OBSERVATIONS ON THE TRANSPORT OF SUGARS IN THE TEMPERATE HOLOTHURIAN, *CUCUMARIA FRONDOSA*

By

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ABSTRACT

Krishnan, S. and Solemdal, P. 1974. Observations on the transport of sugars in the temperate holothurian, *Cucumaria frondosa*. *Fisk. Dir. Skr. Ser. HavUnders.*, 16: 171—176.

Different views have been put forward regarding the function of the perivisceral fluid and coelomocytes in the nutritional transport of echinoderms. To obtain more information in this field an experiment was done with the temperate holothurian, *Cucumaria frondosa*, using carbon labelled glucose, fructose and sucrose. The results showed the main role played by perivisceral fluid in nutritional transport. The different organs such as the alimentary canal, respiratory tree, body wall and gonad showed differences in their uptake.

INTRODUCTION

The roles of the perivisceral fluid and the coelomocytes in the nutritional transport system in different members of the echinoderms have been worked out by other authors (Ferguson 1962, 1963 and 1968; Farmanfarmaian 1963 and 1969; Fontaine and Lambert 1973). However, while considering the role of the perivisceral fluid, some authors (Ferguson 1962, 1963 and 1968; Farmanfarmaian 1963 and 1969) believe that the liquid phase of this fluid plays an important role in the transportation of nutrient materials. In a previous work on a tropical holothurian, *Holothuria scabra* (Krishnan and Krishnaswamy 1970; Krishnan 1971), it was shown that both the perivisceral fluid and the coelomocytes take part in such a function. In the present study *in vivo* experiments were carried out with carbon labelled sugars in the temperate holothurian, *Cacumaria frondosa*, to see the rate of transport of nutrient materials by the perivisceral fluid. The work was also carried out to compare such function in the tropical and temperate forms.

MATERIAL AND METHODS

The specimens of *Cucumaria frondosa* were collected from Strømme near Bergen, Norway, by diving. They were acclimated to the laboratory condition. During the experiments the animals were maintained in continuously circulating sea water with a temperature of 10°C and salinity of $34°/_{00}$. Before treating the animals with isotopes, they were starved for not less than 24 hours (to avoid loss through excretion) and then transferred to the experimental tanks. The carbon labelled sugars used in these experiments were purchased from the Radiochemical centre, Amersham, England. These isotopes were first diluted in the filtered sea water to a concentration of 5 μ Ci/ml. Each animal was injected with 1 ml of the above mentioned sugars having the following specific activity:

D-glucose — 1—C—14: 57 mCi per mmol.
D-fructose — 1—C—14: 58 mCi per mmol.
Sucrose — C—14 (U) 600 mCi per mmol.

In order to follow the role of the perivisceral fluid, the radioisotopes were injected directly into the coelom of the animal with the help of a microsyringe. After an interval of 1 hour, 1 ml of the coelomic fluid was syringed out and dissolved in 9 ml of the instagel for counting. To see the rate of uptake of these sugars by different organ systems, major organs such as the alimenteray canal, respiratory tree, gonad and body wall were removed simultaneously from those individuals and dried in a hot air oven at 75°C to constant weight. Each set of experiments (with glucose, fructose or sucrose) lasted for 10 hours. The results given are the mean values of duplicate sets.

For counting the radioactivity in the dry tissues, the method given by the Beckman application, was followed. All the countings were carried out with the Packard Scintillation counter, model 2002. In the case of the perivisceral fluid the results of the translocation are presented in counts per 100 g wet weight of the animal:

$$\left(\frac{\text{counts for 1 ml of the coelomic fluid}}{\text{weight for the animal}} \times 100 \right).$$

In the case of the organs the results are expressed in counts per 100 mg dry weight. The results are shown in a semilog graph. Since eye fitting was found more informative, it was used for the perivisceral fluid, and for organs they are plotted using the formula $y = ax + b$.

RESULTS

Fig. 1 shows that all three types of sugars (two mono — and one disaccharide) are translocated, more or less uniformly, from the coelomic fluid. However, the figure also shows clearly that glucose and sucrose are transported much more quickly than fructose. It is also obvious that such a function is completed within the first 4—5 hours for all the sugars.
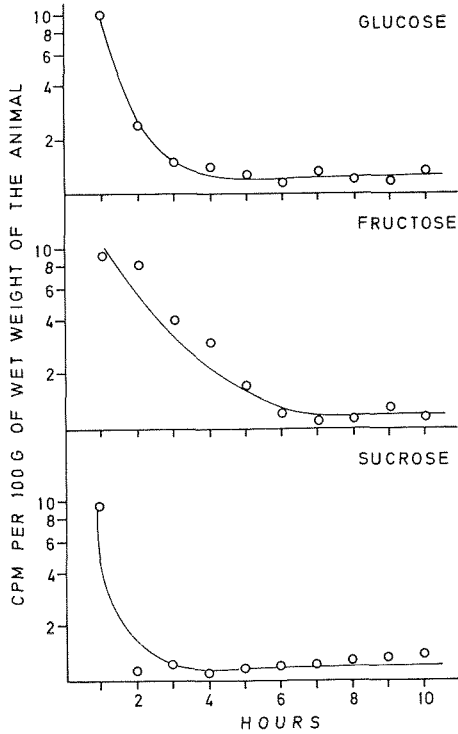

Fig. 1. The rate of translocation of carbon labelled sugars by the perivisceral fluid during 10 hrs. Actual values in Y-axis: 2×10^4 for glucose; 4×10^3 for fructose and sucrose.

Of the four major organs analysed (namely body wall, alimentary canal, respiratory tree and the gonad) for the uptake of the translocated sugars from the perivisceral fluid, the body wall appears to have absorbed a maximum of glucose when compared to other organs (Fig. 2 A); the respiratory tree seems to have taken up mainly fructose (Fig. 2 B); the alimentary canal seems to have absorbed all the sugars in the beginning, but started losing glucose and fructose after some time (Fig. 3 A). In the case of the gonad all three sugars are absorbed in the beginning, but after some time it starts losing all of them (Fig. 3 B). Such diversified results may be due to the varied metabolic stage of the organs.
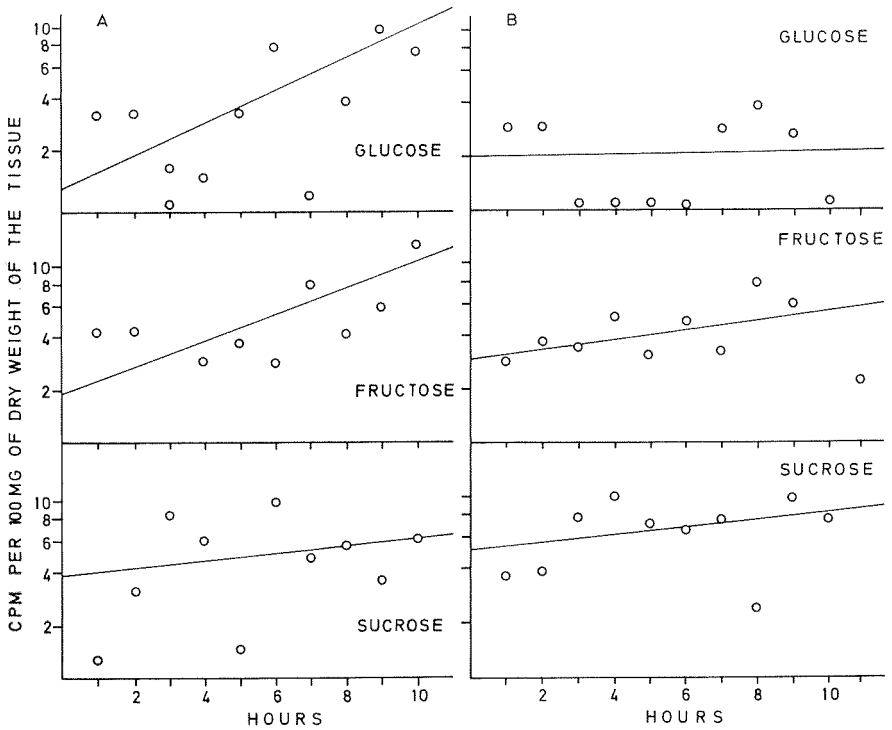
Fig. 2 A. The reaction of the body wall to the C-14 sugars.
Y = 2091x + 0,31 for glucose
Y = 1077x + 0,44 for fructose
Y = 551x + 0,95 for sucrose

Fig. 2 B. Absorption rate of carbon labelled sugars by the respiratory tree.
Actual values in Y-axis: x 10^5 for glucose, x 10^4 for sucrose and fructose.
Y = 979x + 0,20 for glucose
Y = 2189x + 0,29 for fructose
Y = 2736x + 0,52 for sucrose

DISCUSSION

The results of the present experimental studies confirm the view of FARMANFARMAIAN (1963) and KRISHNAN (1971) that the perivisceral fluid is playing a significant role in the transport of both of the sugar types employed. While presenting the rate of uptake of sugars in the holothurian, *H.scabra*, KRISHNAN and KRISHNASWAMY (1970) found that there appeared to be selective absorption of the sugars by different organs analysed. In *C. frondosa*, experiments with the labelled sugars have shown such selective absorption by the various tissues. However, such selectivity differs from what was observed for *H. scabra*. In the present study there seems to be a loss of the absorbed sugars from the
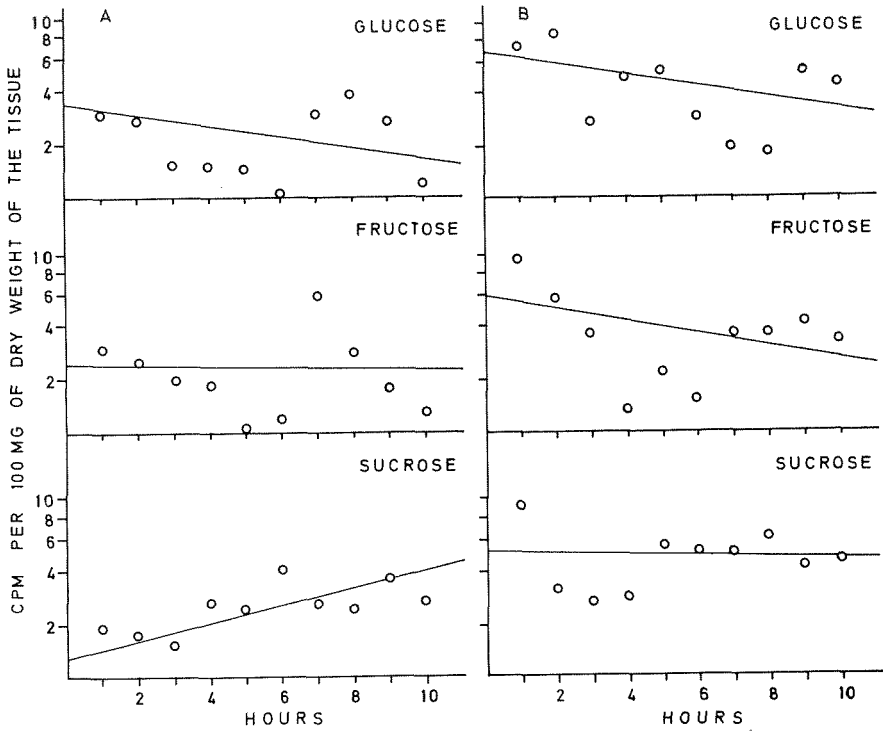
Fig. 3 A. The rate of absorption of C-14 sugars by the alimentary canal.
Actual values in Y-axis: x 10^4 for all sugars
$$Y = -1713x + 0,32 \text{ for glucose}$$
$$Y = - 238x + 0,24 \text{ for fructose}$$
$$Y = 2158x + 0,13 \text{ for sucrose.}$$

Fig. 3 B. Absorption rate of C-14 sugars by the gonad.
Actual values in Y-axis: x 10^3 for glucose; 5 × 10^3 for fructose; 3,5 × 10^2 for sucrose.
$$Y = - 401x + 0,67 \text{ for glucose}$$
$$Y = -1938x + 0,30 \text{ for fructose}$$
$$Y = - 239x + 0,18 \text{ for sucrose}$$

alimentary canal. This is true for the monosaccarides glucose and fructose. However, sucrose is shown to be steadily increasing with time. In another holothurian, *Thyone briareus*, FARMANFARMAIAN (1969) reports that the little glucose absorbed (3—17%) in the beginning was later transferred to the required organs through the perivisceral fluid by active transport. This too is true in the case of *C. frondosa*.

While looking into the uptake of sugars by the respiratory tree and body wall, one can easily understand that all those sugars were absorbed steadily. However, the body wall appears to be utilizing more of the monosaccharides than the disaccharide. It is shown in different species of holothurians that glycogen is absent in the body (BENAZZI-LENTATI

1941; FISH 1967; KRISHNAN 1968). Hence it is tentatively suggested that the body wall may be utilizing the simple sugars available, instead of the glycogen, for its metabolic activity. On the other hand the respiratory tree seems to have more capacity for utilizing sucrose when compared to the other organs. It is quite obvious that this being the most active organ of the body, it is able to utilize all the sugars, sucrose in particular.

The animals taken for the experiments were all in mature condition. It is suggested that since there may not be much synthetic activity in mature gonads, no significant uptake of sugars has been shown by the gonadal tissues. Further, the descending nature of the slope in the regression lines for the three sugars (Fig. 3 B) can be explained as the reabsorption by the coelomic fluid and translocation to other active organs like the respiratory tree and body wall.

ACKNOWLEDGEMENTS

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COMPARISON OF BLOOD PROTEINS OF COALFISH
FROM NORWEGIAN AND ICELANDIC WATERS

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ABSTRACT

MØLLER, D. and NÆVDAL, G. 1973. Comparison of blood proteins of coalfish from Norwegian and Icelandic waters. *FiskDir. Skr. Ser. HavUnders., 16*: 177—181.

Blood samples of coalfish from Norwegian and Icelandic waters were collected and analyzed for hemoglobin, serum protein, and serum esterase variations in order to study the relation between the two coalfish stocks. Clear intraspecific variation was found in the serum transferrins, but the distributions of phenotypes were nearly the same in the sample from Iceland as in the total samples from Norwegian waters.

INTRODUCTION

The coalfish, *Pollachius virens,* spawns on the banks off the west coast of Norway and in the northern North Sea. Other spawning grounds are located at Iceland and the Feroe Islands. The three stocks of coalfish have been regarded as separate selfsustaining populations, but tagging experiments have shown a rather extensive emigration from Norway to Iceland and Feroe waters (OLSEN 1961).

In the investigations reported here an attempt have been made to use frequencies of polymorphic or Mendelian characteristics to study the relation between coalfish from Norwegian and Icelandic waters. The electrophoretic patterns from analyses of coalfish hemoglobins are described elsewhere as a part of a comparative study on hemoglobins of gadoid fishes (MØLLER and NÆVDAL 1969).

MATERIAL AND METHODS

Numbers of specimens in each sample, sampling date and sampling localities are shown in Table 1. The Norwegian sampling localities are also plotted in Fig. 1. Samples 1—3 were collected from coalfish brought alive for commercial sale at Bergen harbour, and detailed catching localities are unknown. Samples 7 and 12 were collected from fishes of the O-group, and samples 8 and 10 were collected from mature fishes (sample 8 in the spawning season). All the other samples were collected from one to three years old immature fishes.

Fig. 1. The location of the Norwegian sampling stations listed in Table 1.

Bloods were collected by cardiac puncture or by cutting the tail (small fishes). Samples 1—6 and 12 were analyzed for hemoglobin variation, and in the these samples heparin was used as anticoagulant. The hemoglobins were analyzed fresh, but most sera were stored for some days or weeks in a deep freeze before analyses.

The hemoglobins were analyzed by agar gel electrophoresis (SICK 1965). Sera were analyzed by the combined starch and agar gel electrophoresis described by MØLLER (1966). The proteins were stained by Amidoblack 10 B or Nigrosin. Autoradiography was carried out as for cod sera (MØLLER 1966) based on the method of GIBLETT, HICKMAN and SMITHIES (1959). Staining of esterase activity was performed by 1 % napthylacetate in aceton using Fast Blue BB Salt as dye coupler.

RESULTS AND DISCUSSION

The hemoglobin analyses did not reval any individual variation in coalfish, except one single specimen which showed two strong fractions while all the other specimens analyzed showed only one strong fraction (MØLLER and NÆVDAL 1969). As intraspecific variations were very rare, further studies on hemoglobins of coalfish were omitted.

Also the results of esterase analyses were discouraging as only a diffuse area of esterase activity with no clear intraspecific differences was found.

Serum protein variation of coalfish has been briefly dealt with in preliminary reports (MØLLER and NÆVDAL 1966, MØLLER, NÆVDAL and VALEN 1967).

Some serum protein electrophoretograms are outlined in Fig. 2. A strong fraction of intermediate anodic mobility was shown by autoradiography to represent serum transferrins and was named Tf A. Tf A occurred in all specimens analyzed. Also the weaker component in front of it was found to possess ironbinding capasity. In a few per cent of all specimens analyzed another strong component occurred at the cathodic side of Tf A, and in two specimens (one in sample 8 and one in sample 12) a corresponding strong component occurred at the anodic side of Tf A, also this component with a weaker component in front of it. Sera in which these components occurred were not available when the tracing experiments were made, but their strength and position imply that they represent rare transferrin components, and they were named Tf B and Tf A' respectively. The phenotype which contained Tf A alone, was named Tf AA, and the phenotypes in which Tf B and Tf A' occurred, were called Tf AB and Tf AA' respectively.

The distribution of the phenotypes Tf AA and Tf AB in the collected

Table 1. Observed distributions of transferrin phenotypes in samples of coalfish from Norwegian and Icelandic waters with calculated gene frequencis and expected Hardy-Weinberg distributions.

Sample no	Locality	Date of sampling	Transferrin type			Numbers in sample	Gene frequency q_B
			TfAA	TfAB	TfBB (exp)		
1	Hordaland ......'....	4 Aug.1965	97	3	—	100	0.015
2	Rogaland	11 » 1965	57	3	—	57	—
3	Hordaland	16 Dec. 1965	108	4	—	112	0.018
4	Smøla, Nordmøre	16 » 1965	108	3	—	111	0.014
5	Sandøy, Romsdal	16 » 1965	86	4	—	90	0.022
6	Veidholmen, Nordmøre	17 » 1965	38	—	—	38	—
7	Gamsvik, Vestfjorden .	26 Sept.1965	13	—	—	13	—
8	Røstbanken	8 March 1966	157	6	—	163	0.018
9	Husøy, Nordland	8 Aug.1966	93	2	—	95	0.011
10	61°00N, 03°E' Viking Bank	22 » 1967	79	3	—	82	0.018
11	Borgenfj. Trøndelag ..	25 Oct. 1967	20	1	—	21	0.024
	Total, Norwegian waters		856	26	—	882	0.0157
	Expected Hardy-Weinbergs distribution		856	25	0.2		
12	Husavik, North-Iceland	13 Aug.1967	190	10	—	200	0.025
	Expected Hardy-Weinberg distribution		190.1	9.8	0.1		

samples are shown in Table 1. The numbers of Tf AA' are lumped with the numbers of Tf AA.

A hypothesis of genetic control of the transferrings involving two co-dominant alleles, Tf^A and Tf^B, has been adopted to explain the observed variation. In Table 1 the frequencies of Tf^B are calculated for each sample, and expected distributions of genotypes are calculated for the total of Norwegian samples and for the sample from Iceland respectively. The expectance of the genotype Tf^B/Tf^B in the present material is low, and the overall accordance between observed and expected distributions is reasonable good, implying that the hypothesis is correct.

Tf A' may have a similar control, but because this component is very rare, this hypothesis can not be tested from population data.

Also in other serum proteins intraspecific variations were observed (Fig. 2), but they occurred as presence or absence of weak fractions and clear-cut typing of the individual specimens was impossible.
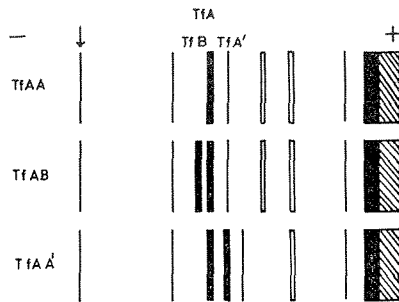
Fig. 2. Outline of serum protein patterns in coalfish obtained by combined starch and agar gel electrophoresis at pH 0.9.
Filled in bars: Strong bands. Open bars: Moderately strong bands. Hatched bars: Diffuse bands. Single lines: Faint bands. Arrow indicate the point of application.

Table 1 shows that no great differences were found among the samples in distribution of transferrin phenotypes. The q^B-value varied between zero and 0.024 in the Norwegian samples, but showed a somewhat higher value, 0.025, in the sample from Iceland. However, χ^2 homogenity test on the distribution of phenotypes showed that the difference between the Norwegian and the Icelandic samples was not significant $\chi^2 = 2.14$, 1 d. f.,0.1 $< P < 0.2$).

Thus no significant difference between Norwegian and Icelandic coalfish was detected in the present study. This may imply that the two stocks are not genetically isolated, but the reason may also be that the transferrin variation is a balanced polymorphism where the controlling factor (probably one or another abiotic ecological factor) shows so similar values in the two environments that similar gene frequencies are established.

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