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INVESTIGATION ON THE ADRIATIC ELASMOBRANCHIA LIVER OILS

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INVESTIGATION ON THE ADRIATIC *ELASMOBRANCHIA* LIVER OILS

IV. Biochemical study about oils from the liver and eggs of *Centrina Salviani* Risso.*)

by

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The oil from the liver of the shark *Centrina Salviani* has been investigated several times in respect of its physical and chemical properties. Huvart(1) reports in one of his notes some chemical constant, Van Gaver (2) and later Kollmann, Van Gaver and Timon-David (3) give a great number of different analytic statements, according to which they find a great resemblance between this oil and the oil of the sea-mammels *Physeter macrocephalus* and *Hyperoodon rostratus*. Marcelet (4) has recently re-examined these statements and determined that it is difficult to search a relationship between the oil from the liver of *Centrina Salviani* and the oil from the body of a mammel, chiefly because of the fact that the fat from the different parts of the body differs considerably in its physical and chemical characters. Among other statements Marcelet alleges different properties of different fatty acids and unsap. matters. Carrière and Bonnemaison (5) engaged themselves particularly in the research of the composition of the unsap. matter of this oil. Besides cholesterol and a hydrocarbon, of which they think to be sqalene, they isolated selachyl, - batyl - and chimyl alcohol from the unsap. yield.

By a comparison of the analytic statements of the quoted authors it is striking that some properties show very great

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*) Synonims: *Squalus Centrina* L.
Centrina vulpecula Bel.
Centrina oxynotus Swain
Oxynotus Centrina Raf.

fluctuations. Having in view the results of Schmidt-Nielsen and Artun (6), who have determined the dependence of the different properties of liver oils the sex on the sample *Spinax niger,* we were interested in making researches about the influence of different physiological stages on the composition of the oil on the sort *Centrina Salviani* especially in regard to the quantitative composition of the unsap. matters.

The variations of the properties in the oils some *Elasmobranchia* in connection with different physiological stages have been investigated by E. O. Aenlle (7). Beecause of the difficulties during the war, unfotunately we had no possibility to obtain this his work.

Thanks to the expedition »Hvar« of this Institute, it has been succeeded to get a certain number of samples of this rather rare sort of sharks and moreover in different stages of their development. Six samples of the oil from the liver have been examined so far. Only one belonged to a male, two from eggs, one from unfructified ones and the other from fructified eggs with young germs. Up to now the oils from eggs have not yet been researched at all.

The characteristics of some particular samples are presented in Table I.

Number of the sample	Sex	Lenght in cm	Weight in kg	Remark
I.	female	64	3,08	
II.	male	64	3,08	
III.	female	78	6,85	pregnant
IV.	„	71	6,0	pregnant with 9 unfruct. eggs.
V.	„	—	2,0	undeveloped ovary
VI.	„	74	7,4	pregnant with fruct. eggs. and foetus in the oviduct.
VII.	oil from unfruct. eggs of number IV.			
VIII.	oil from fruct. eggs from number VI.			

Oils from the liver are gained by cold straining and oils from eggs by extraction with ether. It is interesting to note that the absolute quantity of oil in the fructified eggs is a considerably greater one than in the unfructified eggs:

unfructified eggs: 45,8%
fructified eggs: 53,7%

Comparing the physical properties of the particular samples, shown in table II., it is remarkable that there are considerable differences, especially in respect of the specific gravity and the refraction-index. Among the liver- oils the oil of the male has the highest specific gravity, while the lowest values were found in the oil of pregnant females. The oils from eggs have a much greater specific gravity than the liver oils. It is further to be noted that the specific gravity of the oil from unfructified eggs is considerably smaller than that of fructified ones. It can also be observed in table II. that there are differences in the refraction-index and that they are remarkably great between liver-oils and oils from eggs.

Kollmann, Van Gaver and Timon - David (3) have found that the oil from the ovary has the specific gravity 0,9106 and the refraction- index 1,4744. These ciphers seem to be too small comparing them with our own data.

Physical properties of oils from the livers and eggs of *Centrina Salviani*

Table II.

Sample	Specific gravity d_4^{15}	Refractive index n_D^{20}	Solidification point	Colour
I.	0,9119	1,4754	1	gold coloured
II.	0,9233	1,4694	1	light yellow
III.	0,9022	1,4691	0	yellow
IV.	0,9034	1,4700	3	pale yellow nearly colourless
V.	0,9120	1,4756	4	light yellow
VI.	0,9044	1,4706	0	light yellow
VII.	0,9418	1,4895	5	brown
VIII.	0,9583	1,4883	—3	light brown

Chemical properties of oils from the livers and eggs of *Centrina Salviani*

Table III.

Sample	Unsaponifiable matter %	Total fatty acids %	Free fatty acid	Saponification number	Jodin number	Votalile acids soluble (Reichert Meissl No.)	Votalile acids insoluble (Polenske No.)
I.	22,5	74,5	0,69	168,4	82,2	4,3	1,5
II.	31,38	67,41	0,55	160	65,3	3,62	4,3
III.	29,34	68,36	0,62	152,2	80,3	1,25	6,1
IV.	30,12	66,50	0,76	159,6	75,8	4,48	1,1
V.	25,18	74,61	0,56	164	70,1	3,2	0,9
VI.	28,33	69,65	0,37	158	95,2	2,5	0,65
VII.	26,88	73,11	6,5	160	87,0	—	0,9
VIII.	21,36	63,74	4,1	137,7	94,3	—	0,75

By a comparison of the chemical characters (Table III.) it is straking that the oil from the liver of the male contains the greatest quantity of unsap. matters. This fact has been determined on the sort *Spimax niger* by Schmidt Nielsen and Artun (6). It can further be seen that the content of unsap. matters on pregnant females is a considerably greater one than that on females with an undeveloped ovary. In respect of the quantity of unsap. matters, oils from eggs do not differ from liver--oils. The quantity of free fatty acids is on each oft the six liver-oils. very small. Only in the oil from unfructified eggs there are enough free acids. In the oil from fructified eggs this quantity is a much smaller one.

The saponification number in liver-oils varies between 152,2 - 168,4. Kollmann, VanGaver, Timon-David (3), further Carrière and Bonnemaison (5) have found much smaller values. The oil from unfructified eggs does not differ in its saponification number from these oils either, solely in the oils from fructified eggs it is considerably smaller and accords with the saponification number 133,7,

which Kollmann, VanGraver and Timon-David have determined on oils from the ovary. Between the oils from unfructified and fructufied eggs there exists a rather great difference in their saponification number.

The quantity of unsaturated acids, expressed by the iodine number, is very variable. The iodine number is striking small at males and much greater at females, especially at pregnant ones. It is of interest to note thet there is no difference between the oil from eggs and liver oils in respect of theire iodine number, though the oils from fructufied eggs show an increase of the quantity of unsaturated acids. It is comprehensible that the data of the different authors diverge greatly just in respect of the iodine number. VanGaver (2) has found that it amounts to 97,7, Kollmann, VanGaver and Timon-David (3) 60-73,4, Marcelet (4) 89-105 and Carrière and Bonnemaison (5) 99,5. We could not agree with Kollman and the statements of other authors, according to which the iodine number of the oil from the ovary amounts to 113,9.

The content of volatile acids, soluble in water, also varies on the particular liver oils. The values, we found, are a little greater than those found by Marcelet (4), Carrière and Bonnemaison (5).It is worth notice that oils from eggs do not contain such acids at all. In regard to the vaporable, insoluble acids our results accord with the statements of other authors.

Table IV.

Number of sample	Neutralisation number	Iodine number	Poly-bromide %	Solid acids %	Liquid acids %	Absolute iodine number
V.	221,6	98	14,6	23	74	116
VI.	212,5	82,5	13,4	23.3	73,5	103
VII.	212,4	91,5	11,8	26	69	137,2
VIII.	201,3	109	3,97	24,8	72	153

The chemical properties of fatty acids of some liver oils, as well as of the oil from eggs, are presented in Table IV. No special difference can be observed in the compound of acids between the pregnant female and the female with undeveloped ovaries. There exists only a difference in regard to the iodine

number as it is also determined on oils themselves. The properties of fatty acids from the oil of unfruct. eggs are very alike those of the total acids, as well as of the absolute iodine number is observed on the oil from fructified eggs. Also according to the quantity of polybromide, which was found, it can be concluded that there is a decrease of much unsaturated acids. The relation of solid and liquid acids is rather the same in each of these oils.

Marcelet (4) was the first that engaged himself in the research of the unsap. matters from liver oil on the sample *Centrina Salviani*. By a recrystallization of them he found an alcohol with the melting point 62-63 deg. He gained acetyl derivate from it, with the melting point 61,5 deg., and basing on these data he presumed that it is the question of an alcohol which he isolated from the oil of the seed of the black-berry. Carrière and Bonnemaison (5) have carried out more precise investigations of these matters. By a crystallization of the »unsap« in methanol of 80% they got a crystalline and a fluid part. By a fractional distillation of these acetylised parts in a vacuum they separated chimyl, - batyl - and selachyl alcohol and cholesterol. Besides, they found among the unsap. matters 1,4% of a hydrocarbon, which they think to be squalene. According to the statements of these authors the composition of unsap. matters is circa as follows: selachyl alcohol (d-α-oleyl-glyceril-ether) 40%, batyl alcohol (d-α-octadecyl-glyceril-ether) 38%, chimyl alcohol (d-α-hexadecyl glyceril-ether) 12%, cholesterol 5,6%, hydrocarbons 1,4% unknown matters 3%.

Having in view a report, which was issued by Ziro Nakamya (8), about the possibility of an isolation of the individual components from the »unsap« by means of chromatographic adsorbtion, we tried to employ this method for a quantitative fixation of hydrocarbon, cholesterol and glycerile ethers, using aluminium oxide, activated by Brockmann (9), as adsorbent.

The separation of the individual components from the »unsap« on fish oils by the method of chromatographic adsortion has been tried by Morton (10), Swain (11), Swain and Mc Kercher (12) and Čmelik (13). The quoted authors restricted themselves only to the quantitative deter-

mination of the individual groups, eluated with different solutives.

On our own researches we tried to fix the quantitative relation of the individual components by a decomposition of the fluid chromatogram in a greater number of fractions, as well as by a preparation of different derivates, as that has been done by Prelog, Ružička and Stein (14) and Prelog and Beyerman (15) on the research of the matters from the extract of different organs. Owing to the lack of certain resources it was not possible to carry out the elemntary analysis of the individual compounds.

Comparing the individual chromatograms we see at first that the quantity of hydrocarbons, contained in them, is very small. Only in one case, i. e. on a pregnant female with fructified eggs a great quantity of it has been found, which amountend to 40% of the whole »unsap«. While on the other hand it is interesting to note that the content of hydrocarbons at other pregnant females, as well as the oil from eggs, was a very small one.

It is not probable that the hydrocarbon, which was found, represented squalene as Carrière and Bonnemaison (5) supposed, for it does not give any additional product with brome and besides, it has a much lower refration index (n_D^{20} 1,4470) than squalene (n_D^{20} 1,4967). It is probably the question of pristan, which has been found on different affined squalides.

The quantity of cholesterol can be determined very accurately by eluation by the use of benzen. Very small quantities of carotinoids which gives to cholesterol a yellowish colour, are eluted. As it can be seen by the chromatogram, the quantity of cholesterol in the »unsap« at liver oils varies between 1,1-7,6. It is very striking that the smallest quantity is contained at pregnant females and the greatest one at females with undeveloped ovaries. The quantity of cholesterol is considerably greater in oils from eggs. This fact has also been proved on oils from the eggs of the sample Acanthias vulgaris (13). It can be observed that the quantity of cholesterol is smaller on fructified eggs than on unfructified ones.

By a further eluation by means of benzen, in some cases by a small addition of ether, almost all selachyl alcohol can be

isolated in form of a rather thick, yellowish oil. The identification was carried out according to the refraction index n_D^{20} 1,4698 and the saponif. number of the acetyl product, which amounts to 261.

The percentage of selachyl alcohol on liver oils varies very much, i. e. between 14,07 — 27,1%. According to this fact it can be concluded that Carrière and Bonnemaisons (5) findings are a little too great. In respect of their content of this alcohol the oils from eggs do not differ from liver oiles. It is interesting that the quantity of selachyl alcohol decreases in oils from fructified eggs.

The quantitative separation of the solid glyceril ethers, i. e. batyl- and chimyl alcohol could not be carried out so exactly as on the previously described matters because by the eluation of the last rests of selachyl alcohol a part of the batyl alcohol goes over into the filtrate. The first fractions eluated with ether, on a mixture of ether and benzene, also contain besides batyl alcohol a certain quantity of selachyl alcohol. All ether fractions consist of batyl alcohol, which was identified by the preparation of its bis-phenylurethane (melting point 98°) and di- (p-nitrobenzoate) with the melting point 68,5 deg. The quantity of batyl alcohol, which was found, is considerably greater than Carrière and Bonnemaison (5) have stated and varies at liver oils from 26,5-59% and at oils from eggs 41,6-46%. According to this, batyl alcohol forms the preponderant part of the »unsap« on the sample *Centrina salviani*. It was not possible to prove any relation between the stage of the sexual maturity and the content of batyl alcohol, but it is important to remark that the quantity of batyl alcohol is considerably smaller at two pregnant females, especially at the sample IV, where this quantity decreases in consequence of the increase of the content of hydrocarbon.

From the last fractions, eluted with methyl alcohol, as well as with a mixture of methyl alcohol and acetic acid, the chimyl alcohol has been isolated. It is the most difficult to be eluated. This fact has been established by Prelog, Ružička and Stein (14), further by Prelog and Beyerman (15). The identification has been carried out on ground of its own melting point (61 deg.) as well as by the preparation of its di-(p-nitrobenzoate) with the melting point 58-59 deg. It is striking

that the content of chimyl alcohol in the unsap. part of liver oils is very variable (1,9 — 19,2) and that the greatest quantities were found on pregnant females.

In the last fractions there are besides chimyl alcohol also some other matters which have not been investigated in detailes.

EXPERIMENTAL PART

The preparation of oils for the research.

Liver oils are gained by cold straining. All livers are so rich in oils that they already yield the greatest part of the oil while their mincing. In order to remove the water the oils are filtrated over waterless sodium sulphate. The oils from eggs are gained by extraction by means of ether. Fresh eggs are previously dried in a vacuum at 50-60 deg.

The determination of physical and chemical properties.

The physical and chemical properties of oils and fatty acids are determined by methods described in the previous reports (13).

The preparation of the »unsap«.

About 5-7 gr of oil are saponified over a vapour-bath with 20-25 ccm of a 2n alcoholic KOH solution. After the evaporation of the alcohol the soaps are dissolved in 150 ccm water and shaken in the separation funnel, once with 100 ccm and three times with 50 ccm ether. Because of the neutralization of the remaining alkalies the united ether-solutions are shaken with 3 ccm 0,5n HCl and 20 ccm water and afterwards with 5 ccm 0,5n alcohol solution KOH ond 30 ccm water. After a redistillation of ether on a water-bath the unsap, matters are dried in a vacuum up to their constant weight.

The preparation of the activated Al_2O_3 for the chromatography.

For all chromatographic researches the aluminium-oxide, activated by Brockmann (9) is employed. Aluminium hydroxide is heated in an iron pan on a strong flame for six hours at a constant stir. After the cooling for 30 minutes in a stream

CHROMATOGRAME I.

1,8849 g. were used.

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	3,7	0,2	*hydrocarbons*
3—4	*benzene*	144,4	7,6	*cholesterol*
5—8	*benzene*	288,1	15,2	*selachyl alcohol*
9—10	*benzene-ethyl ether 9:1*	163,6	8,6	
11—14	*ethyl ether*	1123,9	54,3	*batyl alcohol*
15— 6	*ethyl ether*	89,7	4,7	*batyl alcohol*
17	*methyl alcohol*	30,7	1,6	*chimyl alcohol*
18	*methyl alcohol + acetic acid*	27,3	1,4	*chimyl alcohol*

CHROMATOGRAME II.

1, 6482 g. of unsap. were used

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	5,8	0,3	*hydrocarbons*
3	*light petroleum*	traces	—	
4—5	*benzene*	24,4	1,4	
6—7	*benzene*	91,5	5,5	*cholesterol*
8 - 9	*ethyl ether*	320	19,4	*selachyl alcohol*
10	*ethyl ether*	226,1	13,7	*selachyl + batyl alcohol*
11—12	*ethyl ether*	774,3	46,8	*batyl alcohol*
13—15	*ethyl ether*	49	2,9	*batyl alcohol*
16	*ethyl ether*	9	0,5	
17—18	*methyl alcohol*	57,6	3,4	*chimyl alcohol*
19—20	*methyl alcohol + acetic acid*	22,4	1,3	*chimyl alcohol*

CHROMATOGRAME III.

3,25 18 g. were used

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	8,2	0,2	*hydrocarbons*
3—4	*light petroleum*	41,2	1,2	
5—7	*benzene*	47,6	1,4	*cholesterol*
8—9	*benzene*	4'.8	1,3	*cily*
10—16	*benzene_ethyl ether 9:3*	883,2	27,1	*selachyl alcohol*
17—18	*benzene_ethyl ether 9:3*	283,2	8,7	*white, waxy*
19	*benzene_ethyl ether 1:1*	194,4	5,9	*batyl alcohol*
20—25	*ethyl ether*	954,2	29,3	*batyl alcohol*
26—27	*ethyl ether*	145,1	4,4	*batyl alcohol*
28—31	*methyl alcohol*	454,9	13,9	*chimyl alcohol*
32—33	*methyl alcohol + acetic acid*	173,2	5,3	*chimyl alcohol*

CHROMATOGRAME IV.

3,0016 g. of unsap. were used

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	1240,9	41,3	*pristane*
3	*light petroleum*	traces	—	
4—5	*benzene*	20,3	0,6	
6	*benzene_ethyl ether 9:1*	25,1	0,8	*cholesterol*
7	*benzene_ethyl ether 3:1*	36,5	1,2	*cholesterol*
8	*benzene_ethyl ether 1:1*	22,1	0,7	
9—10	*ethyl ether*	422,5	14,07	*selachyl alcohol*
11	*ethyl ether*	61,1	2	*selachyl + batyl*
12—14	*ethyl ether*	691,2	23	*batyl alcohol*
15	*ethyl ether*	105,9	3,5	*batyl alcohol*
16—17	*methyl alcohol*	134,2	4,4	*chimyl alcohol*
18—19	*methyl alcohol + acetic acid*	122,1	4,06	*chimyl alcohol*

CHROMATOGRAME V.

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	15,9	1,3	*hydrocarbons*
3	*light petroleum*	traces	—	
4—5	*benzene*	45,9	3,75	
6	*benzene*	73,8	6,04	*cholesterol*
7—9	*ethyl ether*	210,4	17,2	*selachyl alcohol*
10—13	*ethyl ether*	694,3	56,8	*batyl alcohol*
14	*methyl alcohol*	31,6	1,9	*chimyl alcohol*
15	*methyl alcohol*	traces	—	*chimyl alcohol*
16	*methyl alcohol + acetic acid*	traces	—	*chimyl alcohol*

CHROMTAOGRAME VI.

2.3734 g. of unsap. were used

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	5,7	0,2	*hydrocarbons*
3	*light petroleum*	6,9	0,3	*hydrocarbons*
4	*light petroleum*	traces	—	
5—6	*benzene*	26,9	1,1	*white, waxy*
7—8	*benzene*	26,2	1,1	*cholesterol*
9—11	*benzene_ethyl ether 3:1*	454,7	19,1	*selachyl alcohol*
12—13	*benzene_ethyl ether 3:1*	301,9	12,7	*selachyl + batyl alcohol*
14—15	*benzene_ethyl ether 1:1*	166,8	7	*batyl alcohol*
16—19	*ethyl ether*	1070,1	45	*batyl alcohol*
20—21	*methyl alcohol*	130,5	5,4	*chimyl alcohol*
22—23	*methyl alcohol + acetic acid*	112,2	4,7	*chimyl alcohol*

CHROMATOGRAME VII.

2,0071 g. of unsap. were used

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	7,7	0,3	*hydrocarbons*
3—7	*benzene*	539,9	26,8	*cholesterol*
8	*benzene*	7,8	0,3	*cholesterol*
9—11	*benzene*	37.9	1,08	
12—13	*benzene_ethyl ether 1:1*	322,9	16,1	*selachyl alcohol*
14	*ethyl ether*	65	3,2	*selachyl + batyl alcohol*
15	*ethyl ether*	121,3	6,04	*batyl alcohol*
16—19	*ethyl ether*	717,1	35,7	*batyl alcohol*
20—22	*methyl alcohol*	95,1	4,7	*chimyl alcohol*
23—24	*methyl alcohol + acetic acid*	87,3	4,3	*chimyl alcohol*

CHROMATOGRAME VIII.

1,7931 g. unsap. were used

No.	SOLVENT	Eluted		Objection
		mg.	%	
1—2	*light petroleum*	9,9	0,4	
3	*light petroleum*	traces	—	
4—6	*benzene*	469,7	26,1	*cholesterol*
7—10	*benzene*	255,1	14,3	*selachyl alcohol*
11	*ethyl ether*	262,4	14,6	*semi solid*
12—13	*ethyl ether*	714.2	39,8	*batyl alcohol*
14	*ethyl ether*	9,8	0,4	*batyl alcohol*
15—16	*methyl alcohol*	53,7	2,9	*chimyl alcohol*
17	*methyl alcohol + acetic acid*	12	0,5	*chimyl alcohol*

of air it is preserved in a bottle with a grinded cork. Aluminium oxide, preparated in this way, has according to B r o c k m a n n, a dregree of activity II.

Chromatographic separation of the individual constituents from the unsoap, rest.

2—3 gr of the unsap. matters are dissolved in 100 ccm of light petroleum (boiling point 60-70 deg) and filtrated over a little bar of aluminium oxide, 2 cm wide and 30 cm high. In order to speed the penetration of the liquid a weak vacuum is employed. The eluation is made with light petroleum benzene, a mixture of benzene and ether, pure ether and methanole. After that each 100 ccm are further examined for the identification. After the eluation with methanole the Al_2O_3 is treated several times with methanole which contains 10% acetic acid. The solutions are concentrated to a small volume, diluated with water and shaken with ether. After the washing with a natrium hydrate the solution is filtrated over a waterless natrium sulphate and the ether redistillated on a water-bath. It has always been succeeded to obtain a certain quantity of chimyl alcohol in this way.

THE IDENTIFICATION OF THE INDIVIDUAL COMPOUNDS

Pristan.

The fractions 1-2 of the chromatogram IV contain hydrocarbon which in respect of its refraction index n_{D}^{20} 1,4470 and its boiling point 167-70° (15 mm) corresponds with pristan (C_{18} H_{38}, iso-octadecan) (16).

Cholesterol.

The first benzene fractions of the individual chromatograms contain an entirely pure cholesterol which after a recrystallization, melts at 148 deg. This cholesterol with the cholesterol from gall-stones or with the cholesterol of the firm »Merck« does not give any depression of the melting point.

Selachyl alcohol,

After cholesterol the selachyl alcohol can be eluated by means of benzene or a mixture of benzene and ether. The iden-

tification is made according to the refraction index n_D^{20} 1,4698 and the saponif. number of acetyl-derivate which amounts to 261.

Batyl alcohol.

In all chromatograms the ether- fractions contain almost entirely pure batyl alcohol which after some recrystallizations from methanol and petrolether melts at 64,5 deg.

Di-(p-nitrobenzcate): 0,8172 gr of the substance are dissolved in 10 ccm pyridin and 1 gr p-nitrobenzoylchloride added to it. The mixture is heated on a water-bath for 3 hours, then dissolved in ether and washed with diluted sulphuric acid, water, sodium bicarbonate and again with water. After a redistillation of ether the reactive product is dried in the vacum up to its constant weight. 1,0182 gr of the substance are gained.

A further purification of the substance is made in the way of chromatograms. The matter is dissolved in 50 ccm of light benzene and filtrated over a little bar of aluminium oxide, standardized by Brockmann (Merck) 1,5x15 cm. Benzeneeluates contain pure di- (p-nitrobenzoate) which after a recrystallisation from alcohol melt at 6,5 deg and constains 4,45% N. The ether fractions contain an unchanged batyl alcohol wiht the melting point 64,5 deg.

Di-phenylurethane. 100 mgr of the substance are warmed for one hour at 80 deg with 65 mgr phenyl isocyanate. The reactive product is rezrystallized first from petrol-ether and then from alcohol up to its constant melting point 98,5 deg.

Chimyl alcohol.

From the metanolic fractions chimyl alcohol is gained with the melting point 61°, by the recrystallization in petrol-ether and alcohol.

Di-(p-nitrobenzoate). 0,7 gr of the substance are dissolved in 10 ccm pyridin by adding of 0,9 gr paranitrobenzoylchloride, warmed for 2 hours on a water-bath. A further purification is made in the same way as at the batyl alcohol-p-(nitrobenzcate). From the benzene eluates p-nitrobenzoate is gained by the recrystalization from alcohol. It has the melting point 59-60 deg.

ACKNOWLEDGMENT

A part of the chemical and physical properties of oils have been determined by Ing. M. Krvarić, the assistant of this Institute.

CONCLUSION

By the comparison of the physical and chemical properties of the different liver oils on the sample *Centrina Salviani* it is determined that there exist certain differences between females in the different stages of their sexual maturity. These differences are striking in the specific gravity and the percentage of unsap. matters. It was not possible to fix any differences on other constants.

The content of oil in fructified and unfructified eggs was investigated and their properties compared with liver oils. Great differences were determined in the specific gravity, refraction index as well as in the content of free, further volatile and in water soluble acids. During the period which passes from the fructification and the formation of the germ a great decrease of the percentage of unsap. matters, total fatty acids, free fatty acids, the saponif. number and the iodine number can be observed on the oil from eggs.

The chemical properties of fatty acids from the liver oil of the pregnant female and the female with undeveloped ovaries were researched and the results compared with each other. No essential differences were found. The fatty acids from oils of unfructified eggs do not differ much from the former either. The fatty acids from fructified eggs show an increase of the iodine number and a decrease of the neutralization number and the percentage of polybromide.

A success was attained in the separation of the individual components from the unsap. matters and their quantitative determination in the way of chromatographic adsorbtion. Only in one case a greater quantity of hydrocarbon was found, which is not squalene but probably pristan. It is difficult to fix a relation between the presence of the exceptional great quantity of hydrocarbon in the liver oil of the pregnant female and the development of gonads for at other pregnant females only an insignificant quantity of hydrocarbon was found.

It is further determined that the quantities of cholesterol, selachyl-, batyl and chimyl alcohol are verv variable. In respect of the content of cholesterol and chimyl alcohol in the unsap. yield there exists a difference between pregnant females and females with undeveloped ovaries.

The chemical composition of unap. matters in oils from eggs is similar to the composition of unsap. matters in liver oils and differs from it only by a much greater content of cholesterol.

Basing on these researches it can be concluded that the development of gonads is in a certain connection with the metabolism of the fat in the liver. Essential differences are determined in the composition of the fat during the period which passes from the formation of unfructified eggs till their fructification and the development of the germ in the eggs.

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ISTRAŽIVANJA O ULJIMA IZ JETARA *ELASMOBRANCHIA*

IV. Biokemijska studija o uljima iz jetara i jaja od *Centrina Salviani* Risso

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Kratak sadržaj

Kod uspoređivanja fizikalnih i kemijskih svojstava raznih jetrenih ulja vrste *Centrina Salviani* utvrđeno je da postoje izvjesne razlike između ženki u raznim stadijima spolne zrelosti. Te su razlike upadljive kod specifične težine i postotka neosapunjivih tvari. Kod ostalih konstanata nije bilo moguće utvrditi neke razlike.

Istražen je sadržaj ulja u neoplođenim i oplođenim jajima. Ekstrahirana ulja istražena su i njihova svojstva uspoređena sa jetrenim uljima. Ustanovljene su velike razlike u specifičnoj težini, indeksu refrakcije kao i u sadržaju slobodnih, te hlapivih i u vodi topivih kiselina. U vremenu koje je proteklo od oplodnje i stvaranja zametaka opaža se kod ulja iz jaja opadanje postotka neosapunjivih tvari, ukupnih masnih kiselina, slobodnih masnih kiselina, broja osapunjenja i jodnog broja.

Nadalje su istražena kemijska svojstva masnih kiselina iz jetrenog ulja bređe ženke i ženke sa nerazvijenim ovarijem te međusobno uspoređena. Nisu nađene nikakove bitne razlike, Masne kiseline iz ulja od neoplođenih jaja također se ne razlikuju mnogo od predašnjih. Kod masnih kiselina iz oplođenih jaja opaža se porast jodnog broja, a opadanje broja neutralizacije i postotka polibromida,

Uspjelo je odjeljivanje pojedinih sastojaka iz neosapunjivih tvari putem kromatografske adsorpcije i njihovo kvantitativno određivanje. Pri tome je samo u jednome slučaju nađena veća količina jednog ugljikovodika, koji nije skvalen, nego vjerojatno pristan. Prisustvo izuzetno velike količine ugljiko-

vodika u jetrenom ulju bređe ženke teško je dovesti u vezu sa razvikom gonada, budući da je kod drugih bređih ženki nađena samo neznatna količina ugljikovodika.

Nadalje je utvrđeno da su količine kolesterina, selahil-, batil- i kimil alkohola jako promjenljive. Postoji stanovita razlika između bređih ženki i ženki sa nerazvijenim ovarijem u pogledu sadržaja kolesterina i kimil alkohola u neosapunjivom ostatku.

Kemijski sastav neosapunjivih tvari kod ulja iz jaja je sličan sastavu neosapunjivih tvari kod jetrenih ulja, te se od ovih razlikuje samo mnogo većim sadržajem kolesterina.

Na temelju ovih istraživanja može se zaključiti da je razvitak gonada u izvjesnoj vezi sa metabolizmom masti u jetrima. U vremenu koje je proteklo od stvaranja neoplođenih jaja do oplodnje i razvitka zametaka u jajima ustanovljene su bitne razlike u sastavu masnoće.

БИОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЖИРОВ ИЗ ПЕЧЕНИ И ИЗ ЯИЦ *CENTRINA SALVIANI* RISSO

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Вывод

При сравнении физических и химических свойств различных жиров печени вида *Centrina Salviani* установлено существование известных различий между самками в разных стадиях половой зрелости. Эти различия особенно заметны при рассмотрении удельного веса и процента неомыляемых веществ. Между остальными константами не удалось установить каких либо различий.

Исследован состав жира в неоплодотворенных и в оплодотворенных яйцах. Извлеченные жиры были исследованы и их свойства сравнены со свойствами жиров печени. Были установлены большие разницы в удельном весе, в показателе рефракции, а также в содержании свободных, летучих и растворяющихся в воде кислот. В период времени следующий за оплодотворением и образованием эмбриона замечается в жире, получаемом из яиц, понижение процента неомыляемых веществ, общего количества жирных кислот, свободных жирных кислот, коэффициента омыления и коеффициента иода.

Далее исследованы химические свойства жирных кислот из жира печени беременной самки и самки с неразвитыми яичниками, а затем сравнены между собой. Существенной разницы найдено не было. От этих кислот также много не отличались и жирные кислоты добытые из жира неоплодотворенных яиц. В жирных кислотах полученных из оплодотворенных яиц замечается повышение коэффициента нейтрализации и процента полибромида.

При помощи хроматографической адсорбции удалось выделить некоторые составные части из неомыляемых веществ и сделать их количественный анализ. При этом, только в одном случае найдено значительное количество углевода но не сквалена, а вероятно пристана. Трудно предположить суще-

ствование связи между исключительно большим количеством углевода в жиру печени беременной самки и развитием гонад, ввиду того, что у других беременных самок найдено только незначительное количество углевода.

Далее установлено, что количество холестерина, селахил —, батил — и кимил алкоголя очень непостоянно. Между беременными самками и самками с неразвитыми яичниками существует значительная разница в отношении содержания холестерина и кимил-алкоголя в неомыляемом остатке.

Химический состав неомыляемых веществ в жиру извлеченном из яиц подобен составу неомыляемых веществ в жиру печени и отличается от них только гораздо большим содержанием холестерина. На основании этих исследований можно сделать вывод, что развитие гонад имеет определенную связь с метаболизмом жира в печени. В промежутке времени от образования неоплодотворенных яиц до оплодотворения и развития эмбриона, в яйцах установлены существенные различия в составе жира.

Tisak: Gradsko štamparsko poduzeće „Ante Jonić“ — Split