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INVESTIGATION ON THE ADRIATIC ELASMOBRANCHIA LIVER OILS

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Among the sea-organisms the **Elasmobranchia** represent a very interesting group because of certain compositions found in the unsaponifiable matter of their liver-oils, like squalen, pristan, chimyl,-batyl and selachyl alcohol. The physiological function of these matters is not cleared up yet. Theretore the investigations of other organs in view of their content of these compositions represent a valuable contribution to the knowledge of their part in the metabolism of the fat.

The unripend eggs of the common **Acanthias vulgaris** Risso contain a considerable quantity of a brown oil, which differs by the compound and properties very much from the oil from the liver of this fish.

Ine the fresh eggs, that contain 44,38%, water, 2228% oil have been found by means of an ether extraction, respectively 40,06% calculated over to the dry substance.

In the oil gained by means of an extraction with ether, certain physical and chemical characters are fixed and because of the comparison with the properties of the oils from the liver, they are shown in table I.

Table I.

Constant	Oil from the eggs	Oil from the liver
Specific gravity $d\ \frac{15}{4}$	0,9557	0,9208
Refraction index $n\ \frac{20}{D}$	1,4803	1,4769-1,4786
Colour	dark brown	light yellow
Unsaponif. matters	9,48%	6,28%
Total fatty acids	64,38%	93,94%
Oxy - fatty acids	2,91%	3,62%
Iodine nuber (Hanuš)	127,5	156
Reichert - Meissl' s number	0,05	0,2
Polenske number	2,1	—

The specific gravity of this oil is considerably greater than that of the oil from the liver and so is the refraction index. The quantity of the unsap. matters is also greater than by oils from the liver. This ditterence can probably be a smaller one, for, according to some authors, the unsap. matter in the oil from the liver is a considerably greater one. The small quantity of the total fatty acids is striking. Here are only those fatty acids fixed which are insoluble in water and which could have been extracted with ether after the fixation of the unsap. matter.

The iodine number is also a considerably smaller one and so is the quantity of the vapourable, in water soluble acids. From the vapourable, in water insoluble fatty acids (P o l e n s k e number) there are no datas for the oil from the liver which could be compared.

T s u j i m o t o (1) has found in the eggs of the sort of **Lepidorhinus kimbei** a considerably smaller quantity (17,3%) of some oil which in regard to its properties differs very much from the oil described here. It has a much smaller specific gravity (0,8997) further a great quantity of unsap. matters (33%). Its iodine number is also a much greater one (177,6).

Further the properties of the fatty acids are fixed and compared with the corresponding values of the fatty acids from the liver oils in table II.

Table II.

Constant	Acids from the oil of eggs	Acids from the liver oil
Colour	dark brown	light brown
Melting point	38-39	32
Neutralization point	233,2	179,5
Iodine number (Hanuš)	144,9	133-139
Insoluble polybromides	48,3	28,5

Comparing these datas it is obvious that at the fattö acids of both sorts of oils there are also rather considerable differences in the individual constants. Acids from the oils of the eggs have a rather high melting point. The neutralization point is also a great one and by the researches on the oil of the **Elasmobranchia** up to now it has never been found such a great value. The iodine number is only a little greater but this difference represents nothing of importance. Much more interesting is the fact that the oil from the eggs gives almost a double quantity of indissoluble polybromides than the liver oil.

The results achieved by the investigation of the unsap. matter are very interesting, especially in comparison with the analysis of the unsap. yield from the liver oil. Before we go over to the consideration of the result it is necessary to note another matter. Tsujimoto (2) has divided the liver oil of the **Elasmobranchia** in three different groups, e. i. 1. oils with a very low content of the unsap. matter, which mainly consists of sterine; 2. oils with cca 10-30% of the unsap. matter, consisting of cholesterol and glycerin ether (chimyl, - selachyl, - batyl alcohol) and 3. oils with a very high content of the unsap. matter, which contains a large quantity of squalene. In view of the quantity and the composition of the unsap. matter he places the oil of the dogfish in the

middle between the first and the next two groups. According to Tsujimoto the unsap. part of this oil consists largely of batyl and selachyl alcohol and contains very little or no squalene.

In order to prove the presence of sualene by the sort of **Acanthias vulgaris** he has examined the unsap. yields of the oils from the eggs and the liver oils by the application of the chromatographic adsorbtion method. In the unsap. part from the liver-oil more than 50% of a hydrocarbone have been found by the eluation with light petroleum. Judging by the low iodine number (85,8) here is probably not the question of squalene, which has a high iodine number 371,1 owing to the high degree of its unsaturation. Either by Täufel's method (3) has it been possible to prove the presence of squalene, although this method has proved to be a very precise one. By the application of it the presence of 0,03 g of squalene, dissolved in a 100 cm solution in form of hexahydrochloride, can be proved. Here is probably the question of a saturated hydrocarbon, pristan whose presence Tsujimoto has (4) detected in the liver oil of the **Halsydrus maximus.**

By the research of the unsap. matter from the oil of a fish nearly related to the dogfish **Squalus suckleyi** from the Pacific, neither Swain (5) could find squalene but another hydrocarbon, which he has not characterised particularly. Neither by the chromatographic investigation of the unsap. part of the oil from the eggs has it been succeeded to prove the squalene. In the fraction eluated with light petroleum, that contains hydrocarbons, a very smal quantity of a substance has been obtained, which does not show any reactions on squalene. Moreover the absence of squalene is proved by the very low iodine number of the unsap. yield (71,64). But it is interesting that some searchers have found squalene in the oil from the eggs of other **Elasmobranchia.** So has Tsujimoto (6) proved it in the eggs of some sortes of **Chlamedoselachus anguineus** and **Lepidorhinus kimbei,** and Heilbron, Kamm and Owens (7) in the eggs of the dog **Etmopterus spinax.** Besides Heilbron, Kamm and Owens (8) have found it in the unsap. matter of the oil from the stomach of the **Scymnorhinus lichia.**

The established fact cannot yet be considered as a definite prove that there is no squalene at the dogfish, thus it would ne-

cessary to examine also the fats from the other organs on the eventual content of squalene. It is possible that by some sorts the squalene appears only at certain states of their development. Systematic researches of each organ in particular in this regard could eventually give a better insight in this problem.

The part of squalene in the metabolism of the fat is not cleared yet. One can solely judge on uncertain knowledge. E. André and H. Canal (9) suppose that the fatty acids may turn into squalene e. i. over unsaturated acids and cholesterol. They found that there exists a certain reciprocal relation between the quantity ofholesterol and squalene. By the young shark of the sort of **Cetorhinus maximus** the oil contains 58% fatty acids, 18% squalene and 22,50% cholesterol, as well as its derivations, while by the grown up samples the content of fatty acids is 47%, squalene 48% and only 2% cholesterol. Although this supposition seems to be acceptable ,it would yet be necessary to prove the reason of the connection between squalene and sterine in an experimental way

In Table III the results obtained by means of chromographic investigations about the unsap. matter of the oil from the eggs and liver of the dogfish are shown.

Table III.

Unsap yield of the oil	Iodine number of the unsap.	Percent eluated				Recovery of unsap. %
		Light petr.	Benzene	Ether	Metanol	
eggs	71,64	1,88	45,5	47,28	5,46	100,12
liver	66,9	54,22	18,02	13,74	4,54	90,52

In none of the two oils was it possible to prove squalene neither by means of the iodine number nor in a preparative way by the isolation of its hexahydrochloride. One can discern that the difference in the composition of both unsap. yields is an uncommonly great one. This difference is especially striking in the content of hydrocarbone.

Schmidt-Nielsen and Artun (10) have found by their studies of **Spinax niger,** that the unsap. matters in the oil from the eggs also contain much more hydrocarbons than the unsap. matters from the liver oil. The hydrocarbon, which they found, was squalene.

In the benzen fraction, that contains sterols, there is also a considerable difference, not only in the qualitative but also in the quantitative regard. By the oil from the eggs the entirely pure and white cholesterol, with a melting point 147 degrees C is being gained by the evaporation of the benzen-eluate, that means that the unsap. matter contains 45% cholesterol. By the unsap. matter from the liver oil the benzen-fraction of the chromatogram is of a yellowish colour and a waxen consistence. Only a very small quantity of cholesterol has been gained by an over-crystallisation. The iodine number of this fraction was rather low (49,7) and could also suspect a small quantity of cholesterol, which generally has a much higher iodine numebr (65,70). The quantity of glycerine-ethers, e. i. chimyl,-batyl-and selachyl alcohol, eluated with ether is by the oil from the eggs also a large one. The ether fractions from the unsap. yield of both sorts of oils are at a normal temperature a colourless, solid mass, thus it can be concluded that they chiefly consist of chimyl and batyl alcohol.

Thus the difference in the qualitative and quantitative composition of the unsap. matter of both oils is a very big one. This fact is very interesting in view of Tsujimotos (11) allegations who has settled by his examinations of oils from the body and liver of the dog **Squalus suckley,** that the compositions of their unsap. are corresponding.

CONCLUSION

The oil from the eggs compared with the oil from the liver shows essentially different characteristics in its physical and chemical properties. There exists a big difference in its chemical composition of the unsap. matter, especially with regard to the content of cholesterol and hydrocarbone. Neither in the oil from the eggs nor in the liver oil has it been possible to prove the presence of squalene.

METHODS

Determination of water.

The quantity of water in the fresh material has been determined in the apparatus by Marcusson by means of the distillation with xylen.

Determination of oil.

The determination of oil in the fresh material has been carried out in the following way: about 5 g of fresh and fine homogenised eggs have been weighed in the thimble for the extraction, carefully mixed with the same quantity of anhydrous natrium sulphate and extracted in Soxhlet's apparatus with ethyl ether through 24 hours. After the greater part of the ether has been evapourated on the water bath, the oil has been dried in the vacuum till its constant weight.

The gaining of oil.

Eggs, fine reduced to small pieces have been mixed with anhydrous natrium sulphate and extracted with ether. The greater part of the solvent has been over-distillated over a water bath and the rest removed in the vacuum.

The physical and chemical properties.

The greater part of the analysis has been carried out according to the prescriptions in »Acta Adriatica« (12). The iodine numbers were fixed by Hanuš's method.

Examination of the unsap. matters.

For the chromatographic examinations the unsap. yield is being prepared by Fahrion's method, with ethyl ether. For the analysis the aluminium oxide is being used, standardised by Brockmann (Merck) in tubes of 12 mm width. For the eluation light petroleum with a bolling point till 70 degrees C, benzen, ethyl ether and absolute methanol are being used.

ACKNOWLEDGMENT

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PRILOG POZNAVANJU ULJA IZ JAJA OD ACANTHIAS VULGARIS Risso

Zaključak

Ulje iz nedozrelih jaja od **Acanthias vulgaris Risso** istraženo je obzirom na njegove fizikalne i kemijske osebine. U poređenju sa uljem iz jetara ono pokazuje znatne razlike, naročito u pogledu specifične težine, masnih kiselina i jodnog broja. Svojstva masnih kiselina također su vrlo različita. Visoki heksabromidni broj upućuje na veliku količinu nezasićenih kiselina sa tri dvostruka veza.

Metodom kromatografske adsorpcije ispitane su neosapunjive tvari ulja iz jaja i jetrenog ulja. Utvrđeno je, da se neosapunjivi ostatak od ulja iz jaja pretežno sastoji od holesterina i glicerinskih etera t. j. chimyl i batyl alkohola. Nazočnost squalena nije mogla biti utvrđena, što je zanimljivo obzirom na činjenicu, da su ga neki autori u uljima iz jaja drugih vrsta **Elasmobranchia** uspjeli dokazati. U neosapunjivom dijelu jetrenog ulja također nije uspjelo dokazati prisutnost squalena. On se većim dijelom sastoji iz jednog drugog ugljikovodika. Sudeći po vrlo niskom jednom broju radit će se vjerojatno o pristanu. Iz frakcije eluirane benzolom dobivena je samo vrlo mala količina holesterina.

Istraživanja o sadržaju squalena u uljima raznih organa **Elasmobranchia** mogu predstavljati znatan doprinos za upoznavanje njegove uloge u metabolizmu masti.

ПРИЛОЖЕНИЕ К ПОЗНАНИЮ ЖИРА ИЗ ИКРЫ *ACANTHIAS VULGARIS* Risso

Степан Чмелик

Вывод

Жир из недозрелой икры *Acanthias vulgaris* Risso исследован в отношении его физических и химических свойств. В сравнении с жиром из печени он показывает значительную разницу в особенности по отношению удельного веса, жирных кислот и иодного числа. Свойства жирных кислот также очень различны. Высокое гексабромидное число указывает на большое количество ненасыщенных кислот с тремя двойными связями.

Способом хроматографической адсорбции исследованы неомыляемые вещества из икры и жира из печени. Установлено, что неомыляемый остаток от жира из икры большей частью состоит из холестерина и глицериновых эфиров т. е. *chimyl* и *batyl,* алкоголей. Присутствие squalena не удалось установить, однако надо заметить, что некоторые авторы успели доказать его присутствие в жирах из икры других сортов *Elasmobranchia.* В неомыляемой части жира из печени также не удалось доказать присутствие *squalena.* Он большей частью состоит из другого углеводорода. Судя по очень низком иодном числу вероятно надо предполагать, что это есть пристан. Из фракции елюированной бензолем получено совсем незначительное количество холестерина.

Исследования о содержании squalena в жирах различных органов Elasmobranchia могут представлять большой интерес для изучения его роли в метаболизме жиров.