Analysis of Arsenic in Marine Oils by Neutron Activation. Evidence of Arseno Organic Compounds

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Abstract

The arsenic content of phospholipid fractions separated from codliver oil (*Gadus morrhua*) and herring oil (*Clupea harengus*) was analyzed by means of neutron activation. The fractions were separated on a silicic acid column by chloroform/methanol mixtures as eluting agents. The results indicate that the arsenic appears as arseno organic compounds. Two such compounds were evident in herring oil.

Introduction

ARSENIC HAS EARLIER been observed in marine oils (1–4). There seems to be a certain agreement that the arsenic appears as one or more arseno organic compounds (4). A work by Sadolin may be mentioned in this connection (5). By extracting codliver oil by alcohol, it was possible to enrich the arsenic content from 3.9 mg per kg to 100 mg per kg. After further treatment a fraction was obtained, which contained 1000 mg arsenic per kg. On the basis of these studies it appeared that the compound containing the arsenic has a closer resemblance to the phospholipids than the neutral lipids.

The purpose of this work was to study, in more detail, whether the arsenic replaces phosphorus in the phospholipids or whether it exists as one or more independent arseno organic compounds. Codliver oil (*Gadus morrhua*) and herring oil (*Clupea harengus*) were used in this investigation. The phospholipids from these oils were fractionated, and each fraction was analyzed by neutron activation. A description of the method is given elsewhere (4).

Preparation of Samples

Oil from codliver and herring was extracted by a mixture of chloroform/methanol (2:1 v/v) after homogenization of the samples in a mechanical mixer. After removal of the solid phase the solvent was evaporated at 50–60C. The phospholipids were separated from the neutral lipids by using activated silicic acid (E. Merck AG, Darmstadt), 0.2–0.5 mm, for chromatography. The oil was dissolved in chloroform and mixed with silicic acid. About 4 g of silicic acid to 1 ml of oil were used. In this step the phospholipids were adsorbed to the silicic acid, and the chloroform phase, containing the neutral lipids, was filtered out.

TABLE I

Arsenic Content in Phospholipid Fractions

Fraction	Eluating agent	Eluating volume (ml)	As (ppm) in Phospholipids: Codliver oil	As (ppm) in Phospholipids: Herring oil
1	Chloroform	500	1.0	0.8
2	Acetone	150	18.0	23.0
3	0.5% Methanol in chloroform	200	26.0	34.0
4	7 % Methanol in chloroform	250	11.2	34.0
5	12 % Methanol in chloroform	250	24.3	30.0
6	17 % Methanol in chloroform	250	85.0	225.0
7	22 % Methanol in chloroform	500	308.0	900.0
8	27 % Methanol in chloroform	250	718.0	911.0
9	above 27% Methanol	250	427.0	590.0

TABLE II

Eluating Agents Used in Fraction-Collector Experiment

Fraction		Eluating agent	Eluating volume
1	A[a]	Chloroform	210
2	B	Acetone	150
3	C	5% Methanol in chloroform	240
4	D	15% Methanol in chloroform	300
5	E	25% Methanol in chloroform	300
6	F	40% Methanol in chloroform	300
7	G	65% Methanol in chloroform	240
8	H	90% Methanol in chloroform	270

[a] See Fig. 1.

It was then washed twice with chloroform prior to the filtering of the phospholipids by a chloroform/methanol mixture (1:20 v/v). The phospholipids were fractionated on a silicic acid column (height 50 cm, inner diameter 2.2 cm) by means of chloroform/methanol mixtures.

The fractions were eluted by gradually increasing the methanol content in the chloroform with a speed of about 4 ml per minute. The first elution processes were carried out with chloroform/methanol mixtures, as described by Froines et al. (6) in their studies of the phospholipid distribution in menhaden tissue (Table I). Neutral lipids, not separated during the initial purification stage, were eluted first with pure chloroform. An acetone elution was done to remove oxidized products (7). To obtain a more exact elution diagram, a fractionation with an automatic fraction-collector was carried out. Each fraction was 30 ml, and the chloroform/methanol mixtures (Table II) were almost the same as used by de Koning (8). The phospholipids in the various fractions obtained were not analyzed.

Irradiation of Samples

After evaporation of the solvent the phospholipid fractions were weighed, transferred to polyethylene ampoules, and sealed. The ampoules were irradiated in the nuclear reactor JEEP I (Kjeller, Norway)

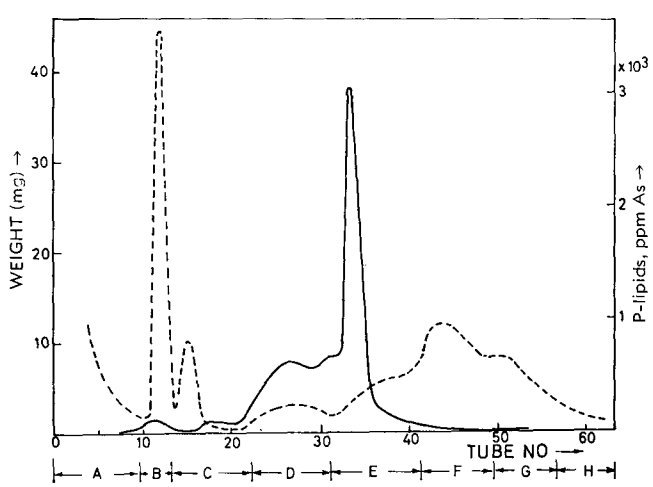

FIG. 1. Phospholipids ------, arsenic ———. Chromatographic separation of phospholipids and arsenic (herring oil). The composition of the eluating mixtures A, B, etc., is given in the text.

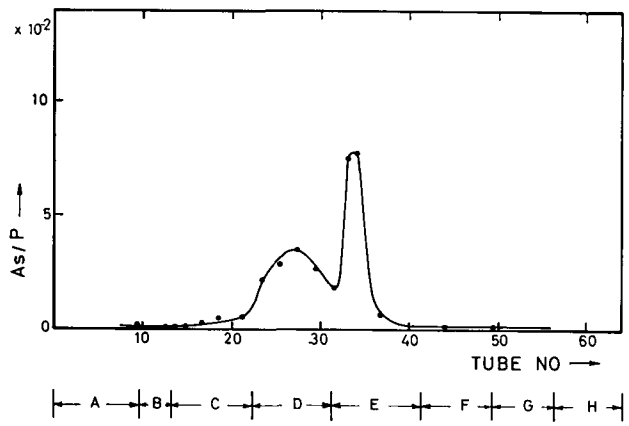

FIG. 2. The ratio between arsenic and phosphorus (w/w) in fractions of phospholipids from herring oil. From the same chromatographic separation as shown in Fig. 1.

with a neutron flux of approximately 2.10^{12} neutrons cm^{-2} sec^{-1} for 24 hours. Arsenic and phosphorus standards (As_2O_3 and $(NH_4)_2HPO_4$ PA-reagents, E. Merck A. G., Darmstadt) were irradiated at the same time. A 400-channel *gamma* spectrometer (Victoreen Scipp 400), with a 2×2-in. sodium-iodide crystal, was used for recording the induced activity in the samples. As it was difficult to avoid sodium contamination of the samples during fractionation of the phospholipids, it was expedient to wait about five days before recording the activated arsenic, arsenic-76. The activity of sodium-24 [$T_{1/2}$ (half life) = 14 hours] was then reduced sufficiently to allow recording of the arsenic-76 ($T_{1/2} = 26$ hours). The arsenic photo peak of 0.55 MeV was used for the quantitative determination of arsenic in the phospholipids. This was compared with the same photo peak from the arsenic standard.

When the measurement of radio-active arsenic is undertaken without removing the lipids from the activated polyethylene ampoules, impurities from the polyethylene ampoule can disturb the recording of the arsenic-76. This applies especially when the arsenic content in the samples is below 0.1 μg. These problems can be avoided by subtracting, during the recording of the samples, the *gamma* spectra from an identical, empty polyethylene ampoule which is irradiated under the same conditions as the samples. After the arsenic-76 activity has decayed, the induced phosphorus isotope, phosphorus-32, is measured directly by means of a proportional counter. As phosphor-32 is a pure β-emitter, this method gives a poor degree of precision owing to the uneven distribution of the lipids in the polyethylene ampoules.

The phosphorus determinations were used for calculating the As/P ratio in the different phospholipid fractions. For this purpose a high accuracy was not necessary.

Results and Discussion

The results of the first experiments appear in Table I. The arsenic content of the various fractions is calculated in ppm. The results indicate that the arsenic content in the fractions is highest in the fractions eluted with 20–30% methanol. From the experiment where the fractions were collected with an automatic fraction-collector (Table II and Fig. 1) it was evident that there were two arseno organic compounds present, one that was eluted with about 20 volume % methanol and the other with about 25–30 volume % methanol. The arsenic content in one of the fractions was about 3,000 ppm. This should have been sufficient to characterize the compound by other analytical methods. The phosphorus content in the fractions (fraction 20–50) was from 1.9–3.9%. By means of the phosphorus determination the As/P ratio was calculated (Fig. 2). The two peaks indicate also the presence of two arseno organic compounds. If the arsenic had replaced the phosphorus in the phospholipids, this would probably have resulted in an approximate constant As/P ratio in the different fractions.

On the basis of the results obtained, two arseno organic compounds or arseno lipids are indicated and their chemical properties resemble the properties of the phospholipids. It is most probable that it is the fish itself or other organisms contained in the food intake of the fish, which synthesizes these arseno organic compounds. The arsenic in sea water (about 0.003 ppm) appears mainly as inorganic anion (9).

ACKNOWLEDGMENT

Authorities of the Central Institute for Industrial Research (Blindern) provided the opportunity to perform this research; various members of the staff at the Central Institute gave advice and valuable discussions.

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[Received November 27, 1967]