Chemistry and Biology of Lipids

XII. Group B Lipoid of Human Pancreas

First Paper

By

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The procedure employed in preparing the Group A lipoid of human pancreas¹⁾ was applied to the same organ of persons belonging to Group B. Here again, an electrophoretically homogeneous product was obtained, which proved identical with the Group A lipoid in so far as N, P and carbohydrate content and solubilities concern.

EXPERIMENTAL

In the successive fractions the group potency (anti-isohemoagglutinative) was measured to follow up the active factor as in the case of the Group A lipoid.

14 pancreases weighing from 40 to 105 g. (977 g. altogether) from individuals belonging to Group B were separately ground and dehydrated by standing with 4 volumes of denatured alcohol, followed by desiccation in a Faust apparatus. 127 g. of dry substance were thus given. It was Group B-active at a dilution of 1:2,000.

1st stage. The material was refluxed with two 650 cc. portions of denatured alcohol (99%), each time extending over 2 hours. The hot extracts (yellow-brown) were each quickly separated, while hot. They were combined and placed in an ice chest for 2 days, the occurring precipitate was filtered off and the filtrate was distilled in vacuo. The yellow-brown mucilaginous residue (5.7 g.; Group B-active at 1:50,000 dilution and Group A-active at 1:100) was washed with acetone and 3.0 g. of a yellowish, non-sticky, fine powder were given which was Group B-active at 1:100,000 and Group A-active at 1:100. It was fractioned with cold ether. The insoluble (2.5 g.) inhibited the isoagglutination of B erythrocytes even at a dilution of 1:200,000, and was further subdivided with cold chloroform. The portion remaining undissolved here (1.8 g., a yellow-brown, hygroscopic, hard clump) counteracted completely the

β -hemoagglutination at the same dilution as the ether-insoluble.

2nd stage. It was taken up in water and the solution was precipitated with lead acetate. The precipitate was suspended in water, bubbled with H_2S gas, and after addition of HCl to pH 2.0, centrifuged. The centrifugate was agitated with many changes of methanol, and the solutions distilled at low temperature to dryness. The still residue was again treated with methanol and the solution evaporated, washed with acetone and dried. The crop at this stage was 333 mg. It was perfectly inhibitory to the isoagglutination of **B** erythrocytes at a dilution of 1:1,000,000.

3rd stage (counter current distribution). Solvent pair: an equilibrated mixture of water, methanol, benzene and toluene (1:4:4:1 by volume). 10 separatory funnels were used and 320 mg. of the product in the foregoing stage were distributed between 150 cc. of the upper phase and an equal volume of the lower phase in Funnel 0, and then the centrifuged upper layer was transferred to a second funnel. After the last transfer the contents of all the funnels were also shaken well, and from all the phases a 0.5 cc. portion was pipetted out and evaporated. The remainders were separately suspended in 0.5 cc. of physiological saline for the assay of the inhibition of the isoagglutination of **B** erythrocytes, which resulted:

	Funnel number									
	0	1	2	3	4	5	6	7	8	9
Upper phase	++	++	++	+	+	+	+	-	-	-
Lower phase	##	##	++	++	++	++	++	++	++	-
Control without any of the substances	##									

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

The A serum had agglutinin titre 80 and was diluted fivefold.

The upper phase of Funnels 7, 8 and 9 in which most of the Group **B** factor had accumulated were distilled together in vacuo to dryness. The residue was taken up in water by slight warming (40—50°C, 5 minutes) and after rejecting a trace of an insoluble part, redistilled and washed with acetone. 120 mg. of a fine powder with yellowish hue were given.

Fig. 1. An electrophoresis pattern of a 3/4% solution (opalescent) of the Group **B** lipid preparation in phosphate buffer, pH 7.8, I 0.29; temperature 5°C; 10 ma. Exposure 50 minutes after starting current; mobility $8.6 \text{ cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1} \cdot 10^5$. Ascending limb to the left.

It inhibited perfectly the isoagglutination of B erythrocytes at a dilution of 1:100,000. Elson & Morgan (tested after acid hydrolysis) and Molisch reaction positive. Biuret, Goldschmiedt, Neuberg & Saneyoshi and Rosenthaler negative.

Electrophoresis. The pattern in Fig. 1 was obtained with its solution in phosphate buffer of pH 7.8, which demonstrates only one boundary.

Regarding the quantitative composition, see Table I. The figures of the present product coincide fairly well with those of the one from pancreases of Group A persons.

The solubilities are listed in Table II.

TABLE I
Composition of the Group B Lipoid-preparation (Human Pancreases)

Analysis	Per cent
N*	2.4
P†	2.8
Reduction on ferricyanide of the hydrolysate as glucose‡	18.4
Hexosamine§	4.8
Ash	5.7

* Micro Kjeldahl. † Haneda, Sinokawa and Yasuoka's modification of Plimmer method. ‡ Masamune and Tanabe method. The hydrolysate (filtrate) contained none precipitable by phosphotungstic acid, so that it was immediately neutralized. § Hamasato-Akakura method.²⁾ || Micro Pregl without use of H₂SO₄.

TABLE II
Solubilities of the Group B Lipoid-preparation (Human Pancreases)

—: insoluble, ±: faintly sol., +: partly sol., ++: mostly sol.

Solvent	Water		Methanol		Ethanol		Glacial acetic acid		Ether		Petrol ether		Benzene		Chloroform		Tetralin	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Solubility	+	* ++	±	+	±	±	—	±	—	—	—	—	+	† +	±	† ±	±	† ++

* The solution was opalescent. † The undissolved particles swelled up and took a jellylike state.

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References

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- 2) Hamasato and Akakura, J. Bioch., 1941, **34**, 159.