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PERMEABILITY OF THE MARSUPIUM OF THE PIPEFISH *SYNGNATHUS FUSCUS* TO [^{14}C]-ALPHA AMINO ISOBUTYRIC ACID

THOMAS W. HARESIGN and SANDRA E. SHUMWAY*
Natural Science Division, Southampton College, Southampton, New York 11968, U.S.A.

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Abstract—1. Male pipefish, *Syngnathus fuscus*, with embryos were injected with a radioactively labelled non-metabolizable amino acid and accumulation of the acid monitored in the developing embryos.
2. Evidence is presented for the transfer of the amino acid from paternal tissue to developing young.

INTRODUCTION

Transfer of nutrients to developing young is, in the vertebrates at least, generally attributed to the female of the species. The family Syngnathidae (pipefishes and seahorses) is a small group of highly specialized fish in all of which the males carry the eggs in a brood pouch which is more or less perfectly developed (Breder & Rosen, 1966).

At the turn of the century two workers, Gudger (1905) and Huot (1902), believed that they had established evidence for nutrient transfer from the male pipefish to developing embryos in its marsupium. These conclusions were based solely on histological evidence.

The following experiments, utilizing a non-metabolizable amino acid, were designed to establish whether or not these nutrient transfers do in fact take place.

MATERIALS AND METHODS

Pipefish, (*Syngnathus fuscus*) with embryos (approximately 170–200 mm in length) were collected in Peconic Bay (Southampton, NY, U.S.A.) and used as soon as possible after capture, usually the same day 250 μCi of the non-metabolizable amino acid [^{14}C]-alpha amino isobutyric acid was obtained from ICN and diluted to 50 ml in teleost ringer. All injections were 0.1 ml and given intra-peritoneally. Males with embryos were injected and later sacrificed. Embryos were then collected along with samples of marsupium and body wall, washed in running water, blotted dry and wet weights were determined. Tissues were then digested for 24 hr at 60°C in protosol (Nuclear Chicago) and a 0.1 ml aliquot of the digest was taken for counting. The aliquot was added to a scintillation counting fluid composed of 20% ethanol and 80% Liquiflour (New England Nuclear). Samples were counted on a Packard Tri-Carb Liquid Scintillation Counter and channels ratio method was used to establish potential quenching. Efficiency was determined by internal standardization. No variable quench was apparent and efficiency was consistently 50%.

Two additional experiments to assess possible contamination and permeability of fry to [^{14}C]-alpha amino isobutyric acid were also carried out and are discussed below.

* Present address: State University of New York, Department of Ecology and Evolution, Stony Brook, New York 11794, U.S.A.

RESULTS AND DISCUSSION

As shown in Table 1, all embryos contained radioactivity although the amount varied considerably. There was no evidence of accumulation of material over a 3 hr time span or longer time spans (see Table 2). The amount of radioactivity in embryos was always lower than activity in body wall and in marsupium tissues. The marsupium, in most animals, contained more activity than body wall. This may be due to relatively greater vascularity in the marsupium than in the body wall. No systematic attempt was made to age the embryos when they were removed from the parent; however, the following general observations were made: Animals No. 3, 7, 9 and 13 had embryos which were free and moving in the marsupium and close to release; these embryos had only vestiges of yolk sac or no yolk sac at all. As seen in Table 1, these embryos had relatively low levels of radioactive material. All other embryos were firmly attached in the marsupium; animals No. 2, 8 and 14 contained very young embryos with no optic cup visible.

Because those embryos showing highest levels of isotope were those most firmly attached in the marsupium it was possible that activity was due to contamination during removal. Therefore, males carrying embryos were injected daily with 0.1 ml of the teleost ringer containing [^{14}C]-alpha amino isobutyric acid. After fry emerged from the marsupium they were collected, weighed, digested and counted as above. These results are shown in Table 2. Some of the activity found in these newly hatched fry is due to ingestion of labelled material excreted into the tank by the parent during the incubation period or taken up across gills or other permeable surfaces.

To assess the amount of label that could be taken up by fry over a short time period, newly hatched fry were placed in seawater containing [^{14}C]-alpha amino isobutyric acid. After 24 hr fry were removed and treated as above for counting. 0.1 ml of seawater contained 20.7 DPM. Two groups of fry receiving this treatment contained 0.41 DPM/mg and 0.458 DPM/mg.

It seems apparent then that low molecular weight compounds such as alpha amino isobutyric acid can pass from paternal tissues to developing young, a

Table 1. DPM/mg in tissues of male pipefish injected with [^{14}C]-alpha amino isobutyric acid and in their embryos

Fish No.	Fish weight (g)	DPM/mg Marsupium	DPM/mg Body wall	DPM/mg Embryos
Fish sacrificed after 1 hr				
1	1.80	119	55	20.0
2	1.15	125	79	28.0
7	1.70	21	22	1.4
8	2.60	62	28	8.1
9	3.90	50	62	1.8
Fish sacrificed after 2 hr				
3	1.70	99	108	1.7
4	2.20	—	108	2.3
10	2.20	47	47	10.0
11	1.60	75	47	11.0
12	2.10	99	69	14.0
Fish sacrificed after 3 hr				
5	1.50	51	34	3.4
6	3.00	48	26	3.5
13	2.10	66	62	4.2
14	1.80	48	25	16.0
15	3.10	34	32	3.6

phenomenon not usually encountered in the vertebrates, although the relative importance of this transfer is as yet unclear. To determine the importance of this marsupial permeability to embryonic nutrition would require accurate calorimetry experiments to assess the total biological mass of fry relative to mass of eggs.

Table 2. DPM/mg [^{14}C]-amino isobutyric acid in fry from ♂♂ injected daily

Fish No.	Wt of embryos (mg)	No. of days after injection	DPM/mg
16	70	7	17.2
17	151	9	7.9
18	75	8	9.5
19	66	9	3.5

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