Biochemical Studies on Carbohydrates

CLXXXII. Chemical Nature of KIK Factors

First Report: KIK Factors in Cancerous Gastric Juice*

By

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In 1937, Kozawa, Iwatsuru and Kawaguchi²⁾ discovered that cancerous gastric juices give rise to anemia in rabbits when injected intravenously, and named it KIK reaction. Since then, Iwatsuru and co-workers³⁾⁴⁾ continued the study of the reaction and stressed on its significance in diagnosis of gastric cancer, but the chemical nature of the substance or substances inducing the reaction remains still unestablished.

The writers fractioned cancerous gastric fluids by means of trichloroacetic acid, acetone and ammonium sulfate in turn, taking KIK reaction as an indication, and obtained two active preparations ("KIK factor I" and "II") which were electrophoretically almost homogeneous. The corresponding fractions of non-cancerous gastric juices were also separated. After examination of them it was concluded that there are two anemia-inducing principles in gastric fluids of stomach cancer patients and that one of them (KIK factor I) is a mucopolysaccharide and the other (KIK factor II) a mucoprotein.

EXPERIMENTAL

Preparation Procedure

Gastric juices were obtained by Dr. O. Ogata in Prof. Muto's surgical clinic from patients of gastric cancer and of non-cancerous diseases according to Katsch, and those, which were not stained with much blood or bile, were separately filtered through several cotton ganses laid one over another and then sucked through a filter paper. The nearly clear filtrates were adjusted to pH 6.4-6.8 with NaOH or HCl, dialyzed against running water for 24 hours and distilled under reduced pressure to syrupy con-

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sistency, followed by drying in a vacuum desiccator over H_2SO_4 . 0.2–0.3 g. of a powder was given from the gastric juice drawn out at one time. It contained usually about 30% ash. The following description concerns a fractionation example each of the batch of the dry powders obtained as above from cancerous gastric fluids and that of the powders from the non-cancerous.

1) *Fractionation of the cancerous material.* First stage. 4.0 g. of the dry material from the cancerous gastric juices were taken up in 100 cc. of water. The insoluble part was centrifuged off and washed with water, and the supernatant and washing united (130 cc. in all; green colored and turbid) were added to with half the volume of 10% trichloroacetic acid under agitation. The yellowish, flocculent precipitate was centrifuged after standing for half an hour, washed with three 10 cc. portions of 3.3% trichloroacetic acid at first and afterwards with 30 cc. portions of acetone and dried. 170 mg. (4.3% of the dry material) of a greenish, gray powder were yielded.—**Ppt. I.**

Second stage. The mother fluid and the 3.3% trichloroacetic acid-washing of Ppt. I were combined. A yellowish green, turbid fluid—**Fl. I**—was given. To it 1.5 volumes of acetone were added in portions. The white, ropy centrifugate was washed with changes of 60% acetone and the washings were added to the supernatant. This fluid mixture will be referred to as **Fl. II**. The centrifugate was further washed with acetone and dried. The powder given weighed 750 mg. (18.8% of the material)—**Ppt. II**. To Fl. II was added additional acetone to 80%. The flocculent precipitate here was separated in a centrifuge, washed with 80% and dry acetone and dried. 155 mg. (3.9% of the material) was the yellowish powder obtained—**Ppt. III**. The greenish, transparent mother fluid of Ppt. III was distilled under reduced pressure at 5–10°C to about 1/6 the volume in order to expel off most of the acetone, and the green precipitate that occurred hereby (36 mg.) was discarded. The clear fluid was dialyzed for 24 hours until the pH was elevated to 6.6 to remove most of trichloroacetic acid and distilled to 65 cc. To the yellowish-greenish condensate, which was of pH 6.4, were added 3 volumes of acetone to 75%. And the yellowish flocculent precipitate was centrifuged, washed with acetone and dried. The powder given weighed 250 mg. (6.3% of the material)—**Ppt. IV**. The yellowish green, clear supernatant was added to further with as much acetone as to increase the acetone:water ratio to 10:1 (Acetone concentration became 91%), whereby a white fine flocculent substance was carried down. After standing for 1 hour, the precipitate was centrifuged, washed with three 30 cc. portions of acetone and with a 30 cc. portion of ether in sequence and dried. 534 mg. (13.4% of the material) was the yellowish powder yielded. It contained 23% ash—**Ppt. V**. The mother

fluid of Ppt. V was distilled *in vacuo* to syrupy consistency, precipitated with 50 cc. of abs. ethanol containing sodium acetate and dried after washing with ethanol and ether. A yellow powder amounting to 113 mg. (2.8% of the material) was given—**Ppt. VI.**

Third stage. Next, 500 mg. of Ppt. V were dissolved in 25 cc. of water (pH of the solution 6.4) and the yellow clear solution was saturated with ammonium sulfate by stirring up at 30°C with 18.6 g. of the salt. pH was lowered to 5.6. The flocculent precipitate was separated on a filter after 4 hours and then dissolved in water to reprecipitate by saturation with the same salt. Redissolution and reprecipitation was carried out once more.

a) The ammonium sulfate-saturated filtrates were united, diluted with 3 volumes of water and dialyzed against running water for two days, followed by vacuum distillation to 40 cc. The condensate was subjected to three repetitions of alternative shaking with a 10 g. (wet weight) of Amberlite IR-120 and with a 14 g. (wet weight) of Amberlite IRA-400, transferring by decantation from one vessel to the other, and after ascertaining no more occurrence of pH change with a new portion of those resins, filtered by means of a jet pump. The filtrate was free from NH_4^- and SO_4^- ions. The resins employed were washed in the order described with three 30 cc. portions of water. The filtrate and washings were distilled together *in vacuo* to a syrup, and precipitated with 50 cc. of ethanol and a few drops of ethanol saturated with sodium acetate. The deposit was washed with ethanol and ether and dried. An uncolored hygroscopic product was given—**Ppt. VII ("KIK factor I")**. Yield 60 mg. (1.6% of the dry material started with).

b) The precipitate by $(\text{NH}_4)_2\text{SO}_4$ was dissolved in 50 cc. of water and dialyzed against running water for two days, then the solution was distilled *in vacuo* to 30 cc. (pH of the condensate 6.4) and treated with the cation- and anion-exchange resins as above, followed by further condensation, precipitation and so on also similar to above. 120 mg. of a yellow powder were obtained—**Ppt. VIII ("KIK factor II")**. The crop is 3.2% of the dry starting material.

2) 5.2 g. of a dry green material from stomach juices of non-cancerous patients were fractioned just as in 1). Its fractions amounted as embodied on next page (The numbers of Ppts. signify the fractions corresponding to those of the same numbers in 1)). Figures in parentheses are the yields in percentages of the fractions above of the cancerous material.

Namely, as compared with the corresponding fractions of the cancerous material, the trichloroacetic acid-precipitate (Ppt. I) and particularly the fractions (Ppts. VII and VIII) corresponding to "KIK factors" were less and Ppt. II, the fraction richest in the group substances, was more in yield.

“ Ppts.” of the Non-cancerous Material (a 5.2 g. lot)

Ppt.	Yield	
	g.	in per cent of the dry material
I	0.12	2.3 (4.3)
II	1.51	29.0 (18.8)
III	0.51	9.8 (3.9)
IV	0.39	7.5 (6.3)
V	0.46	8.8 (13.4)
VI	0.071	1.4 (2.8)
VII	0.012	0.24 (1.6)
VIII	0.044	0.89 (3.2)

Properties of “ KIK Factors ” and the Corresponding
Preparations from Non-cancerous Juices

The same fractions by several runs were mixed together to investigate their properties.

Animal experiment

The anemia-inducing effect was assayed by following Iwatsuru and Kato⁴⁾ in principle.

Various amounts of the fractions were dissolved in physiological saline and injected into the auricular vein of rabbits weighing 2–3 kg. The erythrocytes number of the venous blood was counted before and at intervals of 1 hour during 5 hours after the injection to determine the minimum dose per kg. body-weight, which caused 10% decrease of erythrocytes within that duration of time. In Table I are summarized the results on not only the final products but also the intermediate. The most part of this assay was carried out by Dr. O. Ogata, who will publish the results in detail elsewhere by himself.⁵⁾

Electrophoretic examination

The substance from non-cancerous gastric juices, which corresponded to “ KIK factor I ”, was not examined because of shortage of material. Judging from their electrophoretic patterns (Fig. 1), the remaining substances looked nearly homogeneous in the present aspect, and on the other hand, “ KIK factor II ” and the corresponding fraction of the non-cancerous gastric juices did not coincide perfectly with each other as for the mobility.

Blood group potency

The hemagglutination inhibition test was processed by the procedure described by Masamune *et al.*⁶⁾ on a glass plate and the results are embodied in Table II. The faint A and B potencies of the preparations are most

TABLE I
Anemia-inducing Effect in Rabbits of Fractions of
Cancerous and Non-cancerous Gastric Juices

Gastric juice	Fraction (Ppt.)	mg. substance injected per kg. bodyweight	Number of examinations	Decrease (%) of erythrocytes number*
Cancerous	I	1.0	3	10.2
	II	1.0	3	6.4
	III	1.0	3	3.8
	IV	1.0	5	14.2
	V	0.4	4	14.8
	VI	1.0	3	5.2
	VII ("KIK factor I")	0.4	2	17.5
		0.1	2	17.2
		0.05	5	15.2
		0.03	3	12.0
	VIII ("KIK factor II")	0.5	3	16.5
		0.3	3	13.4
Non-cancerous	I	1.0	3	3.5
	II	1.0	3	7.7
	III	1.0	3	8.5
	IV	1.0	3	6.3
	VI	1.0	3	3.7
	VII	1.0	3	2.4
		0.5	2	5.5
	VIII	1.0	3	5.0
		0.5	2	2.3

* Average decrease.

probably due to minute contamination by the blood group substances, most of which accumulated in Ppts. I-IV.

Solubility

Each pair of the substances from cancerous and non-cancerous gastric juices showed no discrepancy of solubility.

a) "KIK factor I" and the corresponding fraction of non-cancerous gastric juices. They were readily soluble in water of any pH value and pretty soluble in the saturated ammonium sulfate and the saturated sodium sulfate (33°C), but insoluble in glacial acetic acid, ethanol and ether. The watery solution was not precipitated by lead acetate, cupric chloride, barium acetate, baryta and trichloroacetic acid.

b) "KIK factor II" and the corresponding fraction of non-cancerous gastric juices. Insoluble entirely in the saturated ammonium sulfate, ethanol, acetone and ether and partly in the saturated sodium sulfate at 33°C. Soluble in glacial acetic acid and water of pH 1.0-9.6. Not precipitated from water by cupric chloride, barium acetate, baryta and trichloroacetic acid, but precipitated in part by lead acetate.

Fig. 1. Electrophoretic patterns of 1% solutions of "KIK factor I" and "II" and the mucoprotein from non-cancerous gastric juices corresponding to "KIK factor II" in 0.1 *M* veronal-HCl buffer of pH 8.6 and *I* 0.2 (adjusted with NaCl) (A, B and C respectively) and in 0.2 *M* acetate buffer of pH 4.6 and *I* 0.2 (A', B' and C' respectively). Temperature 15°C; current 10 mA. Exposed 60 or 40 minutes after starting current (see below).

Mobility:

$$\text{"KIK factor I" at pH 8.6, } \frac{(-0.2) \times 0.012029 \times 0.3}{3600 \times 0.01} = -2.0 \times 10^{-5} \text{ cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1}$$

at pH 4.6, 0.0 (Exposure 40 minutes after starting current)

$$\text{"KIK factor II" at pH 8.6, } \frac{(-0.5) \times 0.012029 \times 0.3}{3600 \times 0.01} = -5.0 \times 10^{-5} \text{ cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1}$$

at pH 4.6, 0.0 (Exposure 40 minutes after starting current)

$$\text{The non-canc. mucoprotein corresponding to "KIK factor II" at pH 8.6, } \frac{(-0.6) \times 0.011548 \times 0.3}{2400 \times 0.01} = -8.7 \times 10^{-5} \text{ cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1}$$

$$\text{at pH 4.6, } \frac{(-0.1) \times 0.006165 \times 0.3}{2400 \times 0.01} = -0.8 \times 10^{-5} \text{ cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1}$$

(The same distance was travelled by the ascending boundary as by the descending in any of the cases.)

Test-tube tests

Each pair of the substances from cancerous and non-cancerous gastric juices behaved similar to each other toward test reagents excepting that "KIK factor II" showed the positive Millon reaction, though faint, contrary to the corresponding substance from non-cancerous gastric juices which gave the negative reaction. The results will be embodied in a table.

TABLE II

Group Potencies of "KIK Factors" and of the Corresponding Substances from Non-cancerous Gastric Juices

The sera (α and β sera, a normal anti-O goat serum and an anti-OS saliva chicken serum of respective titres 480, 480, 160 and 1280) were diluted to titre 16 for the assay. —: no hemagglutination, $\pm$: faint hemaggl., +: slight hemaggl., ++: marked hemaggl., $\equiv$: strong hemaggl.

Substance		Red cells	Dilution of the substances (1:)								
Gastric juice	Fraction		Serum	10 ²	2×10 ²	2 ² ×10 ²	2 ³ ×10 ²	2 ⁴ ×10 ²	2 ⁵ ×10 ²	2 ⁶ ×10 ²	2 ⁷ ×10 ²
Cancerous	“KIK factor I”	A	α	—	+	+	++	++	++	++	++
		B	β	—	+	+	++	++	++	++	++
		O	Goat-	+	++	++	++	++	++	++	++
		O	Anti-OS saliva	+	+	++	++	++	++	++	++
	“KIK factor II”	A	α	—	+	+	++	++	++	++	++
		B	β	—	+	+	++	++	++	++	++
		O	Goat-	+	++	++	++	++	++	++	++
		O	Anti-OS saliva	+	+	++	++	++	++	++	++
Non-cancerous	Ppt. VII	A	α	—	+	+	++	++	++	++	++
		B	β	+	++	++	++	++	++	++	++
		O	Goat-	++	++	++	++	++	++	++	++
		O	Anti-OS saliva	+	+	++	++	++	++	++	++
	Ppt. VIII	A	α	—	+	++	++	++	++	++	++
		B	β	++	++	++	++	++	++	++	++
		O	Goat-	+	++	++	++	++	++	++	++
		O	Anti-OS saliva	+	+	++	++	++	++	++	++

Substance	Test			
	Molisch	Osaki-Turumi Indirect	Neuberg-Saneyoshi Direct	Lugol (for glycogen)
"KIK factor I"	++	+	+	—
Ppt. VII of non-canc. gastric juices	++	+~++	+	—
"KIK factor II"	++	+	+	—
Ppt. VIII of non-canc. gastric juices	++	+	+	—

Substance	Test				
	Biuret	Millon	Phosphorus	Bial	Dische
"KIK factor I"	$\pm$	—	—	+	—
Ppt. VII of non-canc. gastric juices	$\pm$	—	—	+	—
"KIK factor II"	++ (red violet)	$\pm$	—	+	—
Ppt. VIII of non-canc. gastric juices	++ (red violet)		—	+	

Paper partition chromatography of the sugar and amino acid components

Sugar chromatography. i) Hydrolysis with hydrochloric acid and de-

scending chromatography was made at first according to the principle of Masamune *et al.*⁶⁾ Amounts taken of "KIK factor I" and the corresponding preparation from non-cancerous gastric juices were both 6 mg. and those of "KIK factor II" and the corresponding preparation from non-cancerous gastric juices were 11 and 10 mg. respectively. The hydrolysates were freed from humin and almost all of hydrochloric acid, and the dried up residues were dissolved each in 0.1 cc. of water (pH of the solutions here 3-5). A 0.04 cc. portion of the solutions were sampled excepting the case of the mucoprotein from non-cancerous gastric juices where 0.06 cc. was applied. The paper slivers (Toyo Roshi filter paper no. 2) used were 12×60 cm. and spotted with the solutions 10 cm. apart from the upper margin. Solvent for irrigation: butanol-pyridine-water (5:3:2 by volume). Indicator aniline hydrogen phthalate.

As shown in Fig. 2A, all the four preparations gave spots at the positions corresponding to chondrosamine, glucosamine, galactose, glucose, mannose and L-fucose and/or xylose in the order downward from above. The lowest spots had some red shade suggesting overlapping of the two sugars L-fucose and xylose.

ii) Next, 6.5 mg. of "KIK factor I" were hydrolyzed with 0.7 cc. of 1 *N* H₂SO₄ in a sealed tube at 100°C for 5 hours, and when cold, added to with the sat. Ba(OH)₂ to pH 5.8. The centrifuged supernatant and hot washings of the centrifugate were combined and evaporated in vacuo over CaCl₂ to dryness, and the remainder was dissolved in 0.06 cc. of water. Of the solution, 0.03 cc. was sampled on a paper sliver (5×60 cm.), which was then irrigated (also in the descending way) with a mixture of *n*-butylacetate, acetic acid, ethanol and water in the proportions of 3:2:1:1 by volume. The chromatogram obtained (Fig. 2B) indicated a spot of xylose, though weak, besides the ones of hexosamine, glucose, mannose and L-fucose.

Amino acid chromatography. It was carried out in a usual manner. 6 mg. each of "KIK factor I", the corresponding fraction from non-cancerous gastric juices and "KIK factor II" and 5 mg. of the fraction from non-cancerous gastric juices corresponding to "KIK factor II" were hydrolyzed with 0.6 cc. (in the cases of the first three substances) or 0.5 cc. (in the case of the last one substance) of 6 *N* HCl in a sealed tube at 100°C for 24 hours. The humin was filtered off and washed. The filtrate and washings were evaporated together in vacuo over solid caustic soda to dryness and then liberated from HCl as far as possible by repeated evaporations with water. The final dry residue was taken up in 0.1 cc. of water. 0.02 cc. (in the cases of "KIK factors" and the substance from non-cancerous gastric juices corresponding to "KIK factor II") or 0.025 cc. (in the case of the remaining preparation) of the solutions

Fig. 2. Sugar paper partition chromatograms of "KIK factor I" and "II" and the corresponding substances from non-cancerous gastric juices developed by irrigation with butanol-pyridine-water at $23^{\circ}\pm 1^{\circ}\text{C}$ for 22 hours (A) and that of "KIK factor I" by irrigation with *n*-butylacetate-acetic acid-ethanol-water (B) at $25^{\circ}\pm 1^{\circ}\text{C}$ for 24 hours. Irrigation descending in the both cases. Indicator aniline hydrogen phthalate of Partridge. Reference runs were effected with a 0.02 cc. portion of 1% solutions with respect to individual sugars.

a: "KIK factor I", b: "KIK factor II", c: the mucopolysaccharide from non-cancerous gastric juices corresponding to "KIK factor I", d: the mucoprotein from non-cancerous gastric juices corresponding to "KIK factor II", e: reference run. 1: chondrosamine hydrochloride, 2: glucosamine hydrochloride, 3: galactose, 4: glucose, 5: mannose, 6: xylose, 7: L-fucose.

was sampled on a square 30×30 cm. of Toyo Roshi filter paper no. 2 for two-dimensional chromatography. Solvents for irrigation were butanol-acetic acid-water (4:1:2 by volume) for the primary run and 80% phenol (Ir-

rigation effected in an NH_3 -containing atmosphere) for the secondary run. Indicator ninhydrin. As are shown in Fig. 3 (A, B, C, D), the four sub-

Fig. 3. Amino acid paper partition chromatograms of "KIK factor I" (A) and "II" (B) and Ppts. VII (C) and VIII (D) of the non-cancerous gastric juices. Temperature of irrigation $22^{\circ}\pm 1^{\circ}\text{C}$ for the first two substances and $25^{\circ}\pm 1^{\circ}\text{C}$ for the last two.

1: aspartic acid, 2: glutamic acid, 3: cysteine, 4: serine, 5: glycine, 6: hexosamines, 7: lysine, 8: histidine, 9: arginine, 10: threonine, 11: alanine, 12: proline, 13: tyrosine, 14: valine and/or methionine, 15: phenylalanine, 16: leucine and/or isoleucine, 16': leucine and/or isoleucine and/or phenylalanine, 17: a peptide, 18: an unknown ninhydrin-positive component.

stances contained amino acids enumerated below besides those common to all: aspartic and glutamic acid, serine, glycine, arginine, alanine, proline, valine and/or methionine, leucine and/or isoleucine and an unknown ninhydrin-positive component.

“KIK factor I”: phenylalanine, a peptide

“KIK factor II”: cysteine, histidine, tyrosine, phenylalanine, a peptide

The substance from non-cancerous gastric juices corresponding to “KIK factor I”: lysine, histidine, phenylalanine (?)

The substance from non-cancerous gastric juices corresponding to “KIK factor II”: lysine, histidine, threonine, phenylalanine (?), a peptide

Quantitative composition

As shown in Table III, “KIK factor I” was less in L-fucose and sialic acid contents than the corresponding fraction of the non-cancerous gastric juices, and “KIK factor II” and the corresponding fraction of the non-cancerous gastric juices showed marked differences of analyses between themselves not only in percentages but also in equivalent ratios of the sugar components including sialic acid, although the kinds of sugar constituting the carbohydrate moiety of the four substances were the same.

Optical rotation in water

$$\text{“KIK factor I”}: [\alpha]_D^{25} = \frac{-0.64 \times 1}{0.0101 \times 1} = -63.4^\circ \text{ (pH of the sol. 7.4)}$$

$$\text{“KIK factor II”}: [\alpha]_D^{25} = \frac{-0.77 \times 1}{0.0050 \times 1} = -153.4^\circ \text{ (pH of the sol. 6.8)}$$

$$\begin{array}{l} \text{The non-canc.} \\ \text{specimen correspond-} \\ \text{ing to “KIK factor} \\ \text{I”}: \end{array} [\alpha]_D^{25} = \frac{-0.302 \times 1}{0.0110 \times 1} = -27.4^\circ \text{ (pH of the sol. 7.0)}$$

$$\begin{array}{l} \text{The non-canc. speci-} \\ \text{men corresponding to} \\ \text{“KIK factor II”}: \end{array} [\alpha]_D^{25} = \frac{-0.725 \times 1}{0.0060 \times 1} = -120.8^\circ \text{ (pH of the sol. 7.0)}$$

The substances from cancerous gastric juices were much more levorotatory than the respective corresponding substances from non-cancerous gastric juices.

DISCUSSION

Needless to say, there remains a non-cancerous part of mucosa, whether large or small, in cancerous stomachs. Therefore “KIK factors” (preparations) are possible to have been mixtures of a KIK factor (pure) and the corresponding normal secretion, although “KIK factor II” proved pretty homogeneous electrophoretically probably due to the slight amount

TABLE

Analyses of "KIK Factors" and of the Corresponding

Analysis	In pee cent			
	Canc. gastr. juice		Non-canc. gastr. juice	
	"KIK factor I"	"KIK factor II"	Ppt. VII	Ppt. VIII
N*	6.4	11.2	6.1	13.4
Total hexosamines as glucosamine†	20.0	5.7	17.8	4.6
Galactose‡	6.3	2.9	5.4	1.3
Mannose‡	18.0	3.5	16.0	1.8
Glucose‡	15.2	1.9	15.8	1.2
L-Fucose§	2.5	1.1	5.6	1.4
Xylose//	1.8	0.9	1.5	0.5
Sialic acid¶	1.7	2.6	4.5	3.4
Ash**	3.8	1.7	6.1	4.1

* Micro Kjeldahl. † Total hexosamines were estimated without antecedent separation & Sakamoto.⁹⁾ § A modification of Dische & Shettles.⁹⁾ // Masamune & Sakamoto.¹⁰⁾ use of H₂SO₄.

of the contaminating non-cancerous substance with somewhat different mobility. In other words, KIK factors (pure) must be assumed to rather show more discrepancy in various properties from the respective corresponding substances of non-cancerous gastric juice than "KIK factors" (preparations). Judging from the analytical figures above, KIK factor I (pure) and the corresponding non-cancerous substance are mucopolysaccharides and KIK factor II (pure) and the corresponding non-cancerous substance belong to the class of glucidamins in Masamune's terminology.

SUMMARY

1. Two anemia-inducing substances ("KIK factor I" and "II") were prepared from gastric juices of stomach cancer patients. The corresponding substances were also separated from gastric juices of non-cancerous patients for comparison.

2. "KIK factor I" is assumed as a mixture of two mucopolysaccharides KIK factor I (pure) and the corresponding mucopolysaccharide secreted by the normal part of the gastric mucosa, and "KIK factor II" a mixture of two glucidamins KIK factor II (pure) and the corresponding mucoprotein secreted by the normal part of the gastric mucosa.

3. "KIK factor I" was much more effective than "KIK factor II" in causing anemia.

4. The substances from non-cancerous gastric juices were yielded

III

Substances from Non-cancerous Gastric Juices

In equivalents per equivalent weight			
Canc. gastr. juice		Non-canc. gastr. juice	
"KIK factor I "	"KIK factor II "	Ppt. VII	Ppt. VIII
4.10	25.15	4.39	37.33
1.00	1.00	1.00	1.00
0.31	0.51	0.30	0.28
0.90	0.61	0.89	0.39
0.76	0.33	0.88	0.26
0.14	0.21	0.34	0.33
0.11	0.19	0.10	0.13
0.05	0.26	0.15	0.43

tion of the both hexosamines by a minor modification of Blix method.⁷⁾ ‡ Masamune
 † Werner & Odin (Direct Ehrlich)¹¹⁾; Calculated as $C_{11}H_{19}NO_9$.¹²⁾ ** Pregl without

much less, and were also less in anemia-inducing power or entirely devoid of this power. Furthermore, they diverged from "KIK factors" in quantitative composition and optical rotation.

5. "KIK factors" and the corresponding preparations from non-cancerous gastric juices possessed almost no blood group potencies.

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H. Masamune

References and Notes

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- 7) Masamune & Yosizawa, *ibid.*, in press.
- 8) Masamune & Sakamoto, *ibid.*, this volume, p. 345.
- 9) L-Fucose was kindly analyzed by Dr. M. Sakamoto by Dische and Shettles' procedure

(J. Biol. Chem., 1948, **175**, 595) with modifications as follows.

0.8 cc. (instead of 1 cc.) of a solution of the unknown is taken in a test-tube, and after addition of 3 cc. (instead of 4.5 cc.) of the 6:1 $\text{H}_2\text{SO}_4\text{-H}_2\text{O}$ mixture by volume, heated in a boiling water-bath for 10 minutes, followed by immediate chilling in ice water. The mixture was then further mixed with 0.1 cc. of a 3% cysteine hydrochloride solution, shaken and maintained at 25°C (water-thermostat) for 1.5 hours to measure extinctions at the wave-lengths of 400 and 430 $\text{m}\mu$ (not at those of 396 and 430 $\text{m}\mu$) within subsequent 1 hour. By these minor modifications the reaction could be distinctly characterized for methylpentoses, namely, $E_{400}\text{-}E_{430}$ values for even 10 mg% solutions of hexoses, hexuronic acids and acetylhexosamines became neglectable while larger extinction increments are utilized for methylpentose calculation, for instance, $[E_{400}\text{-}E_{430}]_{0.0025\%}^{1\text{cm}}$ for L-fucose amounts as much as 0.656.

10) Masamune & Sakamoto, Tohoku J. Exp. Med., this volume, p. 357.

11) Werner & Odin, Acta soc. med. Upsal., 1952, **57**, 230.

12) Cf. Blix, Lindberg, Odin & Werner, Nature, 1955, **175**, 340.

Note at proof-reading: Ogata's article (5)) has been published in Tohoku Igaku Zasshi (Jap.), 1955, **52**, 545.