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Vitamins and the Reproductive  
Cycle of Ovaries in Cod  
*(Gadus morrhua)*

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## INTRODUCTION

In a preliminary communication (BRÆKKAN, 1955 a) the general results from an investigation of the relation between the vitamin content and the reproductive cycle of ovaries in fish has been reported. At that time only the problem with regard to pantothenic acid was fairly elucidated, while the investigation of other B-factors was in progress. The present paper reports the results from an investigation of the relation between the contents of the B-vitamins pantothenic acid, riboflavin, niacin, vitamin B<sub>12</sub> and biotin, and the stages of the reproductive cycle of the ovaries in fish.

The study was occasioned by the finding of very high values for pantothenic acid in the ovaries of the tunny (*Thunnus thynnus*) caught off the coast of Norway (BRÆKKAN, 1955 b). Average sample from a representative number of fish showed up to 245  $\mu\text{g}$  per g fresh tissue. The tunny spawns in the Mediterranean and nearby waters in June and early July, whereupon shoals of the fish move in search of food and arrive off the coast of Norway in July–October. At this time the ovaries are very small and in the first stages of a new reproductive cycle. Earlier observations in this laboratory on ovaries («hard roes») from different species had indicated differences in the pantothenic acid content in relation to their degree of «ripening»; the values being lowest in the ovaries ready for spawning. Samples of ripening ovaries from tunny are very rare in the catches along the Norwegian coast, but by chance a fairly developed ovary was obtained, and analysis showed a content of 90.5  $\mu\text{g}$  pantothenic acid per g fresh tissue. These findings indicated a relation between pantothenic acid and the reproductive cycle of ovaries in fish. The possibility of a special importance of some vitamins for the development of the fish ovaries and as chemical constituents of the eggs in the development of the embryo thus had to be considered.

Previous investigations of the vitamin content of ovaries in fish have mainly been concerned with samples of «hard roe», which are ovaries

in the last stages of the sexual cycle. (LUNDE, 1938 a-c, 1939, 1940; LUNDE, KRINGSTAD & OLSEN, 1938 a-b, 1939; LUNDE & KRINGSTAD, 1938 a-b; KRINGSTAD & NÆSS, 1939; KRINGSTAD & THORESEN, 1940; LIE & LUNDE, 1940; KRINGSTAD & FOLKWORD, 1945, 1949). All investigations, however, aimed at an evaluation of the vitamin content of «hard roe» from a nutritional point of view, and give no information as to the relation between the vitamin content and the development of the ovaries. LUNDE et al. (1939 a) actually investigated the thiamin and riboflavin content of cod ovaries taken at different fishing grounds and times of the year, but no important variation could be found. It must be pointed out that even if these ovaries differed in weight, they were probably all from the last stages of the reproductive cycle in fish of different age.

PEARSON & BURGIN (1941) found an extraordinary high content of pantothenic acid and biotin in royal jelly, the special food given to the bee larvae that are destined to become queens, and thus develop ovaries. This made it of interest to include biotin in the present study of the development of ovaries in fish.

The present study was carried out with carefully selected samples of ovaries from cod (*Gadus morrhua*). This fish was chosen for several reasons. SIVERTSEN (1935) has investigated the spawning of cod especially with regard to the yearly cycles of the regeneration of the reproductive organs. Based on his findings, ovaries from cod were collected during the period September-April, thus giving samples covering all stages of the development. Fresh samples from live cod can be collected all the year round at the fish market in Bergen.

When considering the relation between the vitamin content and the development of the ovary, the influence of simple dilution effects must not be overlooked. Apparently the ovary of the cod takes up considerable amounts of water just before spawning. To rule out any anomaly, chemical analysis of the water-, protein- and ash-contents were carried out. Fat determinations were omitted. During the maturation of teleostean eggs there is an exhaustion of depot fat during spawning migration, among others, caused by deposition in the eggs. (DAVIDSON & SHOSTROM, 1936; CHANNON & SABY, 1932; LOVERN, 1953). It would have been of interest to include such observations from cod ovaries in the present study, but the material available from the important samples of the very small ovaries from juvenile fish, often only allowed for one or two vitamin determinations.

The protein determinations, especially of the «ripe» ovaries or «hard roe», further give additional values for the nutritional evaluation of this important fish product.

## METHODS

Samples were collected from live fish brought directly from the fishing grounds to the fish market in well-boats. The fish was weighed and the ovary taken out and brought to the laboratory. The female cod has a single ovary, possibly resulting from a fusion of two in the embryo (BRELAND, 1953). All ovaries were then examined to determine the maturity of the fish. The method and classification used by SIVERTSEN (1935, 1939) were applied. For a general observation of the stage

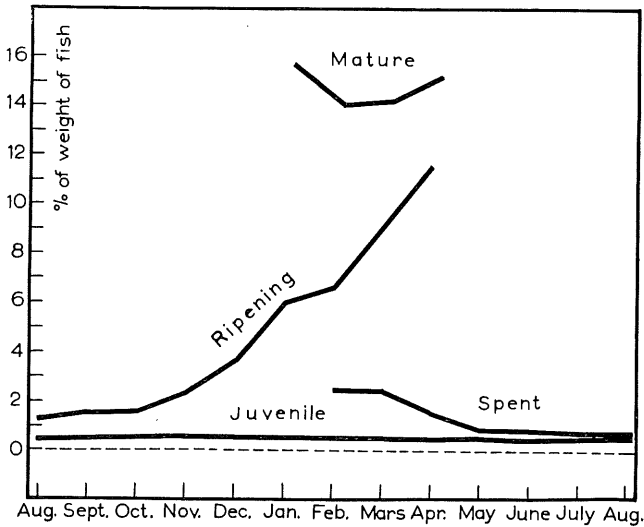


Fig. 1. Weight of ovaries in cod (*Gadus morrhua*) at different stages of ripening or regeneration in relation to the time of the year (Sivertsen).

of development of the ovaries, the percentage body-weight is useful. Juvenile ovaries in cod have a fairly constant relative weight, being below 0.5 % of the total body weight, as shown in the figure (Fig. 1), taken from SIVERTSEN (1935). In the beginning of the present investigation, the size of the eggs were measured under the microscope, but later only external examinations, combined with the observed relative weight, were used as basis for the classification. SIVERTSEN (1939) also points out that this is a fairly accurate method. In juvenile cod the ovaries are small with a thin and transparent wall, the colour of the tissue is yellow-gray to reddish. If, however, the small ovaries are from mature fish which previously have spawned, the regenerated organs have a thicker wall, and the colour is bluish-white and fairly opalescent. All other stages of the reproductive cycle were measured by the weight relation between

ovary and fish, this giving a sufficient measure for the development of «ripening» or regeneration.

After the biological examinations, each ovary was homogenized and either analysed at once in the fresh state, or stored frozen at below  $\div 15^{\circ}\text{C}$  until further examinations could proceed.

The methods for the assays of pantothenic acid, riboflavin, niacin and biotin were essentially those described in «Methods of Vitamin Assay» (1951).

*Pantothenic acid* was determined microbiologically with *Lactobacillus arabinosus* as test-organism. The growth was measured turbidimetrically in a Beckman Model B Spectrophotometer at  $660\text{ m}\mu$  after 20 hrs. incubation at  $30^{\circ}\text{C}$  in a water bath. The extraction of the vitamin was carried out by digestion of 1 g of the homogenized sample with 1 ml of a suspension of 20 mg takadiastase + 20 mg papain in 8 ml 0.2 N sodium acetate buffer of pH 4.5. The mixture was layered with toluene, the flask plugged with cotton, and incubated ca. 20 hrs. at  $37^{\circ}\text{C}$ . At the end of this time the digest was steamed for 10 min., cooled, neutralized to pH 6.8., made up to volume and filtered through a fluted filter. The clear filtrate was used after suitable dilution for the assay response. The enzymes were comparatively free from pantothenic acid, thus a blank could be omitted.

*Riboflavin* was determined microbiologically by the acidimetric method using *Lactobacillus casei* as test organism. Incubation was carried out for ca. 72 hrs. at  $37^{\circ}\text{C}$ , and the response measured by potentiometric titration of the lactic acid produced.

The extraction was carried out as described for pantothenic acid, but after the steaming the digest was cooled, made up to volume and filtered through a fluted filter, thus removing fatty acids which eventually might interfere with the bioassay and cause «drift». The enzymes contained riboflavin and a blank had to be run parallel with the samples to correct the values.

*Niacin* was determined microbiologically with *Lactobacillus arabinosus*. Incubation and response measurements as described for riboflavin.

The extraction was carried out by autoclaving 1 g of the sample with 50 ml 1 N  $\text{H}_2\text{SO}_4$  for 30 min. at 15 lbs. pressure. The digest was neutralized to pH 6.8, filtered and diluted to a suitable concentration.

*Biotin* was determined microbiologically with *Lactobacillus arabinosus*, with incubation for ca. 20 hrs. at  $30^{\circ}\text{C}$  and response measurements as described for pantothenic acid.

The extraction was carried out by autoclaving 1 g of the sample with 25 ml 3 N  $\text{H}_2\text{SO}_4$  for 3 hrs. The digest was neutralized to pH 6.8, filtered and diluted to suitable concentration.

*Vitamin B<sub>12</sub>* was determined by its growth promoting activity on *Lactobacillus leichmannii*, using the method described by THOMPSON, DIETRICH & ELVEHJEM (1950). The incubation was carried out for 20–22 hrs. at 37° C, and the response measured turbidimetrically.

The extraction was carried out by autoclaving 1 g homogenized sample with 50 ml sodium acetate buffer of pH 4.5 + 5 ml 1 % KCN-solution for 15 min. at 15 lbs. pressure.

*Moisture* was determined by drying in an oven at 120° C until constant weight.

*Ash* was determined by ignition of the dried sample, first carefully over a gas-burner, then in an oven at 550° C until constant weight was obtained.

*Protein* was calculated based on Kjeldahl-N multiplied by the factor 6.25.

## RESULTS

Altogether 128 ovaries taken from live cods were investigated and from one to eight analyses carried out on each sample. The quantity of data thus obtained made it inconvenient to report detailed results in tables. Instead, a graphical plotting was found more suitable. A log plotting along both axis was found to give the clearest and most convenient expression of the results found for the relations investigated. Although these graphs give plotting of every single observation, the exact values are difficult to read and above all, averages could not be reported in this way. The results thus were further summarized in a table giving average and minimum and maximum values for the main stages of the sexual cycle. As the size of the fish and ovaries varies considerably (Fig. 2), an accidental grouping had to be used in this summary. Pantothenic acid was the vitamin which showed the most typical variation, and it was first taken into consideration when a grouping was chosen. Based on the analysis of pantothenic acid and the state of development of the ovaries as judged by the method of SIVERTSEN (1938), a weight grouping 0.5–50, 51–500 and 501–2000 g was chosen as most representative averages for the sizes of fish investigated. These groups approximately represent ovaries which are «juvenile and first stage of regeneration», «maturing and regenerating» and «ripe» respectively.

The chemical analyses (Fig. 3 and Table 1) show that the water content decreases from an average of 81.7 per cent in juvenile and small ovaries in early stages of regeneration to 73.3 per cent in ripe ovaries or so called «hard roe». The corresponding increase in dry matter mainly constitutes an increase in the protein content from an average of 13.8

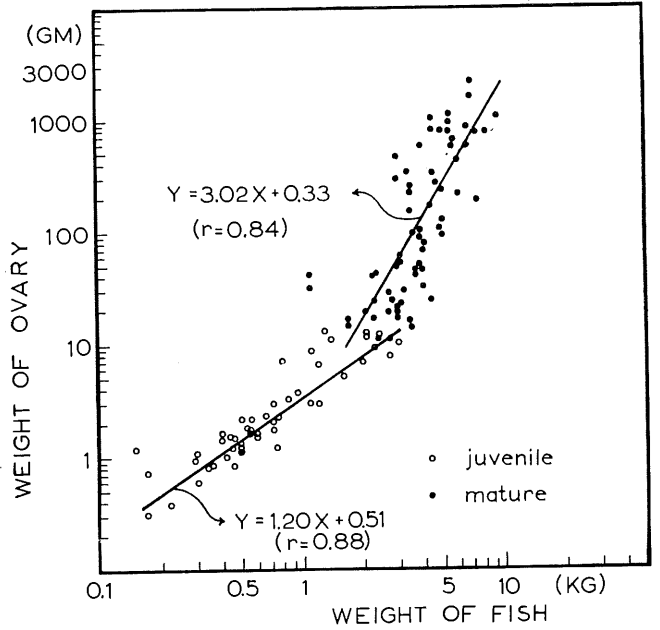


Fig. 2. Relation between weight of ovary and weight of fish in juvenile and mature cod. (*Gadus morrhua*). (Y = log weight of ovary; X = log weight of fish.)

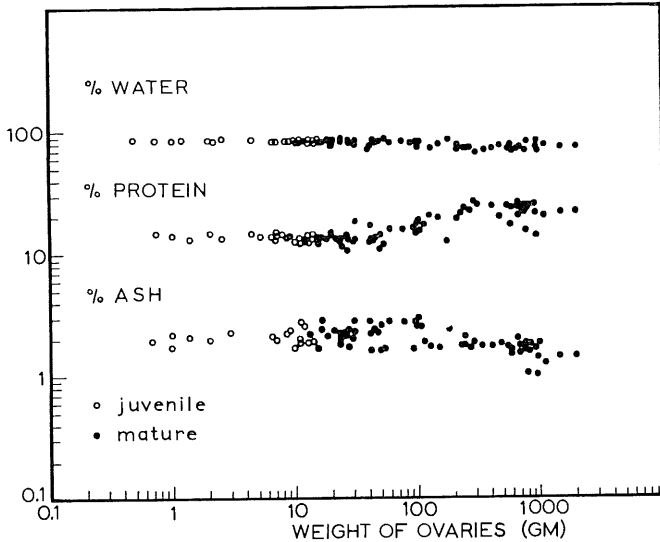


Fig. 3. Relation between water, protein and ash contents and weight of ovary in juvenile and mature cod (*Gadus morrhua*).



Table 1. Summarized results from the investigation of B-vitamins, moisture, protein and ash in ovaries from cod (*Gadus morrhua*).

	Juvenile and first stage of regeneration	Maturing and regenerating	Ripe («Hard roe»)
Weight (g) .....	0.5-50	51-500	501-2000
Moisture (%) .....	81.7 (76.1-85.7)	77.1 68.2-86.3)	73.3 (69.2-84.6)
Protein (%) .....	13.8 (11.0-17.3)	16.5 (11.0-26.5)	21.9 (14.0-25.5)
Ash (%) .....	2.25 (1.67-2.88)	2.10 (1.63-2.84)	1.62 (1.00-2.05)
Pantothenic acid:			
μg/g fresh weight .....	234 (96-377)	115 (56-202)	30 (10-50)
μg/g protein .....	1695	697	137
Riboflavin			
μg/g fresh weight .....	6.9 (5.0-11.2)	9.2 (5.4-13.0)	7.3 (4.6-10.0)
μg/g protein .....	50.0	55.7	33.3
Niacin:			
μg/g fresh weight .....	14.7 (10.0-18.8)	13.6 (11.1-18.8)	13.5 (9.7-16.9)
μg/g protein .....	106	82	63
Vitamin B <sub>12</sub>			
μg/g fresh weight .....	0.31 (0.20-0.42)	0.20 (0.11-0.29)	0.10 (0.08-0.13)
μg/g protein .....	2.25	1.21	0.45
Biotin:			
μg/g fresh weight .....	0.130 (0.081-0.230)	0.153 (0.083-0.190)	0.130 (0.091-0.180)
μg/g protein .....	0.94	0.92	0.61

to an average of 21.9 per cent for the selected groups. The ash content is fairly constant around 2 per cent until just before the completion of the regeneration, when it drops to 1.6 per cent in the present samples.

The values for the vitamin contents per g fresh weight are given in Table 1 and Fig. 4-6. In the table are further included average values for the vitamin content per g protein for the stages selected. These values were calculated on the basis of the average values for the protein content with respect to the vitamin content. This was the only convenient

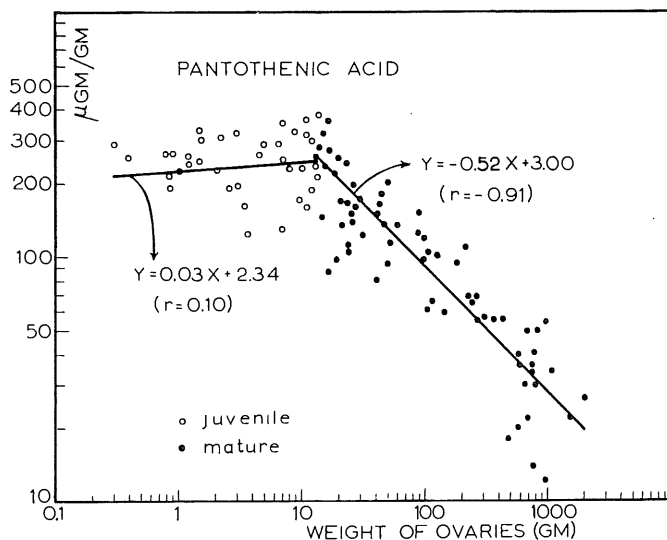


Fig. 4. Relation between pantothenic acid per gm wet weight and weight of ovary in juvenile and mature cod (*Gadus morrhua*). ( $Y = \log$  pantothenic acid pr. g;  $X = \log$  weight of ovary).

basis for this calculation as the size of the ovaries often only permitted one or a few of the eight different determinations involved in the present study. Thus the values, especially those from small juvenile ovaries, were derived from different samples.

Pantothenic acid (Fig. 4 and Table 1) was found present at very high levels in juvenile fish. In mature fish the values varied with the stage of ripening or regeneration of the ovaries as expressed by increased size. In ovaries from juvenile fish and ovaries in the first stages of regeneration from mature fish, the average value was 234  $\mu\text{g}$  pantothenic acid per g of fresh tissue or 1278  $\mu\text{g}$  per g of dry weight. For one sample a value of 377  $\mu\text{g}$  per g of fresh weight, 2430  $\mu\text{g}$  per g of dry weight was found. During the ripening or regeneration of the ovaries the values for

pantothenic acid decreased, and when the process was completed the ovaries (or «hard roes») contained an average of 30  $\mu\text{g}$  per g fresh weight or 149  $\mu\text{g}$  per g of dry weight. From some ovaries in the last stage, from fish which were spawning, eggs were collected by filtration. These eggs contained 5 to 10  $\mu\text{g}$  pantothenic acid per g fresh weight. Seen in relation to the protein content of the ovaries, the values for the selected groupings were respectively 1695, 697 and 137  $\mu\text{g}$  per g protein. The pantothenic acid was almost equally released by water extraction and

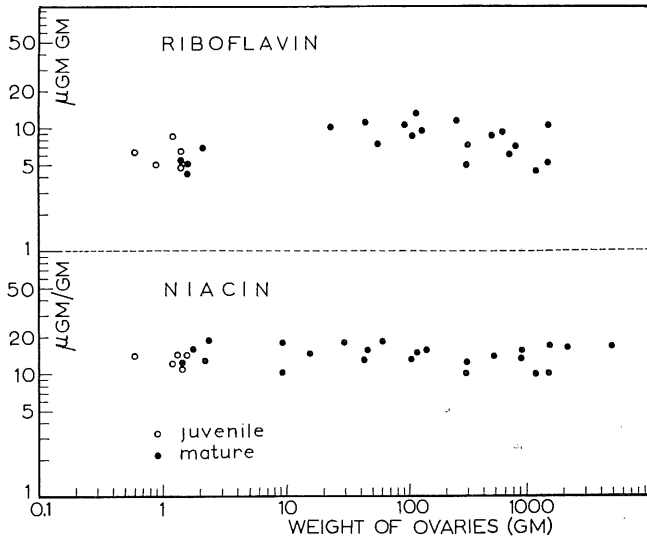


Fig. 5. Relation between riboflavin and niacin per gm wet weight and weight of ovary in juvenile and mature cod (*Gadus morrhua*.)

by digestion with papain-takadiastase or chicken liver + intestinal phosphatase (Table 2), thus indicating that it is mainly present in the ovaries as the free acid.

Riboflavin (Fig. 5 and Table 1) showed no variation with the regenerative cycle. The total average was 7.7  $\mu\text{g}$  per g fresh weight. Calculated in relation to the protein content, the values were respectively 50, 55.7 and 33.3  $\mu\text{g}$  per g.

Niacin (Fig. 5 and Table 1) showed a similar picture to that of riboflavin, the total average being 13.9  $\mu\text{g}$  per g fresh weight. Calculated in relation to the protein content the values for the selected groups were respectively 106, 82 and 63.

Biotin (Fig. 6 and Table 1) neither showed any variation with the regenerative cycle. The total average was 0.137  $\mu\text{g}$  per g fresh weight.

The values calculated in relation to the protein content were respectively 0.94, 0.92 and 0.61  $\mu\text{g}$  per g.

Vitamin B<sub>12</sub> (Fig. 6 and Table 1) showed a total average of 0.23  $\mu\text{g}$  per g fresh tissue. The values, however, decreased from 0.31 to 0.10  $\mu\text{g}$  vitamin B<sub>12</sub> per g fresh weight during the reproductive cycle. Calculated in relation to the protein content, the values were respectively 2.25, 1.21 and 0.45  $\mu\text{g}$  per g.

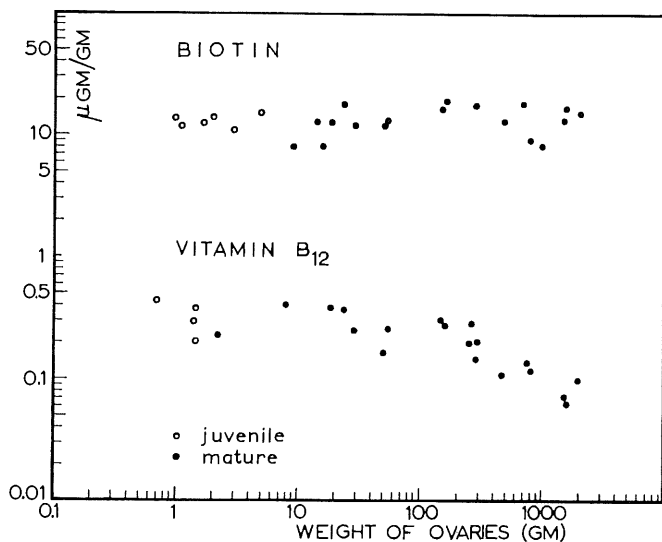


Fig. 6. Relation between biotin and vitamin B<sub>12</sub> per gm wet weight and weight of ovary in juvenile and mature cod (*Gadus morrhua*).

## DISCUSSION

The relation between the vitamin content of the ovaries and the reproductive cycle in fish has not been previously investigated. This study, however, naturally includes investigation of ripe ovaries or «hard roe». Ovaries at this stage have been the subject of several nutritional studies, including vitamin determinations. The present results from «hard roes» thus represent a re-investigation of this problem, and as they to some extent can be compared with earlier studies, they are discussed first.

The values found in «hard roe» for the different vitamins show fairly good agreement with previous findings in samples from the same fishing grounds. Many of these earlier investigations were carried out with canned roe. The canning of roe, however, has been found not to cause

any considerable decrease in the contents of the vitamins in question. It normally takes place without addition of other nutrients, and the values are thus fairly comparable with the present findings. KRINGSTAD & FOLKVORD (1940) found 119 (109–126)  $\mu\text{g}$  pantothenic acid per g dry matter of canned roe, as compared with 113  $\mu\text{g}$  per g dry matter calculated from the values reported in Table 1. For riboflavin values from 6–15  $\mu\text{g}$  per g fresh weight have been reported (LUNDE, 1938 a–c, 1939, 1940; LUNDE, KRINGSTAD & OLSEN, 1938 a–b, 1939, KRINGSTAD & FOLKVORD, 1949) compared with 7.3 (4.6–10)  $\mu\text{g}$  per g fresh weight in the present study. It may be pointed out that these investigators determined riboflavin either according to physico-chemical methods or biologically by the rat growth method.

Niacin has been investigated by KRINGSTAD & NÆSS (1939), KRINGSTAD & THORESEN (1940) and KRINGSTAD & FOLKVORD (1949) who carried out colorimetric determinations according to the bromcyan-method. They found values from 10–25  $\mu\text{g}$  niacin per g fresh weight, compared with 13.5 (9.7–16.9)  $\mu\text{g}$  per g fresh weight in the present investigation.

Values for biotin and vitamin B<sub>12</sub> have not previously been reported. The content of 0.13 (0.09–0.18)  $\mu\text{g}$  biotin per g fresh weight or 0.61  $\mu\text{g}$  per g dry weight makes cod roe a good source of this vitamin, ranging slightly above hens eggs and most fresh vegetables (CHELDELIN & WILLIAMS, 1942). The values for vitamin B<sub>12</sub>, 0.10 (0.08–0.13)  $\mu\text{g}$  per g fresh weight, 0.45  $\mu\text{g}$  per g dry weight, shows that cod roe is a very good nutritive source for this vitamin.

When finally the protein content, 21.9 (14–25.5) per cent, is considered, the overall findings verify the high nutritional value of cod roe as emphasized by KRINGSTAD & FOLKVORD (1949).

The main problem, however, dealt with in this study are the changes in the vitamin contents during the reproductive cycle. Of the vitamins investigated only pantothenic acid shows a characteristic picture (Fig. 3), the values being very high in juvenile ovaries and in the first stages of regeneration. Niacin, riboflavin and biotin did not show any such special relation, the values being almost constant at all stages. Vitamin B<sub>12</sub> shows a slight but significant decrease, however, to such a small degree that further investigation was considered of minor importance.

The number of observations and samples make it possible to analyse further the relations existing between pantothenic acid and the weight of the ovaries as well as the weight of the fish. It is, however, also necessary to clarify the relation between the weight of the ovaries and the weight of the fish for the present samples. This relation is plotted in Fig. 2. It is important to bear in mind that in mature fish widely differing weights

of the ovaries are found for each weight group of the fish, depending on the stage of regeneration or ripening of the ovaries. Actually, ovaries in the same stage of development should be treated as belonging to the same group. Such a classification is, however, very difficult to carry out, and in the present study all results are therefore treated together. There is a significant difference between the regression coefficients for juvenile

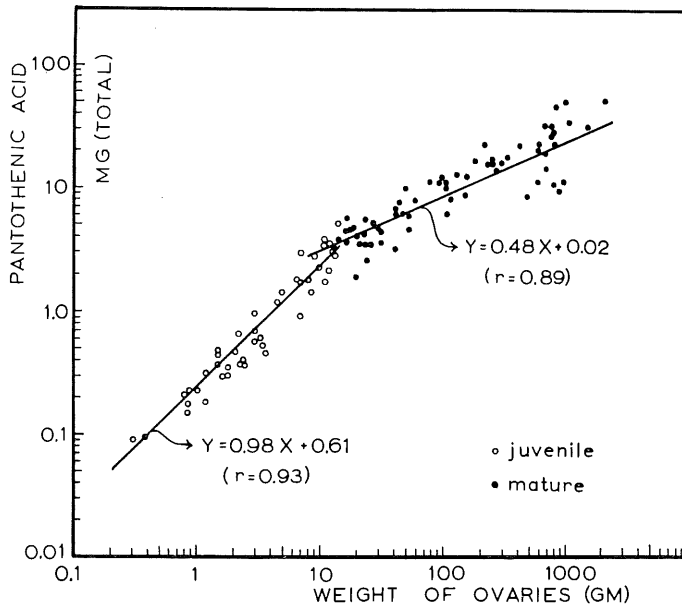


Fig. 7. Relation between total content of pantothenic acid and weight of ovary in juvenile and mature doc (*Gadus morrhua*). ( $Y$  = log pantothenic acid content;  $X$  = log weight of ovary).

and mature fish ( $p < 0.01$ ), thus the relation between the weight of the ovary and the weight of the fish differs in the two groups.

In Fig. 4 is plotted the relation between pantothenic acid per g fresh tissue and the weight of the ovaries. In juvenile ovaries the average value is constant regardless of the size of the organ, as the regression coefficient (0.03) does not deviate significantly from zero ( $0.6 > p > 0.5$ ). For mature ovaries the regression coefficient ( $-0.52$ ) does not deviate significantly from  $-0.50$  ( $0.5 > p > 0.4$ ). In Fig. 7 is plotted the relation between the total content of pantothenic acid and the weight of the ovaries. The regression coefficient (0.98) for juvenile ovaries does not deviate significantly from 1.00 ( $0.9 > p > 0.8$ ), and the regression coefficient (0.48) for mature organs does not deviate significantly from

0.50 ( $0.6 > p > 0.5$ ). The relation expressed by the equations in both Fig. 4 and Fig. 7 actually give the same results differently plotted, and lead to the same conclusion. The total content of pantothenic acid in ovaries of juvenile fish is directly proportional to the weight of the ovaries, while the total amount of pantothenic acid in the ovaries of mature fish is directly proportional to the square root of the weight.

No previous studies of the B-vitamins in relation to ontogenesis of teleostean eggs are reported. The rather high vitamin concentrations in

Table 2. *Pantothenic acid released from cod ovaries by various treatments.*

Sample no.	$\mu\text{g}$ pantothenic acid released per gram sample after treatment with:		
	Chicken liver + intestinal phosphatase	Papain + takadiastase	Water only
7	399	377	375
23	177	170	150
51	249	250	245
64	100	103	98
97	30	32	30
100	15	14	12

the ovaries of cod is however reasonable when the process of egg formation and embryonic development is considered. During the development of the eggs in the ovary, the different substances are supplied by the maternal body. During the spawning migration and the maturation of the teleostean egg there is a fairly large deposition of nutrients such as protein, fat and carbohydrate in the ovary, and an exhaustion of the maternal body with regard to these substances (TANGL & FARKES 1904; MCGLEUDON 1915; DAKIN & DAKIN 1925; CHANNON & SABY 1932; DAVIDSON & SHOSTROM 1936; LOVERN 1936). It may be supposed that the vitamins at the same time are stored to meet the demand arising after transition from the noncleidoic state to oviparity, when the egg is devoid of external supply.

Nevertheless, our present knowledge of processes during the development of the cod ovary does not explain the rather high values for pantothenic acid in juvenile ovaries and in the first stages of the regeneration cycle. NOVELLI (1957) reviewed the preliminary report from the present study (BRÆKKAN 1955 b), and pointed out that the decrease during the reproductive cycle may be caused by a conversion to coenzyme A. This seems not likely, as extraction studies (Table 2) showed

almost the same release by chicken liver enzymes, papain-takadiastase and water, thus indicating that the vitamin is mainly present in the form of the free acid. It may further be pointed out that the total pantothenic acid content shows an increase during the whole period of maturation. Although a variety of metabolic reactions are mediated by coenzyme A, only a very small proportion of the abundance of pantothenic acid would be needed in these processes during ovarian maturation or regeneration. It is more likely that an increased conversion to coenzyme A would take place after spawning to meet the demand of the metabolic processes during the embryonic development.

In the introduction were mentioned the findings of PEARSON & BURGIN (1941) of extraordinary high values for pantothenic acid as well as biotin in royal jelly. The present highest values for pantothenic acid, 377  $\mu\text{g}$  per g wet weight or 2430  $\mu\text{g}$  per g dry weight shows that cod ovary supersedes royal jelly as the natural material containing the highest amount of pantothenic acid. Biotin, however, did not show any such variation in the cod ovaries. PAIN (1951) followed up the findings of PEARSON & BURGIN (1941) to find if pantothenic acid was the substance largely responsible for the development of ovaries in worker bees to make them queens. He found that all vitamins tested, particularly vitamin B<sub>6</sub>, effected ovarian development, pantothenic acid apparently not being of unique importance. Thus a clue to a special effect of pantothenic acid for ovarian development is difficult to find, although its general importance in reproduction is well known. The constant average for pantothenic acid content per gram juvenile ovary of cod regardless of ovarian size, indicates that the vitamin is stored to a capacity limited by the ability of the ovarian tissue to take up the vitamin.

One possible explanation should not be overlooked. Special concentration in the ovaries of pantothenic acid from the food has to be considered. This would be similar to the interesting results observed in hens' eggs. SNELL, ALINE & PEARSON (1941) found that eggs from hens maintained since hatching on a diet low in pantothenic acid showed remarkably lower pantothenic acid contents than normal. This increased rapidly when the diet was supplemented with synthetic pantothenic acid, and the authors believed that within limits the pantothenic acid content of eggs is proportional to that of the diet. PEARSON, MELASS & SHERWOOD (1945) verified these findings and further showed that newly hatched chickens had a pantothenic acid content which showed relation to pantothenic acid in the ration. We may presume that the amount of food eaten by the fish bears a relation to their size, and thus the amount of pantothenic acid supplied and eventually stored should bear relation to the weight of the fish. In Fig. 8 is plotted the relation



between total pantothenic acid in the ovaries and the weight of the fish. There is a significant difference between the regression coefficient for juvenile fish (1.26) and the regression coefficient for mature fish (1.63), and thus a significant difference between the relations in juvenile and mature fish ( $0.05 > p > 0.02$ ). It may be noted that the increase is greater in mature fish than in juvenile fish. This could be caused by a limitation of storage in juvenile ovaries by the size of the organ and the

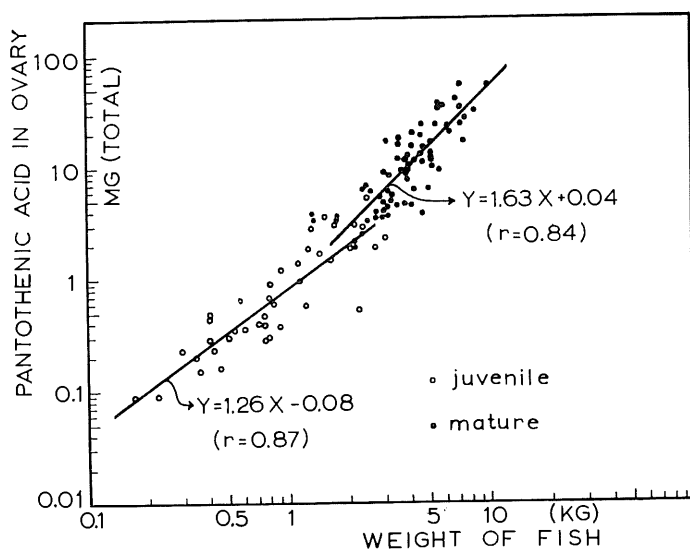


Fig. 8. Relation between total content of pantothenic acid and weight of fish in juvenile and mature cod (*Gadus morrhua*). ( $Y = \log$  pantothenic acid content;  $X = \log$  weight of fish.)

ability of the tissue to store pantothenic acid, while in maturing and regenerating ovaries the total storage capacity is increased. The question then arises as the storage per g is decreasing in mature ovaries, if ontogenesis causes such changes in the ovaries that the ability to store the vitamin is reduced, or if the supply from the maternal body and the food is the limiting factor. A conversion from free pantothenic acid to coenzyme A in increased amount is not likely as judged from the results of the extraction experiments (Table 2).

Calculation of the partial correlations showed that the total content of pantothenic acid was significantly correlated to both the weight of the ovary and the weight of the fish (Table 3).

Freeding experiments with fish should give interesting information as to the possible relation between pantothenic acid in the food and the storage in the ovaries, and eggs of teleosts. Due to the lack of suitable aquarium facilities, such studies have to be postponed.

Table 3. *Total, partial and multiple correlation coefficients between pantothenic acid content (P) as dependent variable and weight of ovary (O) and weight of fish (F).*

Group	Number of Fish n	Correlation coefficients <sup>1</sup>		
		Total d.f. = n-2	Partial d.f. = n-3 (significance)	Multiple
Juvenile fish	44	$r_{PO}=0.93$ $r_{PF}=0.87$	$r_{PF\cdot O}=0.32$ ( $0.05 > p > 0.02$ ) $r_{PO\cdot F}=0.56$ ( $p < 0.001$ )	$r_{P\cdot OF}=0.91$
Mature fish	66	$r_{PO}=0.89$ $r_{PF}=0.84$	$r_{PF\cdot O}=0.43$ ( $0.01 > p > 0.001$ ) $r_{PF\cdot O}=0.72$ ( $p < 0.001$ )	$r_{P\cdot OF}=0.93$

<sup>1</sup> Fisher & Yates: Statistical Tables for Biological, Agricultural and Medical Research, London 1948.

## SUMMARY

The relation between the contents of the B-vitamins pantothenic acid, riboflavin, niacin, vitamin B<sub>12</sub> and biotin and the reproductive cycle of the ovaries in cod has been investigated.

Pantothenic acid showed a unique relation, the values being very high in ovaries from juvenile fish and from mature fish in the first stages of regeneration. The highest value was 377  $\mu\text{g}$  pantothenic acid per g wet weight, or 2430  $\mu\text{g}$  per g dry weight, thus these values are the highest reported yet in Nature. As the ovarian development proceeds, the pantothenic acid content decreases to more normal values. The possible biological importance of this finding has been discussed.

Riboflavin, niacin and biotin did not show any variations of importance, while vitamin B<sub>12</sub> showed a definite though slighter decrease during the regenerative cycle.

Chemical analysis of protein-, ash-, and water-contents have also been reported.

The nutritional value of ripe ovaries or so-called «hard roe» is emphasized, and the results are discussed in relation to previous nutritional studies of cod roe.

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