Blood Group Lipoids from Human Gastric Mucosa and Gastric Cancer.*

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This investigation was undertaken to compare, regarding the composition, not only A, B and O group lipid of the gastric mucosa with one another but also one of the corresponding lipoids of gastric cancer with them. The tissue lipids extractible with hot alcohol were fractioned in a similar manner as already reported,¹⁾ but the final stage by means of hot alcohol was not processed due to lack of material excepting when those from the mucosa of stomachs of Group A persons were dealt with. In this case, however, there was given no subdivision allusive to a pure state of the lipid. Despite of the probable crudeness, the preparations (The subdivisions of the Group A lipid by the final stage operation are excluded) of the different group lipoids gave nearly concordant figures: N 3.6–3.9%, P 2.6–2.8%, total carbohydrate as glucose 9.4–11.9%, ash 7.8–12.4% (3.9–4.3% N, 2.8–3.1% P and 10.3–13.1% total carbohydrate on an ash-free basis). The equivalent ratios of N to P, 2.9–3.1 : 1. On these analyses, the writer inclines to believe that the different blood group lipoids of gastric mucosa and gastric cancer resemble all one another or rather identical in so far as the composition concerns.

Of the lipoids from the normal mucosa, the Group A substance was anti-iso-hemoagglutinative at a dilution of $1:2.56 \times 10^4$ – 5.12×10^4 and the Group B substance at a dilution of $1:2.56 \times 10^4$, and the Group O substance inhibited the agglutination of O erythrocytes by an anti-O immune serum treated in advance with Be erythrocytes at a dilution of $1:1.28 \times 10^4$. The Group A substance from gastric cancer was specifically active at a dilution of $1:6.4 \times 10^3$. That the group lipid from gastric cancer is distinctly less active than the corresponding substance of the normal mucosa reminds the writer of the weak activity of the group lipid which

* 8th report of Masamune and co-workers' "Chemistry and Biology of Lipids."

was separated by Aikawa²⁾ from a metastasis of gastric cancer in a person belonging to Group B and had like contents of N (3.6%) and P (2.6%) (ash, 8.10%) as above. (The author applied indole, orcinol and diphenylamine methods immediately to his preparations for sugar determination, but the values obtained are much larger than the actual as Matuda³⁾ mentioned and, in addition, what they signify is obscure.)

Judging from the analyses of the group lipoids (concentrates) of erythrocytes (Hamamoto⁴⁾) and of liver (Matuda⁵⁾) as well as the finding above, the group lipoids involve N and P in a ratio characteristic to their source. Hence the N:P ratio of the group lipid in gastric cancer furnishes a proof that gastric cancer has a group lipid which is peculiar to its site and differ from the one in the normal mucosa only in the grade of differentiation. And, moreover, it is most plausibly assumed that Aikawa's lipid from the metastasis and the corresponding lipid in the original growth are identical just like the group carbohydrates of those primary and secondary tumors (Cf. Oh-Uti⁶⁾ and Matuda⁶⁾).

What kind of change in structure or molecular form or in some other respect accompanies the biological differentiation must be a very important problem awaiting solution.

EXPERIMENTAL.

1) The Group Lipoid from the Mucosa of Stomachs of Persons Belonging to Group A.

The mucosa torn from muscularis externa was freed mechanically from mucus and minced. 1254 g. of the fresh material thus obtained from 19 stomachs were dehydrated by keeping under 4 volumes of alcohol for many days. 224 g. was the material dried. It was boiled twice with abs. alcohol (5 and 3.5 volumes were used). The extracts were 1800 cc. altogether. After standing overnight the sticky deposits were filtered off and set aside—**Div. II** (1.5 g.). The filtrate (light brown and transparent) was distilled in vacuo and the semifluid residue—**Div. I**—was covered under 100 cc. of acetone for hours. The acetone-insoluble part was separated and after washing with changes of acetone until no colored matter came out, placed in a desiccator. 4.5 g. of a brownish clayey substance (hygroscopic) were yielded. From another batch of tissue material (481 g. in fresh state, 68 g. when dehydrated) was given 0.8 g. of the division corresponding to Div. II above—**Div. II'**—and 1.2 g. of the acetone-insoluble part of the division corresponding to Div. I above (**Div. I'**).

The balances of the acetone-insoluble parts of Divs. I and I', after serological and other assays, were united (total weight 5.5 g., anti-iso-hemoagglutinative at a dilution of 1:3200), exhaustively treated with cold abs. alcohol (Five 30 cc. portions of abs. alcohol were used; the final change

of alcohol remained uncolored). The extracts were distilled together to dryness and placed in a desiccator. Since the distilled remainder was found pretty sero-reactive (active at a dilution of 1:1600), complete separation from it of the part, which involves the group lipoid and is insoluble per se, was tried by repetitions of a similar procedure. All the residues, dried and put together, (3.6 g. in total; the sero-reactive dilution, 1:6400) were washed with acetone, dried and subjected to ether fractionation, namely, they were agitated with 36 cc. of dry ether and centrifuged and the supernatant fluid was distilled in vacuo. The centrifugate (1.7 g.) and the distilled residue here (1.0 g.) possessed nearly equal serological potencies (The dilutions where they were active were 1:12800 and 1:6400 respectively). However, on another treatment of the latter with dry ether, after thorough drying (in a vacuum desiccator for 3 days), the part that remained undissolved (0.3 g.) was found active even at a dilution of 1:32,000, whereas the one which had been recovered from the solution (0.7 g.) was active merely at a dilution of 1:1600. Followingly the both insoluble parts (2.0 g. in total) were refluxed with 20 cc. benzene on a water-bath again and again (five times). Each time the mixture was centrifuged while hot. The supernatant fluids were distilled in vacuo. The amorphous brown gelatine-like substance left (**Prep. I**, 1.2 g.) exhibited the specific anti-iso-hemoagglutinative potency up to 5.12×10^4 times dilution (s. Table I).

TABLE I.

Anti-iso-hemoagglutinative Potency of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

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

—: no agglutination, ±: faint aggl., +: slight aggl.,

++: marked aggl., #: strong aggl.

Group of erythroc.	Dilution of the substance											
	$\times \frac{1}{10^1}$	$\times 2 \times 10^2$	$\times 4 \times 10^2$	$\times 8 \times 10^2$	$\times 1.6 \times 10^3$	$\times 3.2 \times 10^3$	$\times 6.4 \times 10^3$	$\times 1.28 \times 10^4$	$\times 2.56 \times 10^4$	$\times 5.12 \times 10^4$	$\times 1.024 \times 10^5$	$\times 2.048 \times 10^5$
A	—	—	—	—	—	—	—	—	—	—	+	+
B	—	+	+	+	++	++	++	++	++	##	##	##

The final product had solubilities in Table II. Its analyses and optical rotation are shown in Table III.

The substance was considered a mixture when analogized from the finding on the corresponding product from pig gastric mucosa. Hence, a further fractionation by the aid of abs. alcohol was made, but no definite knowledge could be acquired as to the nature of the pure group lipoid.

TABLE II.

Solubilities of Prep. I from the Mucosa of Stomachs of
Persons Belonging to Group A.

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

Solvent	Water		Abs. alcohol		Acetone		Ether		Petrol ether		Benzene		Chloroform		Tetralin		Glacial acetic acid	
	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot	cold	hot
Solubility	≡	≡	—	+	—	—	—	—	—	—	≡	≡	≡	≡	—	≡	—	—

TABLE III.

Composition and Optical Rotation of Prep. I from the Mucosa
of Stomachs of Persons Belonging to Group A.

Analysis	In per cent	In equivalents per equivalent weight
N	3.9	3.1
P	2.8	1.0
Total carbohydrate as glucose*	11.9	
Ash†	8.9	

* Masamune and Tanabe procedure⁷. Since no phosphotungstic acid-precipitable substance was found in the filtrate after hydrolysis, this precipitant was not employed, neutralizing immediately the filtrate for the determination of the reducing force. † Micro analysis.

$$[\alpha]_D^{25} = \frac{+0.023 \times 100}{1.236 \times 1} = +1.9^\circ \text{ (in chloroform).}$$

The operation was effected as follows:—200 mg. of the substance were treated with hot abs. alcohol again and again (five times) by refluxing each time with a 15 cc. portion of the solvent for 20 minutes. The extracts were decanted out, while hot. The brown insoluble part that had adhered to the vessel wall was semifluid while hot, and solidified on cooling—**Fr. I** (30 mg.). The extracts were combined and stood in an ice-chest for one day, whereupon coarse flocculent precipitates (white) appeared. These precipitates were centrifuged and dried—**Fr. II** (a white powder weighing 80 mg.). The supernatant fluid was evaporated to about 10 cc. and again placed in an ice-chest. The occurring deposits were also separated after one day and dried—**Fr. III** (a white powder, 30 mg.)—and the mother liquid was distilled in vacuo to dryness—**Fr. IV** (a yellow powder, 35 mg.). The anti-iso-hemoagglutinative forces and analyses of the fractions are shown in Tables IV and V.

To Divs. II and II' united (2.3 g.) was adapted the above series of fractionation. a) At first they were washed well with 20 cc. portions of

TABLE IV.

Anti-iso-hemoagglutinative Potency of the Fractions of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

Fractions are named as in the text. The B serum used had agglutinin titre 320 and was diluted to 1:20.

—: no agglutination, $\pm$: faint aggl., +: slight aggl., ++: marked aggl.

Fraction	Group of erythrocytes	Dilution of substances								
		$\times 4 \times 10^2$	$\times 8 \times 10^2$	$\times 1.6 \times 10^3$	$\times 3.2 \times 10^3$	$\times 6.4 \times 10^3$	$\times 1.28 \times 10^4$	$\times 2.56 \times 10^4$	$\times 5.12 \times 10^4$	$\times 1.024 \times 10^5$
I	A	—	—	—	—	$\pm$	+	++	++	++
II	"	—	—	—	—	—	—	—	$\pm$	+
III	"	—	—	—	—	—	—	—	—	$\pm$
IV	"	—	—	—	—	$\pm$	+	++	++	++

TABLE V.

Composition of the Fractions of Prep. I from the Mucosa of Stomachs of Persons Belonging to Group A.

Fractions are named as in the text. Analyses are expressed as percentages.

Fraction	Analysis			
	N	P	Total carbohydrate as glucose*	Ash†
I	5.0	2.5	16.0	12.5
II	3.2	1.3	14.6	5.6
III	3.7	1.9	13.0	3.7
IV	4.4	3.6	8.3	9.6

* Calculated from the reducing force of the hydrolysate. Analysis was made as described in the Note of Table III. † Micro analysis.

dry acetone and dried. A hygroscopic brownish powder obtained was sero-reactive at a dilution of 1:8000, and amounted to 0.45 g. b) This was agitated twice with 20 cc. abs. alcohol, each time with following centrifugation. The insoluble part (a hygroscopic white powder) was 150 mg. The supernatant fluids (brownish and transparent) were distilled and the residual, washed with acetone (20 cc.) and dried, (a brownish stuff matter) was again treated with abs. alcohol as above. 100 mg. was the insoluble part here. The substance recovered from the solution (180 mg.) was non-active. c) The parts undissolved (sero-reactive at a dilution of 1:16000) were again washed with acetone and completely dried, and then washed with changes (three 30 cc. portions) of ether. Almost no loss of weight occurred, but the substance (a hygroscopic, yellowish, fine powder) became twice as active as the product in b). d) It was boiled with 20 cc. benzene

under return condenser for 20 minutes and centrifuged when cold, and the centrifugate was treated likewise once again. The extracts were distilled to dryness, 100 mg. of a gelatine-like substance with brown shade were given. This had the same potency as the material before the fractionation, notwithstanding that the benzene-insoluble part (a white powder amounting to about 100 mg.) was almost inactive (anti-iso-hemoagglutinative at a dilution of 1:400). The serological potency, analyses and solubilities of the final product (**Prep. II**) are illustrated in Tables VI, VII and VIII.

TABLE VI.

Anti-iso-hemoagglutinative Potency of Prep. II from the Mucosa of Stomachs of Persons Belonging to Group A.

B and A serum had agglutinin titres of 320 and of 160 and were diluted 20 and 10 times respectively.

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

Group of erythrocytes	Dilution of the substance										
	$\times 1$ $\times 10^2$	$\times 2$ $\times 10^2$	$\times 4$ $\times 10^2$	$\times 8$ $\times 10^2$	$\times 1.6$ $\times 10^3$	$\times 3.2$ $\times 10^3$	$\times 6.4$ $\times 10^3$	$\times 1.28$ $\times 10^4$	$\times 2.56$ $\times 10^4$	$\times 5.12$ $\times 10^4$	$\times 1.024$ $\times 10^5$
A	—	—	—	—	—	—	—	—	—	±	+
B	—	+	++	++	++	++	++	##	##	##	##

TABLE VII.

Composition of Prep. II from the Mucosa of Stomachs of Persons Belonging to Group A.

Analysis	In per cent	In equivalents per equivalent weight
N	3.6	3.1
P	2.6	1.0
Total carbohydrate as glucose*	11.0	
Ash†	7.8	

*, † Signify the same as in Tables III and V.

TABLE VIII.

Solubilities of Prep. II from the Mucosa of Stomachs of Persons Belonging to Group A.

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

Solvent	Water cold hot	Abs. alcohol cold hot	Acetone cold hot	Ether cold hot	Petrol ether cold hot	Benzene cold hot	Chloro- form cold hot	Tetralin cold hot	Glacial acetic acid cold hot
Solub- ility	++ ##	— +	— —	— —	— —	++ ##	++ ##	— ##	— ++

2) The Group Lipoid from the Mucosa of Stomachs
of Persons Belonging to Group B.

The fresh mucosa amounted to 839 g. (13 stomachs) altogether and the dehydrated to 156 g. The hot-alcoholic extracts were 1200 cc. in total.

The precipitates (**Div. II**, 100 mg.) from the tissue extracts when cold were washed with acetone. The substance after the washing (70 mg.; mostly soluble in water) exhibited anti-iso-agglutinative force towards B erythrocytes at a dilution of 1:6400. However, because of small quantity of the material, the group lipoid in it was not traced.

The remainder after distillation of the mother liquid of **Div. II**—**Div. I**—was treated with acetone as in 1), and 1.2 g. of a coarse brownish powder (hygroscopic) were given. Further fractionation was carried out also similar to above, and 0.65 g. of the cold-alcohol-insoluble substance (a yellowish powder), 0.42 g. of the ether-insoluble (a fine white powder) and 0.12 g. of the hot-benzene-soluble (a fine white powder) were obtained in turn. The final product inhibited the iso-agglutination of B erythrocytes at a dilution of $1:2.56 \times 10^4$ and that of A erythrocytes at a dilution of $1:4 \times 10^3$, and had the same solubilities as Prep. I of the Group A lipoid above. Analyses: N 3.6%; P 2.6%; total carbohydrate as glucose 9.4%; ash 8.3%. $[\alpha]_D^{25} = \frac{+0.021 \times 100}{1.023 \times 1} = +2.1^\circ$ (in chloroform).

3) The Group Lipoid from the Mucosa of Stomachs of Persons
Belonging to Group O.

In this case, the deposits from the hot-alcoholic tissue extracts when cold and the substance that remained in solution were fractioned without serological assay of the intermediate products. The final product from the former division exhibited remarkable reaction with an anti-O serum treated with Be erythrocytes, while that of the latter was entirely devoid of the activity.

The fresh mucosa, 927 g. (number of stomachs, 14). The tissue after dehydration, 174 g. The hot-alcoholic extracts altogether, 1260 cc. The deposits in the cold were separated and dried and 0.7 g. of a yellowish brittle clump was given—**Div. II**. From the distilled residue (a syrup) of the mother liquid—**Div. I**, 1 g. of the acetone-insoluble substance (a coarse brownish hygroscopic powder), 0.6 g. of the cold-abs. alcohol-insoluble, 0.45 g. of the dry-ether-insoluble and 0.35 g. of the hot-benzene-soluble (a light, brown powder) were obtained in turn. Yields of the products by successive subdivision of **Div. II** were: the acetone-insoluble (a coarse hygroscopic powder with brown shade), 0.5 g.; the cold-abs. alcohol-insoluble, 0.4 g.; the dry-ether-insoluble (a fine, very hygroscopic,

white powder), 0.35 g.; the hot-benzene-soluble (a brown gelatinous substance), 0.25 g.

The both final products showed solubilities similar not only mutually but also to the corresponding preparations of the Group A and B lipoids. Each of them was Group A-active at a dilution of 1:400 and Group B, active at a dilution of 1:200, but the substance produced from Div. II alone reacted with an anti-O serum [Increasing volumes (8, 10, 12, 14, 16 and 18 cc.) of 10% suspensions of O erythrocytes in physiological saline were injected at intervals of two days into V. jugularis communis of a goat, and after 1 week from the last injection, the blood was shed] adsorbed with Be erythrocytes at a dilution of $1:1.28 \times 10^4$ (s. Tables IX and X).

TABLE IX.

Agglutination of O and Be Erythrocytes by an Anti-O Serum
Treated with Be Erythrocytes.

Adsorption: To 3 cc. of the immune serum diluted 10 times was added 1 cc. of Be erythrocytes and after standing at room temp. (8°C) for 30 minutes, centrifuged.

—: no agglutination, $\pm$: faint aggl., +: slight aggl.

Group of erythrocytes	Dilution of the immune serum				
	$\times 10$	$\times 20$	$\times 40$	$\times 80$	$\times 160$
O	+	+	+	$\pm$	—
Be	$\pm$	—	—	—	—

TABLE X.

Agglutination of O Erythrocytes by an Adsorbed Anti-serum, which had been Treated in Advance with the Final Products from the Mucosa of Stomachs of Persons Belonging to Group O.

The serum diluted 10 times and treated with Be erythrocytes in Table IX was employed for the present assay.

—: no agglutination, $\pm$: faint aggl., +: slight aggl.

Division wherefrom the substance was obtained	Dilution of substances								
	$\times 1. \times 10^2$	$\times 2 \times 10^2$	$\times 4 \times 10^2$	$\times 8 \times 10^2$	$\times 1.6 \times 10^3$	$\times 3.2 \times 10^3$	$\times 6.4 \times 10^3$	$\times 1.28 \times 10^4$	$\times 2.56 \times 10^4$
I	$\pm$	+	+	+	+	+	+	+	+
II	—	—	—	—	—	—	—	—	+

The Group O-active product analyzed: N 3.6%; P 2.7%; total carbohydrate as glucose 9.4%; ash 11.3%. It had the rotation:

$$[\alpha]_D = \frac{+0.015 \times 100}{1.011 \times 1} = +1.5^\circ (\text{in chloroform}). \text{ Its solubilities resembled}$$

those of the corresponding product of the Group A lipid.

4) The Group Lipoid from Gastric Cancers of Persons
Belonging to Group A.

Cancer material: 632 g. (30 cancers) in a fresh state and 98.5 g. when dehydrated.

The deposits, that had come out from the hot-alcoholic tissue extracts when cold, were no more than 50 mg. and, in addition, their part insoluble in acetone (30 mg.) inhibited the iso-agglutination of A erythrocytes only at a dilution of 1:100. The transparent yellowish mother fluid was distilled in vacuo and the remainder treated with acetone as above. The insoluble part here amounted to 0.5 g. The cold-alcohol-insoluble substance separated therefrom was 0.36 g. and on fractioning with ether of its balance after serological assay (0.35 g.) remained 0.3 g. of a fine white powder undissolved. Of this 0.25 g. dissolved in hot benzene. The preparation finally obtained was a brownish brittle clump and exhibited the specific inhibitory power to iso-agglutination of A erythrocytes at a dilution of 1:6400. It had solubilities same as the corresponding preparations from normal gastric mucosa. N 3.6%, P 2.6%, total carbohydrate as glucose 10.9%, ash 12.4%. $[\alpha]_D^{25} = \frac{+0.040 \times 100}{1.028 \times 1} = +3.9^\circ$ (in chloroform).

SUMMARY.

- 1) It was tried to separate the blood group lipoids by the aid of organic solvents from human gastric mucosa and gastric cancers.
- 2) All of the preparations of the A, B and O group-lipoids had similar composition with respect to N-, P- and carbohydrate-content and similar solubilities.
- 3) They rotated D light to the right but slightly.
- 4) Group A lipid from gastric cancer was serologically much less active than the corresponding lipid from the normal mucosa.
- 5) An assumption was expressed that the liver metastasis of gastric cancer contains a group lipid same as the one in its original growth.

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