

hibition by the antagonist, not on the intrinsic activity of the antagonist, from which these interpretations apparently (24) were drawn. This MSH release mechanism, in concert with the inhibitory neuronal input, may modulate fine adjustments in MSH secretion. A "doubly innervated secretory unit" (26), proposed for regulation of MSH release from the frog pituitary, is supported by both morphological (20) and electrophysiological (16, 26) evidence. Our results do not rule out the possibility that some cells possess only α -adrenergic receptors whereas others have only β receptors. The relation of adrenergic mechanisms of MSH release control to possible neurosecretory mechanisms (27) involving postulated hypothalamic factors inhibiting (28) and enhancing (29) MSH release is unclear. Evidence for the structure of these possible neurosecretory MSH releasing and inhibiting peptides remains equivocal (30).

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References and Notes

1. R. J. Wurtman, in *Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry*, J. Meites, Ed. (Williams & Wilkins, Baltimore, 1970), pp. 184-194; *Neurosci. Res. Symp. Summ.* 6, 171 (1972).
2. I. A. Kamberi and S. M. McCann, *Endocrinology* 85, 815 (1969).
3. J. F. Wilber, G. T. Peake, R. D. Utiger, *ibid.* 84, 758 (1969).
4. R. M. MacLeod, *ibid.* 85, 916 (1969).
5. C. A. Birge, L. S. Jacobs, C. T. Hammer, W. H. Daughaday, *ibid.* 86, 120 (1970).
6. F. C. Iturriza and M. Kasal-Iturriza, *Gen. Comp. Endocrinol.*, suppl. 3 (1972), p. 108.
7. I. A. Kamberi, R. S. Mical, J. C. Porter, *Endocrinology* 88, 1003 (1971).
8. ———, *ibid.*, p. 1012.
9. Y. Koch, K. H. Lu, J. Meites, *ibid.* 87, 673 (1970).
10. S. Taleisnik, M. E. Tomatis, M. E. Celis, *Neuroendocrinology* 10, 235 (1972).
11. A. Bower and M. E. Hadley, *Gen. Comp. Endocrinol.* 19, 147 (1972).
12. K. Shizume, A. B. Lerner, T. B. Fitzpatrick, *Endocrinology* 54, 553 (1954); M. R. Wright and A. B. Lerner, *ibid.* 66, 599 (1960).
13. I. R. Innes and M. Nickerson, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, New York, ed. 4, 1970), pp. 478-523.
14. C. L. Ralph and S. Sampath, *Gen. Comp. Endocrinol.* 7, 370 (1966).
15. J. M. Goldman and M. E. Hadley, *J. Pharmacol. Exp. Ther.* 166, 1 (1969).
16. K. Oshima and A. Gorbman, *Science* 163, 195 (1969); D. C. Dawson and C. L. Ralph, *Gen. Comp. Endocrinol.* 16, 611 (1971).
17. G. N. Woodruff, *Comp. Gen. Pharmacol.* 2, 439 (1971).
18. H. G. Baumgarten, A. Björklund, A. F. Holstein, A. Nobin, *Z. Zellforsch. Mikrosk. Anat.* 126, 483 (1972).
19. A. Enemar and B. Falck, *Gen. Comp. Endocrinol.* 5, 577 (1965).
20. Y. Nakai and A. Gorbman, *ibid.* 13, 108 (1969).
21. M. A. Belenky, M. A. Konstantinova, A. L. Polenov, *ibid.* 15, 185 (1970).
22. W. Bargmann, E. Lindner, K. H. Andres, *Z. Zellforsch. Mikrosk. Anat.* 77, 282 (1967).
23. J. F. Wilson and J. M. Dodd, *Gen. Comp. Endocrinol.* 20, 556 (1973).
24. R. Olsson and A. Gorbman, *ibid.* 21, 138 (1973).
25. P. Seeman, *Pharmacol. Rev.* 24, 583 (1972).
26. K. Oshima and A. Gorbman, *Gen. Comp. Endocrinol.* 13, 98 (1969).
27. W. Etkin, *J. Exp. Zool.* 86, 113 (1941); *Gen. Comp. Endocrinol.*, suppl. 1 (1962), p. 148.
28. A. V. Schally and A. J. Kastin, *Endocrinology* 79, 768 (1966).
29. S. Taleisnik, R. Orias, J. de Almos, *Proc. Soc. Exp. Biol. Med.* 122, 325 (1966).
30. N. H. Grant, D. E. Clark, E. I. Rosahoff, *Biochem. Biophys. Res. Commun.* 51, 100 (1973).
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Respiration of Benthopelagic Fishes: In situ Measurements at 1230 Meters

Abstract. *The respiration rate in situ of two common benthopelagic fishes, Coryphaenoides acrolepis and Eptatretus deani, was monitored at 1230 meters in the San Diego Trough. The respiration rate of C. acrolepis was two orders of magnitude lower and that of E. deani was significantly lower ($P < .05$) than rates in comparable shallow-water species.*

Our measurements of the respiration of the rattail *Coryphaenoides acrolepis* and the hagfish *Eptatretus deani* at a depth of 1230 m in the San Diego Trough represent the first successful attempts to determine the metabolic activity in situ of individual deep-sea animals. Previous measurements in situ have shown that the metabolic activities of benthic communities and bacteria are significantly lower in the deep ocean than in shallow water (1). Our respiration measurements show that deep-sea fish respire at a significantly lower rate than comparable shallow-water forms.

Both macrourids (rattails) and myxiniids (hagfish) are common benthopelagic fishes in the deep seas of the world oceans. Macrourids are the most abundant group of deep-sea benthic fishes and are predominantly associated with continental slopes (2). *Coryphaenoides acrolepis* and *Eptatretus deani* are dominant fish species in the San Diego Trough, along with the sable fish *Anoplopoma fimbria*.

The area of investigation during October 1973 was located 14 miles (26.2 km) off San Diego (32°34.75'N, 117°29.00'W) at a depth of 1230 m. Bottom water temperature was

3.5°C, and the dissolved oxygen concentration was 0.71 ml/liter. Sediments were predominantly clay, and large amounts of fecal pellets were present.

Our work was part of a detailed study of the benthos of the San Diego Trough, in which the remote underwater manipulator (RUM) was used. The RUM is a remote-controlled vehicle with a mechanical arm; it is lowered to the seabed by a conducting cable and is monitored continuously with television cameras. On three successive lowerings we secured a fish trap respirometer to RUM. The respirometer consisted of a Plexiglas box (61 by 30 by 30 cm). A spring-loaded door at one end was designed to be opened and closed with the RUM mechanical arm. A polarographic oxygen electrode (3) was inserted in the side of the trap and connected to an amplifier and continuous monitoring recorder which were housed in a glass sphere mounted on the side of the trap. The stirring motor and magnetic stir bar were mounted above the electrode to provide circulation both over the electrode and throughout the fish trap. Approximately 10 g of fresh bonito muscle tissue was enclosed in a wire-mesh box and anchored at the back of

Table 1. Respiration, weight, and length of *Coryphaenoides acrolepis* and *Eptatretus deani*.

Fish	Wet weight (kg)	Overall length (cm)	Respiration (milliliters of oxygen per hour)		Measurement time (min)
			Total	Per kilogram wet weight	
<i>C. acrolepis</i>	1.8	68	4.4	2.4	217
<i>E. deani</i>	0.1	51	0.2	2.2	767

the respirometer to lure scavengers into the enclosure but to make the bait unavailable for consumption. After the RUM was lowered to within 30 m of the bottom, the respirometer was opened and flushed to obtain characteristic bottom water and then closed. A control measurement of oxygen consumption by the enclosed water and bait was made for 1 hour; after this time the RUM was lowered to the seabed and the respirometer door was opened again for flushing and for attracting a fish. The control showed negligible uptake of oxygen.

The first measurement was of *Coryphaenoides acrolepis* which was attracted and enclosed within 8 minutes. Except for occasional bumping of the trap sides, the rattail behaved like unrestrained rattails, as observed by the RUM television. It displayed little interest in the bait after initial capture. Oxygen consumption was monitored on the bottom for 3½ hours, after which the RUM was brought to the surface, and the weight and displacement volume of the fish were measured (Table 1). Increased respiration rates were correlated with relatively rapid movements at the beginning of the experiment. Dissolved oxygen concentration within the respirometer at the end of the experiment was 60 percent of the concentration of the surrounding seawater. There was no evidence of respiration changes with time, suggesting regulatory patterns of metabolism in *Coryphaenoides* at least to 60 percent of ambient oxygen concentration.

A subsequent lowering of the respirometer, following the procedure outlined above, yielded a respiration measurement of a hagfish *Eptatretus deani* (Table 1). The hagfish was captured 30 minutes after the respirometer door was opened. Upon entering the trap, the animal actively swam around and appeared excited by the bait. This period of excitement lasted 21 minutes, and then the animal intermittently swam around the trap and settled to the bottom. No significant changes in respiration rate were noted during the 13-hour recording period, which could be correlated with the intermittent periods of activity.

We compared our respiration measurements with rates of related shallow-water species. The macrourids are phylogenetically related to gadids which have shallow-water representatives such as the Atlantic cod (*Gadus morhua*), which normally inhabits cold water. The

Table 2. Comparative respiration measurements of related shallow-water fishes of the same weight at comparable temperatures.

Species	Respiration (milliliters of oxygen per hour)		Temperature (°C)
	Total	Per kilogram wet weight	
<i>C. acrolepis</i>	4.4	2.4	3.5
<i>Gadus morhua</i> (4)	100.1	55.6	3.0
<i>E. deani</i>	0.2	2.2	3.5
<i>E. stoutii</i> (5)	0.9	9.4	4.0
<i>Petromyzon marinus</i> (6)	7.6	75.5	5.0

respiration of *Gadus* (4) is two orders of magnitude greater than that of *Coryphaenoides* (Table 2).

Shallow-water myxiniids include the hagfish *Eptatretus stoutii* and the lamprey *Petromyzon marinus*. Respiration of both of these species (5, 6), for similar size animals and at similar temperatures, are significantly greater ($P < .05$) than the respiration of *Eptatretus deani* (Table 2).

Comparisons with related shallow-water species revealed that the respiration rates of the macrourid *Coryphaenoides acrolepis* and the myxiniid *Eptatretus deani* are significantly lower. These findings are consistent with other in situ studies which have shown decreased metabolic activity in the deep sea (1) and may be a synergistic func-

tion of food availability, pressure, and temperature. The respiration measurements of these fishes is an overestimate of their true respiration rate if it is true (as hypothesized) that these fishes may exist in a quiescent state until food is available (7). Even as an estimate of maximum respiration, our values show reduced rates of metabolic activity in deep-sea animals.

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References and Notes

1. K. L. Smith, Jr., and J. M. Teal, *Science* **179**, 282 (1973); H. W. Jannasch, K. Eimhjellen, C. O. Wirsen, A. Farmanfarnian, *ibid.* **171**, 672 (1971); H. W. Jannasch and C. O. Wirsen, *ibid.* **180**, 641 (1973).
2. C. F. Phleger, *Am. Zool.* **11**, 419 (1971); N. B. Marshall, *Deep-Sea Res.* **12**, 299 (1965).
3. J. Kanwisher, *Limnol. Oceanogr.* **4**, 210 (1959).
4. R. L. Saunders, *J. Fish. Res. Board Can.* **20**, 373 (1963).
5. F. W. Munz and R. W. Morris, *Comp. Biochem. Physiol.* **16**, 1 (1965).
6. F. W. H. Beamish, *J. Fish. Res. Board Can.* **30**, 1367 (1973).
7. R. R. Hessler and P. A. Jumars, *Deep-Sea Res.*, in press.
8. This study is No. 1 of a series of reports on the results of Expedition Quagmire. It is a contribution of the Woods Hole Oceanographic Institution (3259) and Scripps Institution of Oceanography. K.L.S. was supported by NSF grant GA-39910. R.R.H. was supported by NSF grants GA-31344X and GA-40322. We thank the RUM group, the crew of the R/P *Orb* and George Wilson for assistance, and R. H. Backus, R. B. Campenot, F. G. Carey, J. E. Craddock, G. Grabowski, R. L. Haedrich, H. W. Jannasch, P. A. Jumars, B. H. Robison, R. H. Rosenblatt, J. M. Teal, and C. O. Wirsen for critically reading the manuscript.

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Myelin Basic Protein: Location of Multiple Independent Antigenic Regions

Abstract. Immunization of guinea pigs with homologous myelin basic protein induces antibodies that differ in their ability to bind specific peptide fragments of the protein. Antiserums with differing specificities made it possible to demonstrate at least three mutually exclusive antigenic sites in the protein molecule. One of these sites is located between residues 44 and 89, another between 90 and 116, and the third between 117 and 170.

The present study was undertaken to determine the binding specificity of antibodies induced in guinea pigs by immunization with homologous myelin basic protein. Similar studies have been reported on other proteins of known sequence and structure, for example, ribonuclease (1) and lysozyme (2), but reports on the relation of structure to immunologic activity of myelin basic protein have dealt primarily with its encephalitogenic activity or its ability to induce delayed hypersensitivity reactions in vitro and have not been di-

rected toward an analysis of antibody binding specificity. Binding of antibody to whole protein and to purified and chemically defined fragments was determined by radioimmunoassay (3). Two general immunization techniques were used, one induced antibody accompanied by a disease-resistant state, and the other induced antibody correlated in time of appearance with disease onset.

Guinea pig myelin basic protein (GPBP) and the smaller of the two rat myelin basic proteins (rat S) were