[5], but the cycle's normal pattern can be altered. For instance, the ventilatory activity is inhibited when the salinity of the sea water overlying the burrow is decreased[6], when the burrow contains anoxic water during low tide[7], or when, in experimental conditions, the partial pressure of oxygen (P_{O_2}) in the inspired sea water is lower than 40 Torr[8]. We suggest that, contrary to a generally accepted idea[9], the reduced oxygenation of the respiratory medium could be directly involved in the ventilation inhibition. From data in the literature[10] and from preliminary observations, we also suspected that the tail of the lugworm could be especially sensitive to the level of oxygen. Here, we report physiological and anatomical data which support these two hypotheses.

Material and methods. Lugworms were collected in the vicinity of the Station Biologique, Roscoff, and kept unfed in air-saturated running sea water (14–16 °C). An unrestrained lugworm, 15–20 g fresh weight, was placed in an artificial burrow, a straight glass tube 30 cm long, inner diameter 1 cm. The tail end of the tube was connected to a system of 2 tonometers in which sea water, chlorinity ~19.5‰, was equilibrated against an anoxic ($P_{O_2} \sim 0$ Torr) or a normoxic ($P_{O_2} \sim 155$ Torr) gas phase. Identical values for sea water P_{CO_2}, ~0.25 Torr, and pH, ~8.25, were maintained in the 2 tonometers. The glass tube could be instantaneously switched to either tonometer through a 3-way stopcock. An additional device allowed a permanent flow (3 ml per min) of sea water from the tonometer to be applied directly to the lugworm's tail even when the animal was not ventilating. The head end of the tube was connected to a Gilson flow meter and to a Statham P23BB pressure transducer. The ventilated sea water volumes and the ventilation-induced hydrostatic pressure changes in the glass tube were simultaneously recorded on a Sefram Servotrace recorder. All experiments were carried out at 15 °C.

For examination by scannning and transmission electron microscopy, small samples of tail epidermis in several normoxic animals were prepared by standard techniques.

Results. Shift from normoxic to anoxic water (fig. 1, A and B). When the tube was connected to the normoxic water the lugworm ventilated steadily and the pressure inside the artificial burrow varied with a period (13.4 sec, SEM = 0.4 sec, N = 41) corresponding to the ventilatory period, i.e. to the time lapse between the onset of 2 successive ventilatory waves. A control shift from normoxic to normoxic water had no effect on the ventilatory rhythm (fig. 1A, arrow). Conversely, after a switch from normoxic to anoxic water the ventilatory peristaltism slowed down, then stopped, and the hydrostatic pressure and ventilated water flow dropped to zero (fig. 1A). Figure 1B illustrates the mean effects of 41 normoxic-to-anoxic switches in 17 animals. 63% of the responses occurred within 27 sec, or 2 ventilatory periods. The highest response frequency occurred during the 2nd ventilatory period following the application of the stimulus to the tail of the animal.

Shift from anoxic to normoxic water (fig. 1, C and D): when the tube was connected to the tonometer of anoxic water, the lugworm did not ventilate and the difference of hydrostatic pressure inside and outside the experimental device was almost zero. A control switch from anoxic to anoxic water had no effect. Conversely, after a switch from anoxic to normoxic water (fig. 1C), the lugworm quickly resumed ventilating: pressure and ventilated volumes increased sharply. Figure 1D illustrates the mean effects of 41 anoxic-to-normoxic switches on the 17 animals of figure 1B. 56% of the responses to the normoxic stimulus occurred within about 40 sec, or 3 ventilatory periods. As with the normoxic-to-anoxic switch, the highest response frequency occurred during the time interval corresponding to the 2nd ventilatory cycle after the start of the normoxic stimulus.

Ultrastructure of the caudal epidermis. Epidermal papillae scattered all over the lugworm body are more conspicuous on the tail as rings of prominent structures. They are formed by a

thickened epidermis with supporting cells, large, numerous mucous cells and apical ciliated sensory buds[11] (fig. 2A). Sections of the sensory buds show 2 types of bipolar primary sensory cells, R1 and R2 (fig. 2B). Both have an intraepidermal nucleus and are multiciliated receptors whose short cilia projecting into the sea water have the typical 9 × 2 + 2 tubular pattern. The rarer R2 cells have large striated ciliary rootlets, subsurface cisternae, some dense vesicles and can be considered as mechanoreceptors[12]. Moreover, their ciliary rootlets are surrounded by a very peculiar dense fibrillar sheath. The more numerous R1 cells show ultrastructural features known in chemoreceptors: a clear cytoplasm, one basal foot on the side of each ciliary basal body[13], fine striated rootlets, microtubules and subsurface cisternae, and dense vesicles mainly in the perikaryon and axon[14,15].

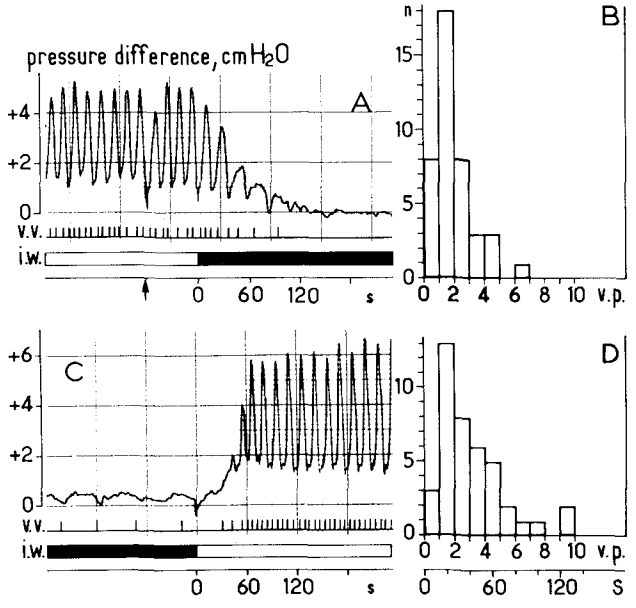

Figure 1. Responses of *Arenicola* to anoxic (A, B) and normoxic (C, D) stimuli applied at time 0. *A* and *C* are records of hydrostatic pressure changes induced by one lugworm's ventilatory activity in the tube, (i.w., inspired water) in normoxia (□) and anoxia (■); each base-line vertical bar represents 1 ml of ventilated sea water (v.v., ventilated volumes). Arrow: control test in normoxia (see text). *B* and *D* Distribution of the latencies of the ventilatory responses. *B* is 41 shifts from normoxic to anoxic water in 17 worms, and *D* is 41 shifts from anoxic to normoxic water in the same 17 animals; time scale expressed as ventilatory periods (v.p.), 1 v.p. = 13.4 sec (see text); time scale in sec is given in D.

Discussion. Our results show that the lugworm is able to sense a variation of the oxygen partial pressure in the respiratory medium. Ventilation can be stopped by an anoxic stimulus applied to the caudal epidermis or reinitiated by a normoxic stimulus. In both cases the response latency is rather short. The response occurs before the change in P_{O_2} could, by diffusion through the body wall, reach the nerve cord and the supposed ventilatory pacemaker. This implies that in *Arenicola* peripheral chemoreceptors are present and detect variations in external P_{O_2} conditions, as proposed in *Limulus*[16] and *Astacus*[17].

The R1-type receptors we describe here are very similar to the ciliated sensory cells of the olfactory epithelium of vertebrates not only in their cytological features and association with mucous cells, but also by their location since, as in the vertebrate olfactory epithelium, they are directly exposed to the inspired medium into which they project their cilia. Although electrophysiological evidence is not yet available, we suggest that the R1-type receptors are involved in the oxygen sensitivity revealed by the ventilatory behavior of the lugworm.

1 This research was partly supported by a C.N.R.S. grant, AI No. 582085. We thank Dr P. Dejours and Dr J. Taxi for helpful comments and discussion, Mrs S. Dejours for editing the English of this paper, M. F. Baucher, A. Dorme, F. Kleinbauer, the staff of the Station Biologique de Roscoff and the Service de Microscopie Electronique, C.N.R.S., boulevard Raspail, Paris, for technical assistance.

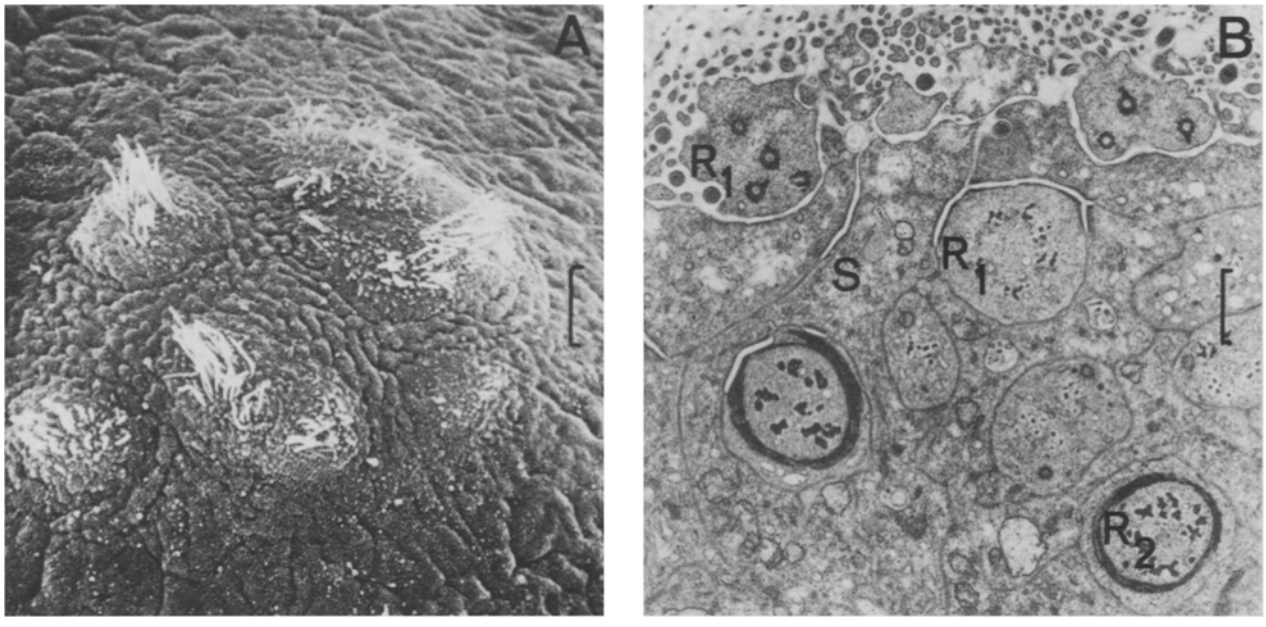

Figure 2. *A* Tufts of cilia projecting above the cuticle at the top of a papilla from the caudal epidermis of *Arenicola*. Each tuft corresponds to a sensory bud. SEM, ×2100. Scale bar 5 μm. *B* Transverse section through a sensory bud shows 2 types of sensory cells separated by sinuous supporting cells (S); note in the putative mechanoreceptor (R2) the dense fibrillar sheath surrounding the heavy rootlets, and in the putative chemoreceptor (R1) the thinner rootlets and the basal bodies with one basal foot. TEM, ×9700. Scale bar 1 μm.

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0014-4754/84/060541-03$1.50 + 0.20/0

The in vitro stability in human plasma of two different technetium-99m-fibrinogen compounds

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Summary. Two ^{99m}Tc-labeling methods of human fibrinogen resulted in different complexes. This was concluded from different dissociation rates in human plasma. The dissociation could be described by a simple exponential function.

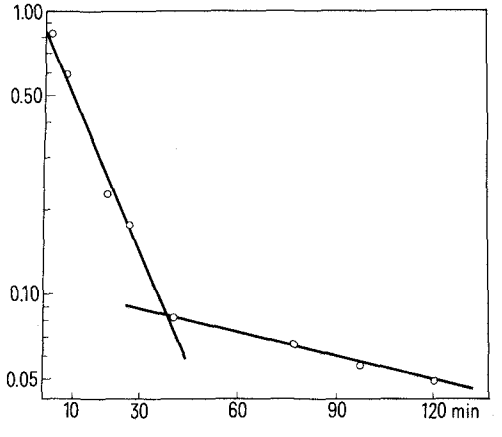
Radiolabeled human fibrinogen (HF) is used in the diagnosis of intra- and extravascular coagulation. While ^{125}I and ^{131}I are covalently bound to the aromatic ring of tyrosine[1], ^{99m}Tc is incorporated into a complex the structure of which has not yet been determined. As the complex may vary according to the labeling conditions, we compared the stability of 2 samples of ^{99m}Tc-HF labeled under different conditions. One method was published recently[2]. The reduction of $^{99m}TcO_4^-$ is obtained by Sn^{2+}. During the whole labeling procedure a physiologic pH (7.4) can be maintained, due to the use of a citrate/carbonate buffer. Column chromatography with Sephadex G 25 is reported to yield 81.1 ± 1.5% radioactivity attributable to HF. The other method replaces citrate by glycine buffer[3]. After adding $^{99m}TcO_4^-$ which is reduced by Sn^{2+} as well, the pH of the mixture is about 10. According to the authors, column chromatography with Biogel P6 yielded 90–95% radioactivity attributable to HF, while the isotopic clottability reached only 65–70%. We investigated the stability in human plasma at 37 °C to obtain comparable results with a view to eventual in vivo investigations.

Methods. One preparation of ^{99m}Tc-HF (complex A) was labeled according to the published method[2]. The other preparation (complex B) was obtained by adding $^{99m}TcO_4^-$ to a commercially available vial containing lyophilized HF, tin chloride and glycine (Sorin SA., Italy). Details of the labeling solutions are listed in table 1. After adding 1 drop of the labeling solution to fresh human plasma the ^{99m}Tc-HF was incubated at 37 °C. The labeled and unlabeled HF were separated from other plasma components by clotting with thrombin. 200 µl plasma samples were brought to 1 ml with Michaelis buffer. After addition of 100 µl thrombin (120 U/ml) the mixture was incubated 2 min at 37 °C. The clot was sedimented at about 2000 × g for 15 min, decanted and washed with 2 ml isotonic NaCl solution. The clot radioactivity was measured by a gamma counter and related to the radioactivity of a plasma sample. Solutions of $^{99m}TcO_4^-$, ^{99m}Tc-human serum albumin (HSA) and ^{125}I-HF (2 mCi ^{99m}Tc in 5 ml respectively 110 µCi ^{125}I in 1.1 ml) were prepared. 1 drop of each solution was added to human plasma and treated as described above.

Results and discussion. The efficiency of our separation method was checked on the one hand by investigating the coprecipitation of $^{99m}TcO_4^-$ and ^{99m}Tc-HSA. These compounds, we thought, have to be considered first after ^{99m}Tc-HF dissociation. $^{99m}TcO_4^-$ might be coprecipitated because about 80% is bound to proteins even if weakly[4]. The table 2 shows neither $^{99m}TcO_4^-$ nor ^{99m}Tc-HSA coprecipitating to an extent worth mentioning with HF. On the other hand, we checked the clot yield with ^{125}I-HF. About 90% radioactivity could be precipi-

Table 1. Details of the labeling solutions

	Complex A	Complex B
Human fibrinogen	3 mg	5 mg
Sodium citrate	3.6 mg	–
Sodium bicarbonate	0.17 µg	–
Glycine	–	q.s.
Tin chloride dihydrate	11 µg	0.2 mg
^{99m}Technetium in isotonic saline	2 mCi (74 MBq)	2 mCi
Total volume	1.6 ml	2 ml
pH	ca. 7.4	ca. 10
Incubation	1 h, 28 °C	20 min



The dissociation of complex A in human plasma at 37 °C, the clot/plasma-radioactivity-ratio incubation time.