XXV. THE RELATION OF THE VITAMIN A POTENCY OF THE LIVER OIL TO THE SEXUAL CONDITION AND AGE OF THE COD.

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It was previously pointed out [Zilva and Drummond, 1922] that a very appreciable disparity exists in the vitamin A potency of cod liver oils. This variation in potency we have shown could not be due to the various modes employed in the preparation of the oils [Drummond and Zilva, 1922]. It must, therefore, be traced to some biological factor. The biological causes which suggested themselves as being likely to influence the potency of the oils were the sexual condition, the age, and the food of the fish. We are now able to bring forward evidence showing that the first two factors do not influence the activity of the oil to any marked extent.

THE POTENCY OF OILS FROM FISHES IN DIFFERENT STAGES OF THE REPRODUCTIVE CYCLE.

It is well established [Hjort, 1914] that the fat of the whole system of the cod, especially of the liver, is being utilised in the process of development of the genital products during spawning. This transmission of fat was expected to be also attended by a transmission of the vitamin, a supposition which was strengthened by the experimental evidence of Coward and Drummond [1922] who demonstrated the part played by the vitamin in the development of the larval trout. This passage of the vitamin to the genitals might either have had no influence on the vitamin concentration of the residual oil of the liver in spite of the diminution of the oil content of the organ or it might have removed more of the vitamin in proportion to the oil. In the former case the potency of the oil during the various stages of the reproductive cycle would not be affected appreciably, in the latter case the livers would yield oils with a diminished vitamin content during the spawning period.

After studying a great number of cod in various stages of their reproductive cycle we have adopted the following standards for their sexual condition:

Males.

"Narrow"	Convolutions of spermatogenic tissue	Immature
"Medium"	"	Mature fish, successive stages of ripening testis
"Broad"	"	
"Opaque"	"	
"Running"	"	

Females.

White	Undeveloped	Stages of gonads in immature fish, the succession of which is uncertain
Cherry	"	
Orange	"	
Vermilion	Developed	Mature fish, successive stages of ripening ovaries
Orange	"	
Yellow	"	
Cream	"	
Mottled	"	
Running	"	

Further details of this differentiation are given by Graham [1923].

The livers and gonads were taken from "live" cod at Flamborough and Scarborough, *i.e.* cod which have been caught by lines so recently that gutting has not been necessary. The livers and gonads were removed immediately on landing and despatched on ice to London. The organs reached the Lister Institute, where the manipulations were immediately proceeded with, not later than 18 hours after the fish had been caught. When the tissues arrived at the laboratory they were immediately weighed, the gonads were preserved in a known quantity of alcohol and the livers were melted out in a steam-jacketed pan. The oils thus obtained and the gonads were then tested out on rats for their vitamin A content by the Zilva and Miura [1921] method. These experiments were carried out on four occasions in 1922, namely, January and

Table I.

Date	Sex and condition of reproductive organs	Minimum growth-promoting dose in mg.
23. i. 22	♂ "Cherry undeveloped"	1.8
	♂ "Orange"	1.8
	♂ "Narrow"	1.4
	♂ "Broad"	1.8
21. iii. 22	♂ "Cream"	1.4
	♂ "Mottled"	1.4
	♂ "Opaque"	Less than 10
	♂ "Spent"	1.8
25. iii. 22	♂ "Spent"	1.4
vi. 22	♂ "Spent"	2.2
	♂ "Spent"	2.2

March utilising unspent fish, and March and June when spent cod were investigated. As will be seen from Table I liver oils from fish of both sexes in different conditions of their reproductive period were studied. Little variation in the potency of the oils could be recorded. Even the liver oils from spent cod in March fish, which must have spawned just before being caught and which had no time to recuperate, did not deviate in potency from the oils of fish before spawning and several months after spawning and consequently after recuperation.

The preserved gonads, after removal of the alcohol in a vacuum at a temperature of about 40°–50° were also tested out for vitamin A and were found to be very potent (minimum growth-promoting dose 0.025 g. in the case of the male, and 0.05 g. in the case of the female, of fresh roe). It is, therefore, evident that as the spawning period approaches the oil and the vitamin from the liver both pass into the gonads. The relative potency of the residual liver oil is, however, not affected.

Further confirmation of this observation that the fluctuation in the potency of liver oils is not controlled by the condition of the fish in the reproductive cycle was obtained by examining liver oils from various fishes caught in the North Sea and in the vicinity of the British Isles at different times of the year, namely, September, 1921 and 1922, November and December, 1921, January, February, April, May, June, July, August in 1922. During this period no serious fluctuation could be observed in spite of the number of samples (about 40 in number) that were examined. We wish to take this opportunity of expressing our very best thanks to Messrs Isaac Spencer and Co. of Aberdeen, and Mr Fairbank Kirby, of Grimsby, through whose kindness we were able to secure all these oils.

THE POTENCY OF OILS FROM FISHES OF DIFFERENT AGES.

This problem was investigated in Newfoundland on two supposed races of cod; one occurring south of St John's (Ferryland) and another north of it (Port Union and Winterton). The fishes were measured for length, gutted and their livers were steamed out under comparable conditions within a few hours of capture. They were then classed according to age, utilising the length and the condition of the development of the sexual organs as criteria. Scales were also taken but the scale readings were inconclusive and the results could not be utilised for age determination. Table II gives the details of the fishes

Table II. *Cod*.

Ferryland			
	No. of fish	Mean length cm.	Range cm.
♂ Immature	59	44.1	37–51
♀ „	44	44.2	39–53
♂ Mature	23	77.9	65–97
♀ „	9	75.3	67–85
Difference between Means of Immature and Mature: ♂ 33.8 cm.; ♀ 31.1 cm.			
Gap between Ranges ♂ 14 „ ; ♀ 14 „			
Port Union and Winterton			
	No. of fish	Mean length cm.	Range cm.
♂ Immature	47	36.5	27–47
♀ „	50	37.3	26–47
♂ Mature	21	74.4	64–85
♀ „	13	73.9	67–79
Difference between Means of Immature and Mature: ♂ 37.9 cm.; ♀ 36.6 cm.			
Gap between Ranges ♂ 17 „ ; ♀ 20 „			

employed. As will be seen from the table the gap in length between the mature and immature groups is 14 cm. in the southern variety and 17 to 20 cm. for male and female respectively in the northern variety. Although the exact rate of growth of the cod is unknown it is nevertheless certain that 14 cm. represents *at least* one year difference. We have thus been able to classify them according to age, maturity and to sex. The livers of the respective groups were pooled for the preparation of the oils. The samples of oil were bottled, care having been taken to exclude air, and despatched to England and were tested out immediately on their arrival at the Lister Institute. These oils have been found to be very potent [Zilva and Drummond, 1923] (minimum growth-promoting dose 1.6 mg.) but no difference was established between the oils of the various batches. The fluctuation in the potency of liver oils cannot, therefore, be attributed to the preponderance of fishes of a certain age at different times.

The above experiments make it plain that neither the sexual condition nor the age of the fish can be responsible for the less potent oils sometimes obtained from livers of *certain* batches and one is forced to turn one's attention to the food. We [Drummond and Zilva, 1922] have shown that the ultimate origin of vitamin A must be traced to unicellular marine organisms. It is not directly from these organisms that the cod receives its vitamin but through several intermediaries such as the copepods and larval decapods and mollusca which are present in plankton and which feed on these unicellular plants. These are in turn consumed by the capelan and other forms of food of the cod. One may, therefore, assume that insufficient synthesis of the vitamin by the unicellular marine organisms owing to adverse natural conditions would ultimately be reflected on the vitamin content of the liver and gonads of the cod and would most probably also influence the mortality of the larval cod. The problem is of great interest especially to the marine biologist and invites further investigation.

SUMMARY.

The sexual condition and age of the cod do not influence the vitamin A potency of the liver oil.

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