Chemical Nature of Toxohormone (Nakahara)*

Second Report

The KIK Factor (V) from Cancerous Urine and the Corresponding Component of Normal Urine Act as "Toxohormones"

By

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This KIK factor was isolated from united urines of stomach-, liver-, lung- and mamma-cancer patients (49, 17, 8 and 18 l., respectively) by the procedure of Kaketa¹⁾. Yielded was 55 mg. It was examined electrophoretically in a Tiselius apparatus (Type HTD-1, Hitachi) and ultracentrifugally²⁾ in a Spinco ultracentrifuge Model E, proving homogeneous or essentially homogeneous as shown in Fig. 1 and 2. N (micro Kjeldahl) 11.9%; hexosamine (Masamune and Yosizawa³⁾) 5.9%; ash (Pregl method without use of H₂SO₄) 3.1%. The anemia-inducing potency as measured according to Masamune and Kawasaki⁴⁾ is detailed in Table I.

Fig. 1. Electrophoresis patterns of 1% solutions of the preparation from the united cancerous urines; current 4 mA; temperature 20°C. (a) Phosphate buffer, pH 7.8, I=0.2; exposure 120 min. after starting current. (b) Acetate buffer pH 4.6, I=0.2; exposure 60 min. after starting current. Mobility:

$$(a) \frac{(-1.92) \times 0.0092 \times 0.18}{7200 \times 0.004} = -11.1 \times 10^{-5} \text{ cm}^2/\text{volt/sec}$$

$$(b) \frac{(-0.4) \times 0.0067 \times 0.18}{3600 \times 0.004} = -3.4 \times 10^{-5} \text{ "}$$

As the component of normal urine corresponding to that KIK factor, the balance of the preparation informed of in the Kaketa's article¹⁾ was ap-

* 212th report of Masamune and co-workers' "Biochemical Studies on Carbohydrates".

Fig. 2. Sedimentation diagrams of 1% solutions of the preparation from the cancerous urines (**I**) and that from normal urine (**II**) in phosphate buffer, 7.8, $I=0.2$ (*a*) and acetate buffer, pH 4.6, $I=0.2$ (*b*). (**Ia**) 110 min. after reaching full speed 57,792 rev./min.; rotor temperature 13°C; bar angle 65°. (**Ib**) 110 min. after reaching full speed 57,798 rev./min.; rotor temperature 13°C; bar angle 55°. (**IIa**) 60 min. after reaching full speed 59,946 rev./min.; rotor temperature 9.6°C; bar angle 70°. (**IIb**) 64 min. after reaching full speed 59,800 rev./min.; rotor temperature 9.8°C; bar angle 64°.

TABLE I
Anemia-inducing Potency of the KIK Factor Preparation (I)

Assay No.	Rabbit			Dosis per kg. body wt. (mg.)	Number of red cells						Maximum decrease(%)
	No.	Body wt. (kg.)	Sex		Before injection	Time after injection (hrs.)					
						1	2	3	4	5	
1	1	2.0	♂	0.1	672	619	630	529	544	591	21
2	2	2.5	♂	”	629	630	601	506	629	643	20
3	3	2.8	♂	0.05	573	630	577	532	489	502	15
4	4	1.9	♂	”	684	665	623	593	668	680	13

plied. Fig. 2 shows also its sedimentation diagrams. To further scrutinize its homogeneity, 10 mg. of the preparation was dissolved in 0.5 cc. of the borax-HCl buffer of pH 8.4 (0.05 *M* sodium tetraborate: 0.1 *N* HCl=6:4 by volume), added to a 0.9×26 cm. (20 cc.) column of Dowex 1 of

mesh 50–200 equilibrated with the buffer above and developed by the aid of the same buffer at a flow rate of 1 drop per 35 seconds, collecting the effluent in fractions of 15 drops (about 1.5 cc.). The fractions were analyzed by the quantitative biuret reaction of Gornall *et al.*⁵⁾ on 1 cc. of them, using the buffer solution for the blank, as well as by quantitative Molisch reaction which was carried out as follows:— A mixture of 0.5 cc. of the unknown with 0.1 cc. of 5% α -naphthol in alcohol is added to with

Fig. 3. Column chromatogram of 10 mg. of the fraction of normal human urine corresponding to the urinary KIK factor developed with Dowex 1 and the borax-HCl buffer, pH 8.4. ○ Molisch reaction. ● Biuret reaction.

TABLE II

Liver Catalase Depression *in vivo* by Preparations, Anemia-inducing (I) and Non-anemia-inducing (II)

The average volume of O_2 evolved for each dose in each test-set is expressed in per cent of the corresponding volume in the blank control.

No. of test-set	Amount of substance injected (mg.)							
	Prep. I (anemia-inducing)				Prep. II (non-anemia-inducing)			
	1	0.5	0.3	0.1	1	0.5	0.3	0.1
1	58.2	66.8	64.8	94.4	65.5	64.7	79.0	93.2
4		66.7	68.0	92.6		71.7	82.3	84.1
2		52.3	85.7	87.7		64.8	78.5	
5								
3								
6								
Depressed catalase activity		0.62	0.73	0.92		0.67	0.80	0.89

TABLE III

Effect *in vivo* on Kidney- and Blood-catalase of Preparations,
Anemia-inducing (I) and Non-anemia-inducing (II)

0.5 mg. amounts of Prep. I and of Prep. II were injected into mice. Liver catalase activities were also determined for comparison.

No. of mouse	Prep. I			Prep. II		
	Kidney	Blood	Liver	Kidney	Blood	Liver
Oxygen evolved (cc.) in blank experiments						
101	4.1	2.0	17.0			
107				4.6	2.3	9.5
102	3.4	1.4	17.7			
108				4.4	2.2	12.9
103	2.6	1.0	18.2			
109				3.3	0.8	16.0
Average	3.4	1.5	18.0	4.1	1.8	12.8
Oxygen evolved (cc.) in main experiments						
104	3.8	2.0	8.9			
110				4.5	2.7	6.9
105	3.2	1.3	9.8			
111				4.4	1.6	9.1
106	2.6	1.2	9.6			
112				3.2	1.4	9.2
Average (% of the control value)	3.2 (94)	1.5 (100)	9.6 (53)	4.0 (98)	1.8 (100)	8.4 (64)

TABLE IV

Effect *in vitro* on Liver Catalase of Preparations, Anemia-inducing (I) and Non-anemia-inducing (II)

The liver homogenates were incubated with 0.5 mg. of Prep. I or II.

No. of mouse	Liver catalase activity (oxygen evolved in cc.)					
	Prep. I			Prep. II		
	Control I (No incubation)	Control II (Incubated without the substance)	Main (Incubated with the substance)	Control I	Control II	Main
113	16.3	14.3	16.2			
116				7.6	4.5	6.0
114	17.8	15.5	17.3			
117				11.7	6.0	8.2
115	16.7	13.0	13.9			
118				11.4	3.5	4.5
Average	16.9	14.3	15.8 (111% of Control II value)	8.9	4.7	6.2 (133% of Control II value)

3.5 cc. of conc. H_2SO_4 under cooling on ice, heated in boiling water for 5 minutes, and quickly cooled in iced water, followed by measurement of the extinction at the wave-length of $565 \text{ m}\mu$. As shown in Fig. 3, the whole substance appeared forming a peak between the 6th and 10th fractions.

Toxohormone activity. It was revealed that the two substances, anemia-inducing and non-anemia-inducing, are both toxohormone-active, namely, they depress liver catalase (Table II) but not blood- and kidney-catalase *in vivo* (Table III), whereas they do not inhibit or do rather activate or protect from destruction the liver catalase *in vitro* (Table IV). The assays were processed as described by Masamune *et al.*⁶⁾

To add, the substances were found to lack blood group activity within ABO classification.

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