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The Fate in Guinea-Pigs of Intravenously Injected I131- γ -Globulin

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Abstract

1) The fate and rate of degradation of I131 labelled rabbit γ -globulin, which retained its native antigenicity and antibody specificity was studied in the guinea-pigs. 2) Blood elimination rate of heterologous γ -globulin is higher than that of homologous γ -globulin. 3) Denatured and digested γ -globulin departs from the blood more rapidly than the native one, and urinary excretion rates of denatured and digested γ -globulin are higher than that of the native one. It is inferred, therefore, that the denatured and digested γ -globulin is more liable to be resolved and decomposed in the reticulo-endothelial organs than the native one. And the value obtained from the urinary excretion reflects the rate of protein break down in some cellular compartments. 4) Following the plasmapheresis the increase in antigen elimination was lessened and delayed as compared with control animals. 5) The organ distribution of heterologous I131- γ -globulin is to the lymphnode > the spleen > the liver > the lung > the kidney > the intestine in descending order. Heterologous I131- γ -globulin is deposited in greater quantity in the reticulo-endothelial organ than other single organ. 6) Following the intravenous injection of I131 labelled antigens, the ratio of the specific activity of mitochondria and microsomes to that of whole liver homogenate was determined over a period from 15 minutes to 3 hours in guinea-pigs, and following results were obtained. a) Organ and intracellular distribution of I131 labelled homologous γ -globulin shows no great difference compared to that of heterologous one. b) The intracellular distribution of heterologous γ -globulin is in mitochondrial > microsomal > nuclear fraction in descending order. c) The heterologous γ -globulin quantity of mitochondrial fraction or microsomal fraction in the spleen is higher than that of the liver. 7) The antibody distribution of intracellular granules measured in terms of radioactivity with a Geiger-Muller counter, after the reaction of I131 labelled antigen. The quantity of distribution of intracellular granules decreases in mitochondrial fraction > microsomal fraction > nuclear fraction in descending order.

STUDIES ON THE INHIBITORY ACTION OF SUGAR AND POLYATOMIC ALCOHOL FOR THE HEAT- INACTIVATION OF COMPLEMENT

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Introduction

When complement is warmed to about 56° C, it becomes opalescent. If the heating is prolonged or the temperature is raised still higher, the cloudiness and viscosity increase and the entire sample may become gel.

Van der Scheer showed¹⁾ that the horse serum, when warmed to about 65° C, becomes denatured to form a colloidal component C, having approximately the same electrophoretic mobility as that of the β -globulin.

It was published that the sugar and polyatomic alcohol had an inhibitory action for the heat denaturation of biological active substances, i. e., diphtheria toxin²⁾, antitoxin³⁾, enzyme⁴⁾, hemagglutinin⁵⁾⁶⁾⁷⁾ and complement⁸⁾⁹⁾.

*L. Silber*⁹⁾ found in 1932 that thermostability of complement was increased by sugar. This discovery was confirmed and extended by *M. Ogata*, *T. Ohkawa*, *M. Ogata*¹⁰⁾ and *M. Hatano*¹¹⁾. The purpose of the present study is to show the effect of sugar and polyatomic alcohol on the formation of a colloidal component C, by means of electrophoretic analysis and measurement of turbidity.

Materials and Methods

The complement used in this experiment was that contained in fresh serum taken from guinea-pigs.

Complementary activity was measured by the initial hemolysis method advocated by *Ecker*, *Pillimer*, *Wertheimer* and *Gradis*¹²⁾. Each sugar and amino acid, was tested for its inhibi-

tory action on heat denaturation by the following method.

The calculated weight of sugar and amino acid was added to 0.5 ml complement, then the mixture was shaken and incubated at respective temperature (54°C, 56°C, 58°C, 60°C, 62°C, 64°C, 65°C) for 15 minutes in a water bath.

Electrophoretic examination ;

Electrophoretic examination has been made with the "Hitachi A type" electrophoretic apparatus. Photographic records were taken by *Svensson's* method¹³⁾. All observations have been made on the migration of protein in the buffer. Before electrophoresis, the samples of guinea-pig serum were diluted with equal volume of a buffer solution and then thoroughly dialyzed against large volume of buffer. The ionic strength of this buffer was 0.2 with respect to phosphates; its pH was 7.7. After the first dialysis of 48 hours, the electrophoretic examination has been made.

Turbidity analysis ;

The strength of light scattering has been measured with nephro-colorimeter.

Experimental results

A) The electrophoretic examination ;

The electrophoretic patterns obtained from the heat-inactivated guinea-pig serum and that after being added glucose, were shown in Table I and Fig. I. In Fig. I (B) and (D) the most striking feature is a component C, that dominates the patterns of sera heated at 65°C. The C is a colloidal aggregation product resulting from denaturation of guinea-pig serum (complement). The C peak moves in the electric field at practically the same rate as the α -peak appearing in native serum. The electrophoretic examination shows that the serum heated at 65°C after being added the glucose is devoid of C component. (Fig. 1 E)

(cf.)

In order to remove the sugar from the serum, the serum was precipitated by saturation with sodium sulfate, and was measured by electrophoretic method. From Fig. 1 (A), (C) and

Fig. 1 (B), (D), the percentage of serum components (albumin, α -, β -, and γ -globulin) measured by electrophoresis were approximately the same value as that of serum components precipitated by sodium sulfate.

Table 1. The percentage composition of the components of guinea-pig serum.

	Percentage of components.			
	Albumin	α -glob. or C	β -glob.	γ -glob.
(A) Native complement (Normal guinea-pig serum)	61.5	13.8	13.1	11.6
(B) Complement heated for 15 minutes at 65°C.	23.0	73.0	—	4.0
(C) Complement precipitated by saturation with sodium sulfate.	64.6	12.3	10.8	12.3
(D) Complement heated for 15 minutes at 65°C and precipitated by saturation with sodium sulfate.	25.0	71.4	—	3.6
(E) Complement when added glucose heated for 15 minutes at 65°C and precipitated by saturation with sodium sulfate.	59.6	15.8	11.4	13.2

Phosphate buffer, pH 7.7, Ionic strength 0.2,

From the above results it was inferred that glucose inhibited the molecular aggregation of serum protein and the formation of colloidal component C. These phenomena were also observed by turbidity measurement.

B) The turbidity measurement ;

Five portions of complement heated to various temperatures (54°C, 56°C, 58°C, 60°C, 62°C) for 15 minutes, were tested for their activity and turbidity.

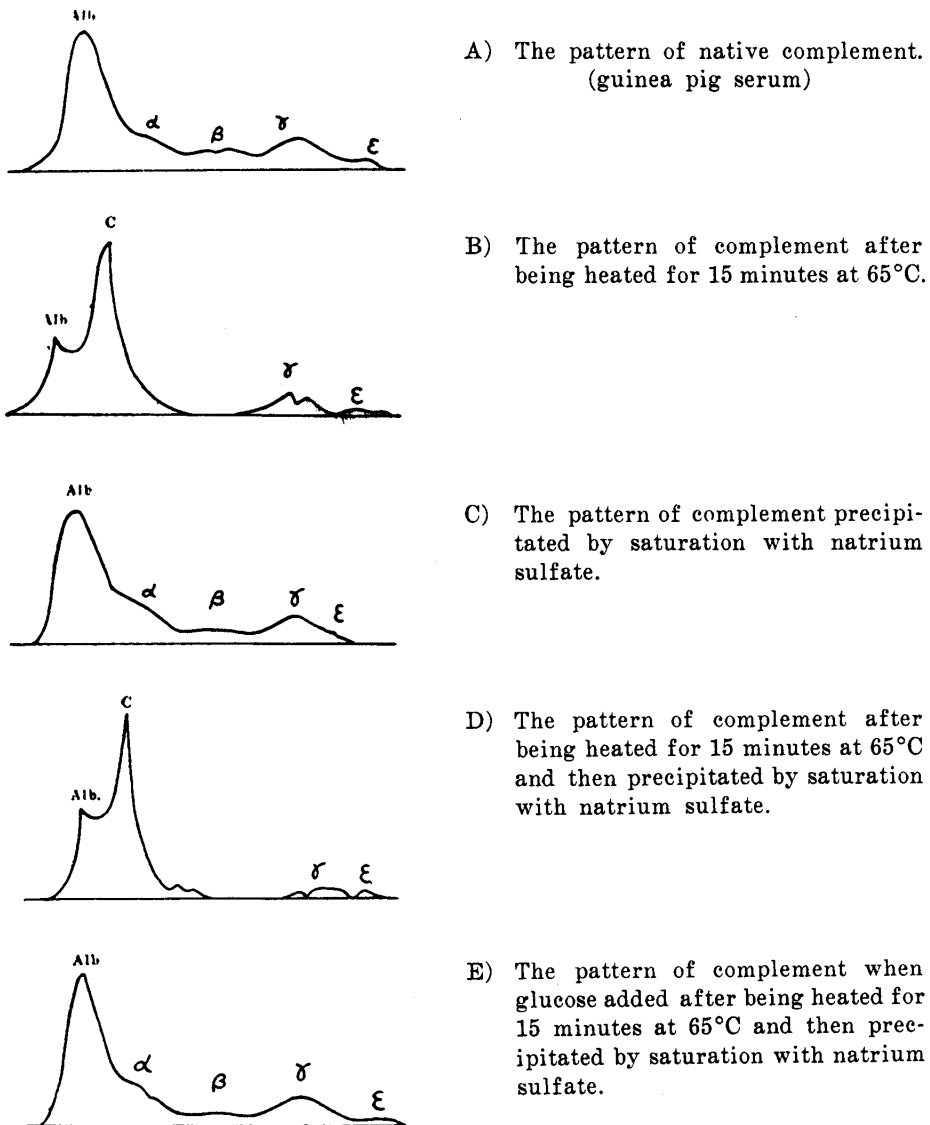
Turbidity was expressed in terms of relative turbidity, i. e.,

$$\text{relative turbidity} = \frac{\text{turbidity reading of heat inactivated serum.}}{\text{turbidity reading of native serum.}}$$

Calculated weight of each sugar and amino acid was added to complement, heated at respective temperature (54°C, 56°C, 58°C, 60°C, 62°C), and the turbidity was measured with nephro-colorimeter.

The results were shown in Table 2 and Fig. 2. Column V -

Fig. 1. Electrophoretic patterns of guinea-pig serum.



A) The pattern of native complement. (guinea pig serum)

B) The pattern of complement after being heated for 15 minutes at 65°C.

C) The pattern of complement precipitated by saturation with natrium sulfate.

D) The pattern of complement after being heated for 15 minutes at 65°C and then precipitated by saturation with natrium sulfate.

E) The pattern of complement when glucose added after being heated for 15 minutes at 65°C and then precipitated by saturation with natrium sulfate.

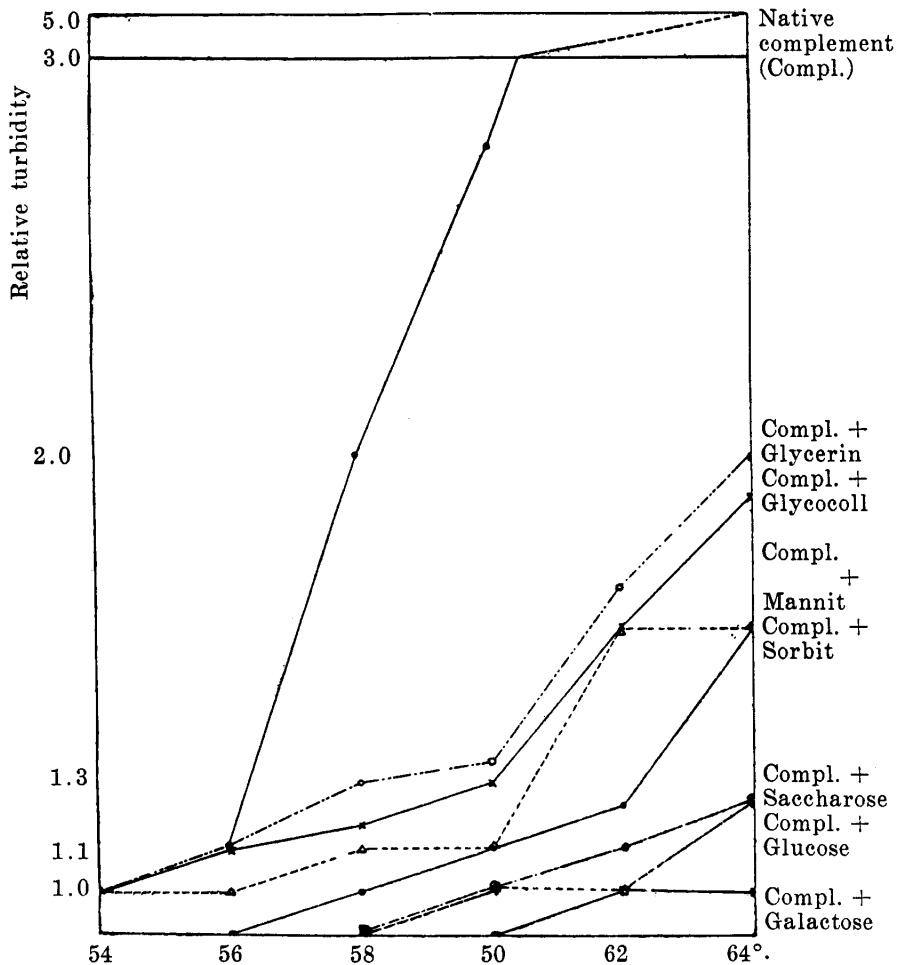
X, the last series (VIII) in table 2 shows the relative turbidity of complement heated at various temperature. Column V - X, series I - VII shows that of complement after adding the calculated amount of sugar and poly atomic alcohol so as to be 10 times as much as the serum osmotic pressure. Column IV shows the maximum temperature allowed for the complement activity. The Fig. 2 shows the results of Table 2. Heating temperatures

Table 2. The relative turbidity of complement and complement heated at various temperature after adding sugar and polyatomic alcohol.

Complement + Sugar, Polyatomic alcohol and Amino acid	Gram added to 0.5 ml of complement	Minimum hemolytic unit (ml)	Minimum hemolytic unit after heating 56°C for 15 min.	Maximum inhibitory temperature for inactivation of complement	54°C	56°C	58°C	60°C	62°C	64°C
					15 min	15 min	15 min	15 min	15 min	15 min
					Relative turbidity	Relative turbidity	Relative turbidity	Relative turbidity	Relative turbidity	Relative turbidity
Complement + Galactose	0.3	0.03	0.03	63°C	1.0	1.0	1.0	1.0	1.1*	1.1*
Complement + Saccharose	0.45	0.04	0.04	62°C	1.0	1.0	1.0	1.1	1.2*	1.3
Complement + Glucose	0.3	0.03	0.03	62°C	1.0	1.0	1.0	1.1	1.1*	1.3
Complement + Sorbit	0.3	0.03	0.03	61°C	1.0	1.0	1.1	1.2*	1.3*	1.7
Complement + Mannit	0.3	0.03	0.04	59°C	1.1	1.1	1.2*	1.2*	1.7	1.7
Complement + Glycocoll	0.1	0.03	0.03	57°C	1.1	1.2*	1.25*	1.35	1.7	2.0
Complement + Glycerin	0.15(ml)	0.03	—	54°C	1.1*	1.2	1.35	1.4	1.8	2.1
Native complement	—	0.03	—	55°C	1.1	1.2*	2.1	2.8	4.3	5.0

* shows the maximum inhibitory temperature for inactivation of complement.

Fig. 2. The relative turbidity of complement and complement heated various temperature after adding sugar and polyatomic alcohol.



Vertical axis shows the relative turbidity. Horizontal axis shows the heating temperature.

were shown on horizontal axis, and relative turbidity shown on vertical axis. The increase of turbidity caused by heat inactivation has not been observed in the serum heated after adding the sugar and poly atomic alcohol. These results correspond to the colloidal C peak formed by heat inactivation by electrophoretic method. The inhibitory action of sugar and polyatomic alcohol for molecular aggregation by heating is galactose > saccharose > glucose > sorbit > mannit > glycocoll > glycerin in descending order and same order as inhibitory action of heat

inactivation of complement activity.

The experiments show thermostability of complement increases by inhibitory action of sugar and polyatomic alcohol on molecular aggregation by heating.

Summary

1) When guinea pig serum was warmed at about 65°C, part of its protein became denatured resulting in the formation of a colloidal component C, which has approximately the same electrophoretic mobility as that of α -globulin.

2) The electrophoretic examination shows that the serum heated at 65°C after adding the glucose is devoid of colloidal component C.

3) The inhibitory action of sugar and polyatomic alcohol on the heat inactivation of complement is galactose > saccharose > glucose > sorbit > mannit > glycocoll > glycerin in descending order.

The inhibitory action of sugar and poly atomic alcohol on molecular aggregation by heating is of the same order as complement activity.

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