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THE BIOSYNTHESIS OF THE EMULSIFIERS OF THE CRAB *CANCER PAGURUS* L.

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Abstract—1. The incorporation of acetate-1-C^{14} into the emulsifiers (fatty acylsarcosyltaurines) occurring in the gastric juice of the crab *Cancer pagurus* L. was studied. The isotope was incorporated into the fatty acid and sarcosine constituents of the emulsifier, not into the taurine part.

2. It was concluded that the emulsifiers are of endogenous origin.

INTRODUCTION

IN THE gastric juice of some decapod Crustacea and some other invertebrates surface active compounds were shown to be present (Vonk, 1935, 1962). Recently the emulsifiers of the crab *Cancer pagurus* L. were reported to be a series of fatty acylsarcosyltaurines (van den Oord *et al.*, 1965). The possible physiological role of the surfactants in fat digestion in invertebrates was discussed (Vonk, 1962; van den Oord, 1965). This role makes a search for the origin of the emulsifiers desirable.

The present paper describes the biosynthesis of the emulsifiers from the gastric juice of the crab *Cancer pagurus* L. Acetate-1-C^{14} was administered to crabs, the emulsifiers were isolated and the constituent components assayed for isotope content.

EXPERIMENTAL

Animal experiments

The crabs, mainly females, were caught in November in the North Sea near the coast of Holland. During the experiments, conducted in November, the animals were kept in aquaria with running sea water at a temperature of 13°C in the Netherlands Institute for Sea Research, Den Helder, Holland. Two series of ten crabs were used. Each crab in the first series was given 70 μc of acetate-1-C^{14} in two portions with a 24 hr interval. Twenty-four hours after the second injection the gastric juice was drawn off and pooled (S1). The animals in this series were not fed. In the second series gastric juice was taken away immediately before the first injection of acetate-1-C^{14} was given. Each crab in this series was administered

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80 μc in four doses with intervals of 48 hr. The gastric juice was collected 12 days after the first injection (S2). The crabs in the second series were fed.

Sodium acetate-1-C^{14} (specific activity 0·114 mc/mg, obtained from Philips–Duphar, Amsterdam) was given in a solution made up to 1% with carrier acetate. Volumes injected never exceeded 0·5 ml.

The gastric juice was collected as described by Vonk (1960).

Isolation of emulsifiers and their constituent components

Emulsifiers were purified by processing the gastric juice as described by van den Oord *et al.* (1965), who also described the hydrolysis of the emulsifiers and isolation of their constituents: fatty acids, sarcosine and taurine.

Analysis of fatty acids

Gas–liquid chromatography (GLC) was performed as described elsewhere (van den Oord *et al.*, 1965). The methyl esters of fatty acids were prepared as described by McGinnis & Dugan (1965).

Isotope determinations

Radioactivity was assayed with an I.D.L. liquid scintillation counter, type 6012. Aliquots of isolated fractions were solved or suspended in the liquid scintillator (Hayes *et al.*, 1956a, b).

Radioactivity in single fatty acids was determined by condensing the acids from the effluents of a GLC column in cartridges filled with silicone oil-coated anthracene (Karmen *et al.*, 1962). One single cartridge was used per peak or for a number of minor peaks between larger ones.

RESULTS

From the animals in series S1 27 ml of a brownish juice was collected. The crabs in series S2 yielded 20 ml of a pale yellow-coloured juice. The amounts of emulsifiers isolated were 139 mg in S1 and 53 mg in S2.

TABLE 1—INCORPORATION OF ACTIVITY INTO CRAB EMULSIFIERS AND THEIR CONSTITUENTS AFTER INJECTION OF ACETATE-1-C^{14} INTO CRABS

	Series S1		Series S2	
	mg	Counts/min/mg*	mg	Counts/min/mg*
Mixture of emulsifiers	139	2770	53	3020
Taurine	39	$-0·2 \pm 0·3$†	15·5	$0·1 \pm 0·2$†
Sarcosine HCl	40	383	17	594
Mixture of fatty acids	69	5560	25	5070

* Calculated values for 100 per cent counting efficiency.

† Standard deviation of the mean.

The emulsifiers, isolated as a mixture, were radioactive. The isotope was located in the sarcosine and fatty acid parts of the emulsifier. The results are summarized in Table 1.

Gas chromatographic analysis of the fatty acids showed that the composition was quantitatively and qualitatively identical in series S1 and S2. The results were in good agreement with results obtained earlier (van den Oord *et al.*, 1965), except for fatty acids higher than palmitic acid, which do not occur in the present mixtures. The labelling of the fatty acids shows the same pattern in series S1 and S2. In Table 2 the results concerning the quantitatively important acids are given. The isotope content is in the same order of magnitude for all acids, except for decanoic acid and dodecenoic acid, their relative abundance being taken into account.

TABLE 2—INCORPORATION OF ACTIVITY INTO FATTY ACIDS FROM CRAB EMULSIFIERS AFTER INJECTION OF ACETATE-1-C^{14} INTO CRABS

Fatty acid*	% total fatty acid	Counts/min†	
		Series S1	Series S2
5:0–9:0	4·5	178	80
10:0	7	1050	364
10:1	3	205	90
11:1	1·5	27	20
12:1	63	6645	4968
13:0 + 14:0	4	166	176
14:3	5	51	102

* Shorthand notation: 14:0, 14:3, fatty acids having 14 carbon atoms with 0 and 3 double bonds respectively.

† Calculated values for 100 per cent counting efficiency. 5 μl of fatty acid methyl esters were put on the GLC column.

The removal of gastric juice before the administration of acetate-1-C^{14} to the crabs does not yield highly labelled emulsifiers (S2). The surfactants are readily synthesized and probably possess a high turnover rate.

DISCUSSION

Acetate is incorporated readily into the fatty acids of the crab (van den Oord, 1964), and, apparently, also in the lower fatty acids occurring in the emulsifiers.

Sarcosine and taurine most probably originate from the same parent amino acid, viz. serine (cf. Fruton & Simmonds, 1959). The conversion of serine into glycine is a well-known pathway in mammals and micro-organisms. A methylation of glycine yields sarcosine. Serine is converted into taurine *via* cysteine.

On administration of acetate-1-C^{14} to blowfly larvae, the isotope was located in the carboxyl carbon of serine and glycine (Sedee, 1961), and in a similar experiment with a decapod Crustacea, *Astacus astacus* L., the same result was obtained for

glycine (serine was labelled but not further analysed) (Zandee, 1964). In the formation of taurine from cysteine the carboxyl group of serine is lost.

Thus taurine may be found unlabelled after administration of acetate-1-C^{14}, which is the case in our experiment. Allen & Awapara (1960) isolated labelled taurine from molluscs after injection of S-35 labelled methionine and cysteine. Awapara (1962) states that taurine is a metabolic product of invertebrates.

It follows from the present experiments that the emulsifiers occurring in the gastric juice of the crab *Cancer pagurus* L. are of endogenous origin. The site of formation probably is the hepatopancreas, but this remains to be confirmed.

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