Comp. Biochem. Physiol. Vol. 78B, No. 3, pp. 761–767, 1984
Printed in Great Britain

0305-049 1/84 $3.00 + 0.00

TURNOVER RATES OF FATTY ACID AND AMINO ACID IN THE COELOMIC FLUID OF THE SEA STAR *ASTERIAS RUBENS*: IMPLICATIONS FOR THE ROUTE OF NUTRIENT TRANSLOCATION DURING VITELLOGENESIS

F. B. BEIJNINK, I. VAN DER SLUIS and P. A. VOOGT
Laboratory of Chemical Animal Physiology, Research Group for Invertebrate Reproductive Physiology, State University of Utrecht, 8 Padualaan, 3508 TB Utrecht, the Netherlands (Tel: 030-532578)

(*Received* 19 *January* 1984)

Abstract—1. The turnover of fatty acid and amino acid in the coelomic fluid of the sea star *Asterias rubens* was quantified by intra-coelomic injection of radiolabelled oleic acid and L-leucine which had half times of 2 and 10 min, respectively.

2. The molar composition of the amino acids of the cell-free coelomic fluid was determined. The total concentration of amino acids was 4.33 ± 1.93 μg/ml; the total concentration of fatty acids was 0.34 μg/ml.

3. In a period of 4 weeks, 74 mg of fatty acid and 201 mg of amino acid is transferred through the coelomic fluid of a standardized 100 g animal.

4. *In vitro* experiments allow an estimation of the apparently minor role of coelomocytes in lipid transport.

5. Transport of fatty acid and amino acid through the perivisceral coelomic fluid can by far not meet the demands of the ovaries during vitellogenesis. Therefore, other systems (haemal and perihaemal) must play an important role.

INTRODUCTION

Reproduction in asteroid echinoderms generally implies a substantial transfer of nutrients to the developing gonads. Although this nutrient flow is mainly sustained by active feeding (Harrold and Pearse, 1980), mobilization of reserves—mainly lipids and proteins—from stores in the pyloric caeca is also involved (Ferguson, 1975b; Oudejans and Van der Sluis, 1979a; Oudejans *et al.*, 1979; Van der Plas and Voogt, 1982).

While evidence for a role of the haemal sinuses and perihaemal coelomic cavities is accumulating (Broertjes *et al.*, 1980a,b; Beijnink *et al.*, 1984), it is still generally assumed that the bulk of material needed for gametogenesis is translocated through the perivisceral coelomic cavity (for review, see Ferguson, 1982). In this concept water-soluble compounds diffuse from the digestive and storage organs into the coelomic fluid and are subsequently absorbed actively by the target organs.

The level of organic constituents in the coelomic fluid of sea stars is extremely low (Boolootian, 1961; Endean, 1966). Therefore, a substantial transfer of these compounds can only be accomplished with high turnover rates. Van der Heyde (1922) already demonstrated that glucose and glycine (injected in large quantities) were rapidly absorbed from the coelomic fluid. Similar results were obtained by Ferguson (1964a) using radiolabelled nutrients. However, these studies do not supply the *in vivo* turnover times or concentrations to allow a proper quantitative estimation of the turnover rates of the studied compounds.

A substantial part of the lipids, accumulated in the ovaries during vitellogenesis, consists of polyunsaturated fatty acids (Ferguson, 1976; Sargent *et al.*, 1983). Little is known with regard to the translocation of these mainly essential compounds. Lipoproteins could not be detected in the coelomic fluid of *Asterias rubens* (Broertjes *et al.*, 1980a). The numerous amoeboid cells (coelomocytes) in the coelomic fluid supposedly play a role in lipid translocation since oral administration of radiolabelled lipid results in a high incorporation of label into coelomocyte lipids (Allen, 1974; Oudejans and Rutten, 1982).

In order to quantify the turnover of coelomic fluid compounds, we have injected tracer amounts of radiolabelled oleic acid and L-leucine in the perivisceral coelomic cavity of *Asterias rubens*. The role of coelomocytes has been investigated further in both *in vivo* and *in vitro* experiments.

MATERIALS AND METHODS

Animals

Mature specimens of *Asterias rubens* were collected in the Dutch Wadden Sea throughout the year. The animals were kept up to several months under natural daylength in a flow-through sea water system at 12°C and were fed *ad libitum* with the sea mussel *Mytilus edulis*.

Radioactive precursors

[9,10-^{3}H(n)]Oleic acid (0.19 to 0.35 TBq/mmol, depending on the batch) and [4,5-^{3}H(n)]L-leucine (1.93 TBq/mmol) were obtained from New England Nuclear (Dreieich, W. Germany).

In vivo *experiments*

Animals, with body weights ranging from 80 to 150 g,

were injected intra-coelomically with 1 μl/g fresh weight of a solution of radiolabelled oleic acid (dissolved in 100% ethanol and diluted 10–20 times to a final radioactivity of 111 kBq/μl with an aqueous solution of 3.5% NaCl containing 0.2% Triton X-100 as a detergent) or L-leucine (dissolved in an aqueous solution of 3.5% NaCl, final radioactivity 25.0 kBq/μl). After injection with oleic acid, each animal was placed on the bottom of a beaker with sea water. This beaker was fixed on a rotating device (axis under angle of approx. 60° with the horizontal plane). In this manner, the animals were gently rotated for 5 min in order to achieve a homogeneous distribution of the injected tracer over the coelomic fluid. At set intervals samples were drawn from the coelomic fluid with a 50 μl microsyringe, take 30 μl from each arm tip to obtain a total sample of 150 μl. Cysteine hydrochloride (1% in an aqueous solution of 3.5% NaCl) was added to a final volume of 1 ml to prevent clotting (Boolootian and Giese, 1959). After centrifugation (3 min, 15,000 *g*), aliquots from supernatant and pellet (resuspended in distilled water) were taken for measurement of radioactivity. In some experiments, the protein content of the pellet was estimated with the Coomassie Brilliant Blue G-250 method (Bradford, 1976) with bovine serum albumin as standard.

In vitro *experiments*

Animals with body weights ranging from 40 to 70 g were used. Each animal was held perpendicularly to allow the coelomic fluid to accumulate in the downward hanging arm. The tip of this arm was cut off and a volume of at least 10 ml of coelomic fluid was collected in a measuring cylinder which already contained 90 ml of a calcium- and magnesium-free salt solution (CMFSS) according to Kanungo (1982) to prevent clotting (CMFSS: 0.44 M NaCl, 10.61 mM KCl, 21.1 mM Na_2SO_4, 16.7 mM D-glucose, 12.0 mM Hepes (*N*-2-hydroxy-ethylpiperazine *N*-2-ethanesulfonic acid), 15.0 mM EDTA (ethylenediamine tetraacetic acid), adjusted to pH 7.4 with NaOH). If the volume after collection exceeded 100 ml, an additional amount of CMFSS was added immediately to obtain a ten-fold dilution of the coelomic fluid. From this solution, which even after several hours did not shown any sign of clotting, 9 ml aliquots were taken and added to test tubes containing 0.1 ml radiolabelled oleic acid (1.85 kBq in CMFSS containing 0.1% Triton X-100) or L-leucine (3.7 kBq in CMFSS) and 0.9 ml CMFSS with or without non-radioactive oleic acid or L-leucine in amounts up to 1 mg. Incubation of this mixture (volume 10 ml) was performed at room temperature under continuous agitation. After incubation and subsequent centrifugation (10 min, 5000 *g*), aliquots of supernatant and pellet (resuspended in distilled water) were taken for measurement of radioactivity. Part of the diluted coelomic fluid was centrifuged (10 min, 5000 *g*) for the determination of dry and fresh weight of the coelomocytes.

Thin-layer chromatography of lipids

Thin-layer chromatography was applied to lipids from coelomocyte samples of both *in vivo* and *in vitro* experiments. Lipids were extracted with chloroform–methanol (Bligh and Dyer, 1959) and separated on Silicagel G (Freeman and West, 1966). For identification of lipid classes, spots were visualized under uv-light (366 nm) after spraying with a 0.005% aqueous solution of Rhodamine-6G. Identified areas were scraped from the plates into minivials for measurement of radioactivity. For photodensitometry, the plates were sprayed with a solution of 3% cupric acetate in 8% aqueous phosphoric acid (Fewster *et al.*, 1969) and charred at 180°C for 20 min. Optical densities of the charred spots were measured with a densitometer (Vitatron TLD-100) equipped with an integrator.

Determination of fatty acid and amino acid concentration

Coelomic fluid was collected by cutting off the arm tips of several sea stars. After spinning down the coelomocytes (10 min, 5000 *g*), the cell-free coelomic fluid was extracted with chloroform–methanol (Bligh and Dyer, 1959). The chloroform fraction was dried *in vacuo*; fatty acids were isolated from the lipid residu by thin-layer chromatography. The methanol–water fraction was centrifuged (5 min, 15,000 *g*) and methanol was evaporated under nitrogen. The remaining water-fraction was lyophilized; the residue was dissolved repeatedly in decreasing volumes of 70% ethanol with subsequent centrifugation (5 min, 15,000 *g*) to precipitate excess salt. Analysis of amino acids in the remaining residue was performed with an automatic amino acid analyser (LKB-320) as described by Worm and Beenakkers (1980).

Measurement of radioactivity

All samples were dissolved in Packard Emulsifier (Scintillator 299). Radioactivity was measured with a Packard 2420 Liquid Scintillation Counter. The external standard channel ratio method was used for quench correction.

Calculation of turnover data

Semilogarithmic plots were used to estimate half times. Turnover times were calculated as $T_t = T_{1/2}/\ln 2$ (Zilversmit, 1960). Turnover rates are expressed as milligrams of fatty acid or amino acid translocated in a period of 4 weeks.

RESULTS

Half time of oleic acid

Throughout the reproductive cycle, both male and female animals were injected intra-coelomically with tracer amounts of radiolabelled oleic acid. Sex and gonad index hardly had any influence on the estimated half times; therefore, all data were pooled. Figure 1 shows an initial steep decline in the recovered radioactivity. From this part of the curve, a half time of approx. 2 min can be deduced (see inset Fig. 1).

Due to the variable and low amount of coelomocytes in the samples, it was difficult to estimate the uptake of radiolabel by these cells. One representative curve is given in Fig. 2. About 11% of the injected amount of radioactivity is associated with the coelomocytes at times up from one hour after injection.

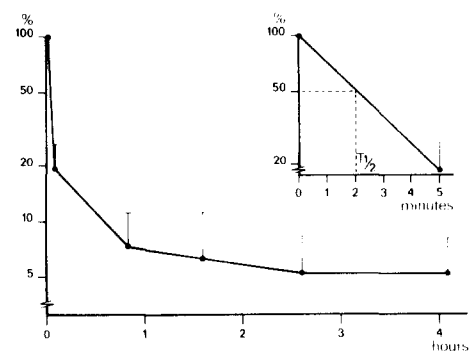

Fig. 1. Proportional radioactivity of the cell-free coelomic fluid of *Asterias rubens* after intra-coelomic injection with radiolabelled oleic acid, given on a logarithmic scale as percentage of the injected dose. In the inset, the half time ($T_{1/2}$) is estimated from the first part of the curve on a different time scale. Values are means ± SD (n = 5).

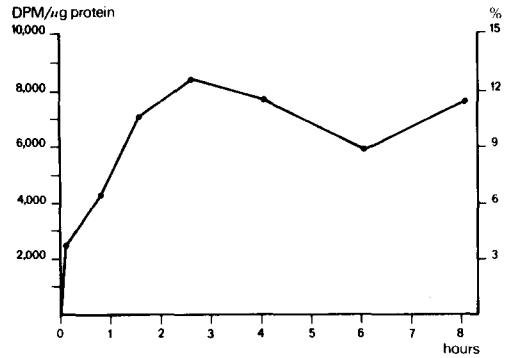

Fig. 2. Radioactivity of the coelomocytes of a female *Asterias rubens* after intra-coelomic injection with radiolabelled oleic acid, given as d.p.m. per μg protein and as percentage of the injected dose.

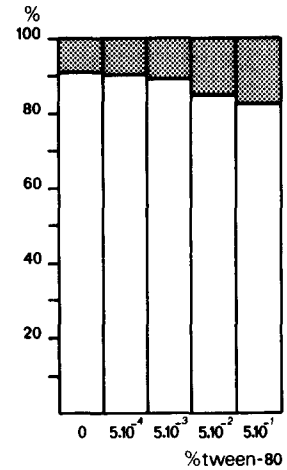

Fig. 3. Proportional distribution of radioactivity over supernatant (cell-free coelomic fluid, hatched area) and pellet (coelomocytes, white area) after intra-coelomic injection of a female *Asterias rubens* with radiolabelled oleic acid and subsequent treatment of the coelomic fluid (isolated after 3 hr) with increasing concentrations Tween-80 followed by centrifugation (3 min, 15,000 *g*).

Table 1 shows the distribution of radioactivity over the individual lipid classes from coelomocytes of a female sea star after injection with radiolabelled oleic acid. The recovery in the coelomic fluid is very low (about 2% after 1 hr) and consists mainly of methanol–water-soluble products (data not given). The radioactivity of the coelomocytes increases with time and is mainly present in fatty acids and in small amounts in mono- and triacylglycerols and polar lipids.

Finally it was investigated whether oleic acid is actually taken up by coelomocytes or is only adsorbed at the cell membrane. Therefore, 3 hr after injection of radiolabelled oleic acid into a female sea star, the coelomic fluid was collected by cutting off the tip of one arm. After addition of cysteine hydrochloride (final concentration 1%), the coelomic fluid was shaken gently for 1 min with the detergent Tween-80, added in concentrations ranging from 5×10^{-4} to 5×10^{-1}%. Figure 3 shows that almost no radiolabel can be removed from the coelomocytes with the detergent used.

Half time of L*-leucine*

One male and two female sea stars were injected with radiolabelled L-leucine at one moment of the reproductive cycle (January 1982). The recovery of radioactivity is plotted in Fig. 4. A half time of approx. 10 min is deduced from the obtained regression line. As in the case of oleic acid, the amount of coelomocytes in most samples was too low to allow an exact determination of radioactivity. However, the uptake of radiolabelled L-leucine by these cells appears to be considerably lower than the uptake of oleic acid in the above experiment.

Calculation of turnover rates

The concentrations of total lipids and fatty acids were determined in one batch of coelomic fluid (410 ml from 11 animals of both sexes, collected in August, 1982). The lipid concentration in the cell-free coelomic fluid was 4.39 μg/ml. The concentration of fatty acids was 0.34 μg/ml. In the same period, the coelomic fluid from female animals was used for

Table 1. Proportional distribution of radioactivity over individual lipid classes from coelomocytes of a female *Asterias rubens* after intra-coelomic injection with radiolabelled oleic acid. In the two upper lines, the total recovery in cell-free coelomic fluid and coelomocytes is given as percentage of the injected dose

Minutes after injection	5	50	95	155	245
% in cell-free coelomic fluid	6	3	2	2	2
% in coelomocytes	6	6	10	10	13
MeOH-H_2O	11.7	6.3	6.6	4.2	4.0
PL	2.7	13.0	2.9	3.1	4.7
MAG	5.1	15.3	5.1	4.4	3.6
FA	50.6	55.0	77.6	80.2	75.0
ST	2.0	1.6	1.3	2.0	1.8
1.2 DAG	3.4	1.1	0.7	0.7	2.1
1.3 DAG	3.2	2.0	0.9	0.7	1.6
TAG + ME	14.8	3.6	3.1	2.8	5.0
SE	4.0	1.6	1.3	1.5	1.4
HC	2.3	0.6	0.4	0.3	0.8

MeOH-H_2O: methanol–water-soluble; PL: polar lipids; MAG, DAG and TAG: mono-, di- and triacyglycerols; FA: fatty acids ST: sterols; ME: fatty acid methyl esters; SE: steryl-esters; HC: hydrocarbons.

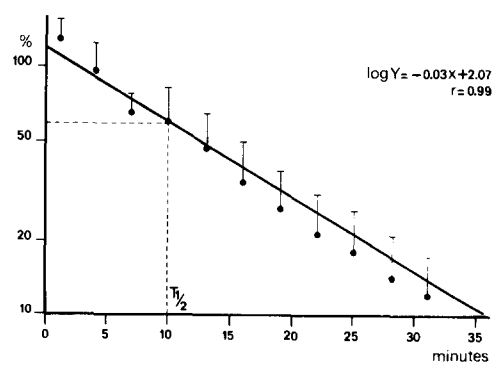

Fig. 4. Proportional radioactivity of the cell-free coelomic fluid of *Asterias rubens* after intra-coelomic injection with radiolabelled L-leucine, given on a logarithmic scale as percentage of the injected dose. The broken line indicates the half time ($T_{1/2}$) after extrapolation of the obtained regression line to 0 min. Values are means ± SD ($n = 3$).

Table 2. Molar composition of the amino acids in the cell-free coelomic fluid of female *Asterias rubens*. Values (nmol/ml) are means ± SD ($n = 4$)

Tau	1.4 ± 1.1
Asp	0.1 ± 0.1
Thr	1.4 ± 1.2
Ser	5.9 ± 2.2
Glu	0.8 ± 0.6
Gln	2.2 ± 2.6
Pro	0.8 ± 0.6
Gly	27.7 ± 6.4
Ala	3.1 ± 1.9
Val	1.6 ± 1.2
Met	0.1 ± 0.1
Cys	1.3 ± 1.3
Ile	1.0 ± 1.0
Leu	1.3 ± 1.1
Tyr	0.5 ± 0.5
Phe	0.3 ± 0.3
total	45.6 ± 20.3

amino acid analysis (Table 2). With a mean amino acid mol. wt of 95, the cell-free coelomic fluid contained 4.33 ± 1.93 μg amino acid per ml ($\bar{x} \pm SD$, $n = 4$).

The volume of the coelomic fluid of female sea stars was measured at regular intervals in the period of May to October 1981. Calculated as organ index (= v/w percentage of total body wt), a value of 16.2 ± 9.6% ($\bar{x} \pm SD$, $n = 10$) was found. This value is used in the calculation of the turnover rates (Table 3). In a period of 4 weeks, 74 mg of fatty acid and 201 mg of amino acid is translocated in a standardized animal of 100 g if the measured half times of oleic acid and L-leucine are applied to all fatty acids and amino acids, respectively.

In the same period, the amount of coelomocytes in the coelomic fluid of female sea stars was measured. One millilitre of coelomic fluid contained 21 ± 15 mg coelomocytes (mean fresh wt ± SD, $n = 10$). Of this amount, 9.7 ± 1.8% represented dry wt ($\bar{x} \pm SD$, $n = 5$).

In vitro *labelling of coelomocytes*

The coelomic fluid from four animals of both sexes was collected and incubated in CMFSS with radiolabelled precursor. Each test tube contained 1.1 ± 0.8 mg coelomocytes (mean dry wt ± SD, $n = 4$) and 2.7 ng tritiated oleic acid or 0.25 ng tritiated L-leucine in 10 ml CMFSS. The uptake of radioactivity by coelomocytes is plotted in Fig. 5. Coelomocytes take up oleic acid at a very high rate; L-leucine is incorporated much more slowly.

Lipid samples from coelomocytes, incubated for 90 min with oleic acid, were analyzed by thin-layer chromatography. Table 4 shows that a great portion of the radiolabel is incorporated into triacylglycerols and to a less degree into polar lipids. The lipid composition of the coelomocytes, pooled from five female animals used in these experiments, is shown between brackets. Sterols, polar lipids and fatty acids are the predominant components. Lipids comprise 12.7% of the total coelomocyte dry weight.

Table 3. Calculation of turnover rates of fatty acids and amino acids in a standardized *Asterias rubens* of 100 g containing 16.2 ml coelomic fluid

	Fatty acids	Amino acids
Half time $T_{\frac{1}{2}}$ (min)	2	10
Turnover time T_t (min)	3	14
Concentration (μg/ml)	0.34	4.33
Turnover rate (mg/4 weeks)	74	201

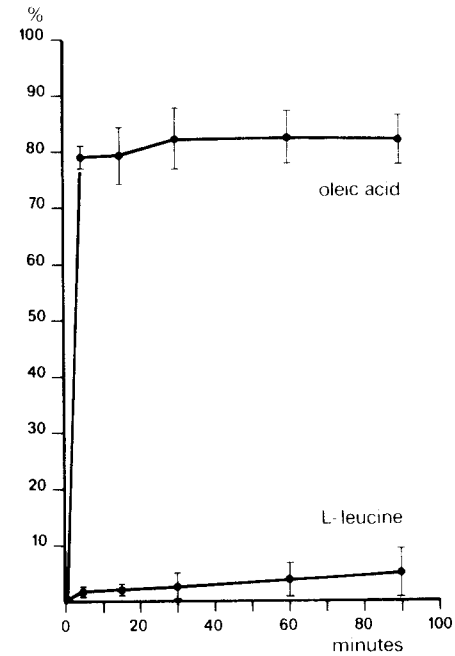

Fig. 5. *In vitro* incorporation of radiolabelled oleic acid and L-leucine into coelomocytes of *Asterias rubens*, expressed as mean percentage of the total dose (± SD, $n = 4$).

The concentrations of oleic acid and L-leucine were raised above tracer levels with increasing amounts of non-radioactive material in a parallel experiment (Fig. 6). The radioactivity of the coelomocytes was measured after 90 min. Each test tube contained 2.2 ± 0.8 mg coelomocytes (mean dry wt ± SD, $n = 4$) and up to 1 mg of non-labelled oleic acid or L-leucine in 10 ml CMFSS. Coelomocytes become saturated with oleic acid above concentration of approx. 1 μg/ml. Saturation with L-leucine occurs at lower concentrations.

DISCUSSION

Turnover rate of fatty acid

Injected oleic acid rapidly disappears from the

Table 4. Proportional distribution of radioactivity over individual lipid classes from ceolomocytes of *Asterias rubens* after *in vitro* incubation with radiolabelled oleic acid during 90 min. Values are mean percentages ± SD ($n = 4$). The lipid composition of the coelomocytes, pooled from five female sea stars, is shown between brackets as percentages of total lipid. For short-hand indications of lipid classes, see Table 1 (n.d. = not detectable)

MeOH-H_2O	n.d.	
PL	12.4 ± 1.3	(19.4)
MAG	0.7 ± 0.2	(5.0)
FA	20.6 ± 6.1	(15.3)
ST	0.3 ± 0.1	(23.3)
1,2 DAG	2.1 ± 0.8	(3.8)
1,3 DAG	1.5 ± 0.6	(2.2)
TAG	61.7 ± 6.5	(6.0)
ME	0.8 ± 0.4	(n.d.)
SE, HC	0.5 ± 0.1	(7.1)

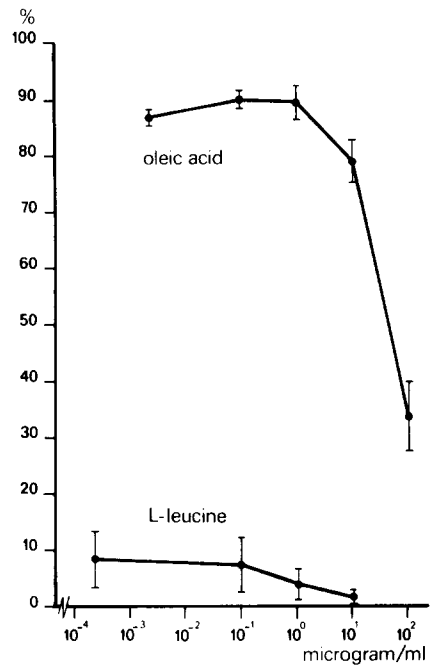

Fig. 6. Incorporation of radiolabelled oleic acid and L-leucine into coelomocytes of *Asterias rubens* in the presence of increasing amounts of non-radioactive material (horizontal scale) after 90 min *in vitro* incubation. Values are mean percentages ($\pm$SD, $n = 4$) of the total dose.

coelomic fluid and is only partly recovered in the coelomocytes. Apparently, most oleic acid is absorbed by the coelomic lining of the internal organs and the body wall. The high rate of this phenomenon is not surprising. In general, the uptake of fatty acid is a very rapid process which initially involves non-metabolic adsorption at the cell membrane (Nikillä, 1971).

In vitro experiments have shown that asteroid ovaries are capable of *de novo* synthesis of lipid from [1-^{14}C]acetate (Allen and Giese, 1966; Allen, 1968). However, the enzymes directly involved in fatty acid synthesis (acetyl CoA carboxylase and fatty acid synthetase) can hardly be detected in the ovaries of *Asterias rubens*, while their activities in the pyloric caeca have been established convincingly (Oudejans *et al.*, 1984). Although decisive quantitative data are lacking, it seems likely therefore that the major part of the ovarian lipids originates either directly from dietary sources or indirectly from caecal depots. This is also indicated by the presence of large amounts of polyunsaturated fatty acids in the ovaries of several asteroids (Ferguson, 1976; Sargent *et al.*, 1983; Broertjes *et al.*, in press).

The present study shows that approx. 74 mg of fatty acid can be absorbed from the coelomic fluid within a 4 week period. In addition, coelomocytes, derived from the coelomic lining of the digestive system (Vanden Bossche and Jangoux, 1976), may transport fatty acids also. A role of these cells in nutrient transport has been suggested by several authors (for review, see Ferguson, 1982) who often observed increased levels of organic (radiolabelled) compounds in coelomocytes after oral administration of such materials. A quantitative approach has been made only by Allen (1974) who calculated a minimum rate of lipid transfer of 1 mg/day to the ovaries of the echinoid *Strongylocentrotus purpuratus*. According to our measurements, a standardized 100 g sea star contains 16.2 ml coelomic fluid (cf. 16.7 ml reported by Oudejans *et al.* (1983)) with approx. 340 mg coelomocytes (fresh wt), corresponding to 33 mg dry wt or 4.2 mg lipid. The last value is in good agreement with data from other studies that indicate that the same amount of coelomic fluid (including coelomocytes) contains 1.5–6.5 mg lipid depending on the asteroid species studied (Greenfield *et al.*, 1958; Giese, 1966; Oudejans and Rutten, 1982). If the supply of fatty acids is not limited as in the described *in vitro* experiments, this amount of coelomocytes can accumulate approx. 0.2 to 1 mg fatty acid maximally before being saturated.

Echinoderms in general are capable of considerable production of coelomocytes (Schinke, 1950; Vanden Bossche and Jangoux, 1976; Binyon, 1982). *In vitro* experiments with isolated arms show that *Asterias rubens* is able to renew its population of coelomocytes in the perivisceral coelomic cavity within a period of approx. 3 days (Binyon, 1982). If this high turnover rate in coelomocytes would be a normal phenomenon, an amount of approx. 7 mg fatty acid would become available by lysis of "old" coelomocytes within a 4 week period (corresponding to 7 times a total population of coelomocytes containing 1 mg fatty acid maximally).

Conclusively, about 81 mg fatty acid can be translocated in a 100 g animal by turnover of coelomic fluid fatty acid and by coelomocytes within a 4 week period. It should be emphasized that the ovaries are not the only organs able to benefit from this supply. The body wall and other organs bathed by the coelomic fluid will also take up fatty acid and other nutrients from this fluid (Ferguson, 1964b, 1970). Moreover, the amount remaining for the ovaries will also be used in sustaining the normal metabolism of somatic parts of these organs. Therefore only a very small fraction of the calculated 81 mg fatty acid will ultimately be available for vitellogenesis. Since the average increase in lipid content of the ovaries of *Asterias rubens* is 250 mg/4 weeks for a 100 g animal (Oudejans and Van der Sluis, 1979a), it seems impossible that translocation of fatty acids through the perivisceral coelomic cavity can fulfil these requirements.

It can not be excluded that also other lipid compounds (e.g. triacylglycerols) may play a role in lipid translocation. However, oral administration of radiolabelled trioleoylglycerol results in labelling of coelomocyte triacylglycerol only with almost no labelling of the coelomic fluid (Oudejans and Rutten, 1982). A similar calculation as above for the potency of coelomocytes to transport triacylglycerols does not lead to any substantial amount, particularly since these cells contain only small amounts of these compounds (Table 4). The lipid composition of coelomocytes of female animals is in agreement with brief data reported earlier for these cells (Oudejans and Rutten, 1982) and is rather similar to that of female pyloric caeca as reported by Oudejans and Van der Sluis (1979b) with the exception of sterols

(about twice as much in coelomocytes) and triacylglycerols (4 to 20 times as much in pyloric caeca). The low triacylglycerol content of coelomocytes is also indicated by an ultrastructural study of the coelomocytes of *Dermasterias imbricata* (Kaneshiro and Karp, 1980) that reveals only a few lipid droplets in micrographs of these cells.

After intra-coelomic injection of radiolabelled oleic acid, most of the label associated with coelomocytes is recovered in the fatty acid fraction. Since the label can not be removed with detergent and is incorporated *in vitro* into triacylglycerols, fatty acid evidently is not merely adsorbed at the cell membrane but is incorporated into intracellular pools. The high labelling of triacylglycerol *in vitro* (Table 4) is surprising. Although the incubation system used may represent a powerful tool in studying the physiological functioning of coelomocytes, important differences evidently exist with the *in vivo* situation that can not be readily explained.

Turnover rate of amino acid

Van der Heyde (1922) injected glycine in amounts ranging from 150 to 700 mg in the coelomic fluid of *Asterias rubens*. Although these quantities are no tracer amounts, his experiments roughly indicate a turnover time of approx. 20 min. Ferguson (1962, 1964b) used isolated pyloric caeca submersed in sea water or cell-free coelomic fluid. After extrapolation for a total intact sea star he deduced a turnover time of approx. 40 min for glycine. The same author also studied uptake constants of several amino acids and reported that all neutral amino acids are absorbed at approximately the same rate by pyloric caeca *in vitro* (Ferguson, 1968).

Giordano *et al.* (1950) reported the presence of five amino acids (arg, gly, phe, ser, trp) in the cell-free coelomic fluid of *Pisaster brevispinus*. Glycine was the most abundant one (67% of total amino acid) and serine the second most prominent one. These data are in good agreement with the results presented in Table 2. The total amino acid content of the cell-free coelomic fluid of *Asterias rubens* (4.33 μg/ml) is low compared with values reported for *Pisaster brevispinus* (15 μg/ml; Giordano *et al.*, 1950) and *Echinaster modestus* (6 to 29 μg/ml; Ferguson, 1980). This may be due to differences between species or condition of animals. Also, the last authors measured total amino acid directly, thus including tryptophan and the basic amino acids too. However, their and other studies (Ferguson, 1975a, 1979) indicate that these amino acids are present in only very small amounts.

We have measured a turnover rate of 201 mg amino acid in a 4 week period in a 100 g animal. Again, only a small fraction of this amount will be available for vitellogenesis since also the body wall and other internal organs take up amino acid from the coelomic fluid (Ferguson, 1970). The average increase in protein and free amino acid content of the ovaries of *Asterias rubens* is 500 and 110 mg respectively in a 4 week period for a 100 g animal (Oudejans and Van der Sluis, 1979a). If the increase in protein content is the result of autosynthetic yolk formation, translocation of amino acids by the coelomic fluid is not sufficient.

Conclusively, translocation of both amino acids and fatty acids through the perivisceral coelomic cavity can not fulfil the needs of the growing ovaries. Therefore other systems that connect the ovaries and the digestive system, will have to be in use. In this respect the haemal sinuses and perihaemal coelomic cavities have been advanced in both morphological (Walker, 1979; Schoenmakers *et al.*, 1981; Beijnink *et al.*, 1984) and experimental studies (Broertjes *et al.*, 1980a,b). Recent biochemical studies (Broertjes *et al.*, in press a,b) indicate that heterosynthesis may occur in *Asterias rubens* involving the supply of glycolipoproteins through the haemal system. To obtain more detailed information on the possible routes of nutrient transfer, autoradiographic experiments currently are in progress in our laboratory.

Acknowledgements—We thank Mr. H. J. K. Ravenstein for performing the amino acid analysis and Drs. R. C. H. M. Oudejans and J. J. S. Broertjes for many suggestions during the experiments and the preparation of the manuscript. The investigations were supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

Allen W. V. (1968) Fatty acid synthesis in the echinoderms: *Asterias rubens*, *Echinus esculentus* and *Holothuria forskali*. *J. mar. biol. Ass. UK* **48,** 521–533.

Allen W. V. (1974) Interorgan transport of lipids in the purple sea urchin, *Strongylocentrotus purpuratus*. *Comp. Biochem. Physiol.* **47A,** 1297–1311.

Allen W. V. and Giese A. C. (1966) An *in vitro* study of lipogenesis in the sea star, *Pisaster ochraceus*. *Comp. Biochem. Physiol.* **17,** 23–28.

Beijnink F. B., Walker C. W. and Voogt P. A. (1984) The microenvironment of vitellogenic oocytes in the sea star *Asterias rubens*; interactions between the ovarian haemal system, follicle cells and primary oocytes. Submitted to *Cell Tissue Res.*

Binyon J. (1982) The number and origin of the coelomocytes in the perivisceral fluid of the starfish *Asterias rubens* L. In *Echinoderms: Proceedings of the International Conference, Tampa Bay* (Edited by Lawrence J. M.), pp. 353. A. A. Balkema, Rotterdam.

Bligh E. G. and Dyer W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37,** 911–917.

Boolootian R. A. (1961) Physical properties and chemical composition of perivisceral fluid: Echinodermata In *Biol. Handbook: Blood and Other Body Fluids*, pp. 339–344. Federation of American Societies for Experimental Biology.

Boolootian R. A. and Giese A. C. (1959) Clotting of echinoderm coelomic fluid. *J. exp. Zool.* **140,** 207–221.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein, utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72,** 248–254.

Broertjes J. J. S., Posthuma G., den Breejen P. and Voogt P. A. (1980a) Evidence for an alternative transport route for the use of vitellogenesis in the sea-star *Asterias rubens* (L.). *J. mar. biol. Ass. UK* **60,** 157–162.

Broertjes J. J. S., Posthuma G., Beijnink F. B. and Voogt P. A. (1980b) The admission of nutrients from the digestive system into the haemal channels in the sea-star *Asterias rubens* (L.). *J. mar. biol. Ass. UK* **60,** 883–890.

Broertjes J. J. S., de Waard P. and Voogt P. A. (a) On the presence of vitellogenic substances in the starfish *Asterias rubens* (L.). *J. mar. biol. Ass. UK* **64** (in press).

Broertjes J. J. S., de Waard P. and Voogt P. A. (b) Purification and characterization of vitellogenic substances in the starfish *Asterias rubens* (L.). *Mar. biol. Lett.* **5** (in press).

Endean R. (1966) The coelomocytes and coelomic fluid. In *Physiology of Echinodermata* (Edited by Boolootian, R. A.), pp. 310–328. Interscience, New York.

Ferguson J. C. (1962) Nutrient transport in the starfish, *Asterias forbesi*, as studied with isolated digestive glands. *Biol. Bull., Mar. Biol. Lab., Woods Hole* **123,** 482–483.

Ferguson J. C. (1964a) Nutrient transport in starfish. I. Properties of the coloemic fluid. *Biol. Bull. Mar. Biol. Lab., Woods Hole* **126,** 33–53.

Ferguson J. C. (1964b) Nutrient transport in starfish. II. Uptake of nutrients by isolated digestive glands. *Biol. Bull. Mar. Biol. Lab., Woods Hole* **126,** 391–406.

Ferguson J. C. (1968) Transport of amino acids by starfish digestive glands. *Comp. Biochem. Physiol.* **24,** 921–931.

Ferguson J. C. (1970) An autoradiographic study of the translocation and utilization of amino acids by starfish. *Biol. Bull. Mar. Biol. Lab., Woods Hole* **138,** 14–25.

Ferguson J. C. (1975a) The role of free amino acids in nitrogen storage during the annual cycle of a starfish. *Comp. Biochem. Physiol.* **51A,** 341–350.

Ferguson J. C. (1975b) Fatty acid and carbohydrate storage in the annual reproductive cycle of *Echinaster. Comp. Biochem. Physiol.* **52A,** 585–590.

Ferguson J. C. (1976) The annual cycle of fatty acid composition in a starfish. *Comp. Biochem. Physiol.* **54B,** 249-252.

Ferguson J. C. (1979) Ingestion and assimilation of dissolved amino acids by a starfish. *Comp. Biochem. Physiol.* **62A,** 343–346.

Ferguson J. C. (1980) The non-dependency of a starfish on epidermal uptake of dissolved organic matter. *Comp. Biochem. Physiol.* **63A,** 461–465.

Ferguson J. C. (1982) Nutrient translocation. In *Echinoderm Nutrition* (Edited by Jangoux M. and Lawrence J. M.), pp. 373–393. A. A. Balkema, Rotterdam.

Fewster M. E., Burns B. J. and Mead J. F. (1969) Quantitative densitometric thin-layer chromatography of lipids using copper acetate reagent. *J. Chromatogr.* **43,** 120–126.

Freeman C. P. and West D. (1966) Complete separation of lipid classes on a single thin-layer plate. *J. Lipid Res.* **7,** 324–327.

Giese A. C. (1966) On the biochemical constitution of some echinoderms. In *Physiology of Echinodermata* (Edited by Boolootian R. A.), pp. 757–796. Interscience, New York.

Giordano M. F., Harper H. A. and Filice F. P. (1950) The amino acids of a starfish and a sea urchin (Asteroidea and Echinoidea). *Wasmann J. Biol.* **8,** 129–132.

Greenfield L. J., Giese A. C., Farmanfarmaian A. and Boolootian R. A. (1958) Cyclic biochemical changes in several echinoderms. *J. exp. Zool.* **139,** 507–524.

Harrold C. and Pearse J. S. (1980) Allocation of pyloric caecum reserves in fed and starved sea stars, *Pisaster giganteus* (Stimpson): somatic maintenance comes before reproduction. *J. exp. mar. Biol. Ecol.* **48,** 169–183.

Kaneshiro E. S. and Karp R. D. (1980) The ultrastructure of coelomocytes of the sea-star *Dermasterias imbricata. Biol. Bull. Mar. Biol. Lab., Woods Hole* **159,** 295–310.

Kanungo K. (1982) *In vitro* studies of the effect of cell-free coelomic fluid, calcium, and/or magnesium on clumping of coelomocytes of the sea star *Asterias forbesi* (Echinodermata: Asteroidea). *Biol. Bull. Mar. Biol. Lab., Woods hole* **163,** 438–452.

Nikkilä E. A. (1971) Transport of fatty acids. In *Biochemistry and Pharmacology of Free Fatty Acids* (Edited by Holmes W. L. and Bortz W. M.), pp. 102–129. S. Karger, Basel.

Oudejans R. C. H. M. and Van der Sluis I. (1979a) Changes in the biochemical composition of the ovaries of the sea star *Asterias rubens* during its annual reproductive cycle. *Mar. Biol.* **50,** 255–261.

Oudejans R. C. H. M. and Van der Sluis I. (1979b) Storage and depletion of lipid components in the pyloric caeca and ovaries of the sea star *Asterias rubens* during its annual reproductive cycle. *Mar. Biol.* **53,** 239–247.

Oudejans R. C. H. M. and Rutten V. P. M. G. (1982) Fate of the glycerol and acyl moieties from dietary trioleoylglycerol in the sea star, *Asterias rubens. Comp. Biochem. Physiol.* **73B,** 685–693.

Oudejans R. C. H. M., Van der Sluis I. and Van der Plas A. J. (1979) Changes in the biochemical composition of the pyloric caeca of female sea stars, *Asterias rubens*, during their annual reproductive cycle. *Mar. biol.* **53,** 231–238.

Oudejans R. C. H. M., De Ruyter A., Van der Plas, A. J. and Voogt P. A. (1983) Fate of ingested leucine and glucose in the sea star, *Asterias rubens*, during its annual reproductive cycle. *Comp. Biochem. Physiol.* **76B,** 591–597.

Oudejans R. C. H. M., Boot J. H. and Voogt P. A. (1984) *De novo* synthesis of fatty acids in the sea star *Asterias rubens* during its annual reproductive cycle. *Advances in Invertebrate Reproduction*, Vol. III. Elsevier, Amsterdam (in press).

Sargent J. R., Falk-Petersen I. B. and Calder A. G. (1983) Fatty acid compositions of neutral glycerides from the ovaries of the asteroids *Ctenodiscus crispatus, Asterias lincki and Pteraster militaris* from Balsfjorden, Northern Norway. *Mar. Biol.* **72,** 257–264.

Schinke H. (1950) Bildung und Ersatz der Zellelemente der Leibeshöhlen Flüssigkeit von *Psammechinus miliaris* (Echinoidea). *Z. Zellforsch.* **35,** 311–331.

Schoenmakers H. J. N., Colenbrander, P. H. J. M., Peute, J. and Van Oordt P. G. W. J. (1981) The anatomy of the ovaries of the starfish *Asterias rubens* (Echinodermata). A histological and ultrastructural study. *Cell Tissue Res.* **217,** 577–597.

Vanden Bossche J. P. and Jangoux M. (1976) Epithelial origin of starfish coelomocytes. *Nature, Lond.* **261,** 227–228.

Van der Heyde H. C. (1922) On the physiology of digestion, respiration and excretion in echinoderms. Ph.D. Thesis, pp. 119. University of Amsterdam, the Netherlands.

Van der Plas A. J. and Voogt P. A. (1982) The biochemical composition of the pyloric caeca and its relation to DNA levels in the female starfish *Asterias rubens* during the reproductive cycle. *J. Comp. Physiol.* **148,** 49–55.

Walker C. W. (1979) Ultrastructure of the somatic portion of the gonads in asteroids, with emphasis on flagellated-collar cells and nutrient transport. *J. Morph.* **162,** 127–162.

Worm R. A. A. and Beenakkers A. M. T. (1980) Regulation of substrate utilization in the flight muscle of the locust, *Locusta migratoria*, during flight. *Insect. Biochem.* **10,** 53–59.

Zilversmit D. B. (1960) The design and analysis of isotope experiments. *Am. J. Med.* **29,** 832–848.