Chemistry and Biology of Lipids

X. Group Lipoid of Hog Liver

(First Paper)

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The procedure of Matuda¹⁾ for concentrating the group lipoids of human liver was applied to hog livers. Diverging from such tissues as the gastric mucosa²⁾ and lungs³⁾ from animals of the same species, the livers were found to contain neither Group A lipid nor Group B lipid. However, the products (final and intermediate) showed Group O specificity. The most sero-reactive specimen that had been obtained after the whole course of fractionations was investigated regarding its properties, physical and chemical. Electrophoresis at pH 7.8 of that specimen gave a pattern with two boundaries and trials to obtain a pure substance are now being in progress.

EXPERIMENTAL

Fractionation of Hot-alcohol Extractible Lipids of Hog Liver

The procedure whereby Sp. I was prepared (First Run) will be described.

A fresh hog liver weighing 1,687 g. was masticated and after washing with 5 l. of physiological saline (on a close linen cloth), covered under 5.4 volumes of 95% denatured alcohol containing 3.5% methanol for 5 days, followed by drying in a Faust apparatus. Of the material dried (370 g.), 270 g. were refluxed with 5 and 3.5 volumes of alcohol in succession, continuing each time for 3 hours. (The tissue residue was set aside for the ether-treatment below.) The hot extracts (dark red, transparent) were allowed to stand for 24 hours in air and filtered. The bulk on the funnel was washed with boiling alcohol and the hot washing was also stood for 24 hours, removed from the precipitate (2.4 g.) and put together with the filtrate above. The fluids combined were distilled, and the residual was dried (a dark brown, extremely viscid fluid, 30 g.) and then exhaustively washed with acetone. 2) The dark brown sticky solid

obtained was treated with four 200 cc. portions of ether. The insoluble part (a white powder) amounted to 4.6 g. 3) The soluble was recovered similar to above and dried and followingly fractioned by repeatedly washing with cold benzene (500 cc. in total). Only 20 mg. of it remained undissolved. 4) The benzene solutions (dark red, limpid) were again distilled and the residue dried (a deep brown clump, 10 g.).

On the other hand, the tissue residue after treatment with boiling alcohol above was subjected to continual ether extraction (with two 400 cc. portions of ether) and the ether extracts (yellowish, clear) were united and evaporated. And the remainder was further subdivided by the aid of acetone (two 40 cc. portions) and benzene similar to above. The distilled residue (a dark brown solid) of the benzene solutions amounted to 0.3 g.

5) The benzene-soluble portions (10.3 g. altogether) were agitated with 5 changes of petrol ether (in total 320 cc.). Almost all the substance went into solution (5 mg. was the insoluble part). 6) The remainder after evaporation of the solution (dark brown, fragile) was washed with portions of 400 cc. acetone. (The yellow washings left 0.3 g. of a light brown semifluid after distillation.) The insoluble part (light brown) amounting to 9.8 g. was divided by means of cold abs. alcohol, employing 800 cc. of the solvent in portions. The alcohol-insoluble part (a dark brown clump, 1.2 g.) was set aside (See 8) below). Again the alcoholic solutions were distilled together in vacuo. 7) On treatment of the residue (a brown fragile substance, 8.5 g.) with hot acetone (450 cc. in total), more than half of it dissolved. The insoluble part here (3.5 g.) was exhausted with cold benzene (300 cc. were used in all) and after rejecting a trace of insoluble substance, the extracts were condensed to a thick solution (about 10 cc.) and precipitated with 300 cc. of acetone—**Ppt. I**, 3.2 g. (This was not further fractioned).

8) The alcohol-insoluble part in 6) was well washed with acetone and thereafter refluxed with acetone repeatedly and when a renewed portion of this solvent remained unstained, the insoluble was dried. This was then precipitated from benzene (All the substance was soluble in benzene) with acetone in the way described in 7) and after washing with acetone (The washing remained colorless), dried—**Ppt. II** (a dark brown powder, 700 mg.). 9) Ppt. II was dissolved in 20 cc. of water, alkalized to pH 9.6 with 0.1 N NaOH and shaken with two changes of benzene, each equal in volume to the solution. The watery layer was separated, acidified to pH 1.2 with 50% sulfuric acid (Some precipitate was not separated off) and treated with benzene just as above. The benzene solutions here united (light brown, transparent; pH 2.4) were washed with an equal volume of water three times (The pH of the benzene solution

was raised to 6.2) and distilled under diminished pressure. 10) The product in 9) was now subjected to partition between 20 cc. of water at pH 2.0 and 20 cc. of chloroform and the chloroform solutions (Two chloroform portions were used) were washed with water, condensed to 5 cc. and precipitated with 10 volumes of acetone. The mother fluid had yellow tint. The deposit was washed with acetone thoroughly and dried. 180 mg. of a light brown powder were given—**Ppt. III** (Sp. I).

5 runs of the fractionations were carried out, but the other runs than the one, whose detailed description is given above, were stopped halfway.

Serological Potency of the Fractions

Anti-iso-hemoagglutinative force of the intermediate and final products (1.: the fraction that had precipitated in the cold from the hot-alcoholic extract of tissue, 2.: the ether-insoluble fraction of the hot-alcoholic tissue extracts which had been freed from 1. and washed with acetone, 3.: the part separated from 2. (ether soluble), 4.: the fraction that had dissolved in petrol ether, 5.: the substance after washing 4. with cold acetone, 6.: the substance obtained by distilling the washings of 4., 7.: Ppt. I, 8.: Ppt. II, 9.: Ppt. III) were assayed by our usual procedure. Products 1.–7. were found not to completely inhibit the isoagglutination of either of A and B red cells at dilutions of 1:25–800 and products 8.–9. at dilutions of 1:100–1600 (Table I).

Ppts. II's and III's were further examined if they have Group O-specificity. A goat anti-O immune serum⁴⁾ (titre 3000) was diluted to 1:10 and adsorbed by standing with 1/10 volume of Be erythrocytes for 20 minutes, followed by centrifugation. After the adsorption its O-agglutinin titre was reduced to 160 but it did not react with Be erythrocytes at all. The results are shown in Table II.

Physical and Chemical Properties of Ppt. I in First Run

Solubilities

See Table III.

Chemical tests

The product gave the positive Molisch reaction but the negative biuret, Osaki & Turumi (both direct and indirect) and Elson & Morgan reaction.

Composition

It analyzed: N (micro Kjeldahl) 2.84 %, P (Haneda, Sinokawa & Yasuoka's modification of Plimmer method) 3.17%, total carbohydrate as glucose (Masamune & Tanabe, First method) 1.24%, ash (micro analysis) 0.6%.

TABLE I

Anti-iso-hemoagglutinative Force of Fractions of the Hot-alcohol
Extractable Lipids from Hog Liver

A and B serum had agglutinin titre 160 and were diluted to 1:10.

±: faint agglutination, +: slight aggl., ++: marked aggl.,
###: complete aggl.

Fraction No.*	Group of erythroc.	Dilution of substances						
		×25	×50	×10 ²	×2 ×10 ²	×4 ×10 ²	×8 ×10 ²	×1.6 ×10 ³
1 (Run 1)	A	+	++	++	++	###	##	
	B	++	++	++	##	##	##	
(Run 2)	A	++	++	++	##	##	##	
	B	++	++	++	##	##	##	
2 (Run 1)	A	+	++	++	++	++		
	B	++	++	++	++	++		
(Run 5)	A	+	++	++	++	++		
	B	+	+	++	++	++		
3 (Run 2)	A	+	++	++	##	##	##	
	B	+	++	++	##	##	##	
(Run 3)	A	+	+	+	++	##	##	
	B	+	+	++	++	##	##	
(Run 5)	A	+	++	++	##	##	##	
	B	+	+	++	##	##	##	
4 (Run 2)	A	++	++	++	##	##	##	
	B	++	++	++	##	##	##	
5 (Run 2)	A	+	+	++	++	++	++	
	B	+	+	+	++	++	++	
6 (Run 2)	A	+	+	+	++	++	++	
	B	+	+	++	++	##	##	
7 (Run 1)	A	+	+	+	+	+	++	
	B	+	+	+	+	+	++	
8 (Run 1)	A			+	++	++	##	##
	B			+	++	++	++	##
(Run 2)	A			+	+	+	++	++
	B			+	+	+	++	++
(Run 3)	A			+	+	+	+	+
	B			+	++	++	++	##
(Run 4)	A			+	+	++	++	##
	B			+	+	++	++	##
9 (Run 1)	A			±	+	+	++	++
	B			+	+	++	++	##

* Consult the text.

TABLE II

Behaviour of Ppts. II's and III toward the Anti-O Goat Immune Serum Diluted and Treated with Be Erythrocytes

Regarding details see also the text.

—: no agglutination, +: slight aggl., ++: marked aggl.

Ppt.	Dilution of substances						
	×10	×20	×50	×10 ²	×2 ×10 ²	×4 ×10 ²	×8 ×10 ²
II (Run 2)	—	—	—	—	+	+	+
(Run 3)	—	+	+	+	+	++	++
(Run 4)	—	—	—	—	+	+	+
III (Run 1)	—	—	—	—	—	+	+

TABLE III

Solubilities of Ppt. III in First Run

—: insoluble, +: partly sol., ++: mostly sol., ##: completely sol.

Solvent (cold)	Plain water	Water (alkaline)	Methanol	Abs. alcohol	Acetone	Glacial acetic acid	Ether	Chloroform	Benzene	Tetralin	Petrol ether
Grade of dissolution	++	++*	—	—	—	+	—	##	##	++	—

* The mixture was alkalinized to pH 9.4.

Electrophoresis

Electrophoresis of its 0.7% solution at pH 7.8 showed two boundaries (Fig. 1).

Fig. 1. An electrophoresis pattern of a 0.7% solution of a preparation of the Group O lipid from hog liver in phosphate buffer, pH 7.8, $I=0.29$; 20°C ; mobility of the faster moving boundary $16.4\text{ cm}^2\text{ sec}^{-1}\text{ volt}^{-1}\cdot 10^5$. Ascending limb to the left.

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References and Notes

- 1) Matuda, Tohoku J. Exp. Med., 1951, **53**, 333.
 - 2) Readers are referred to Masamune and Siozima, *ibid.*, 1951, **54**, 319.
 - 3) Readers are referred to Masamune and Hirata, *ibid.*, this volume, p. 233.
 - 4) Kindly furnished by Dr. T. Murakami.
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