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PURIFICATION OF HEPATITIS TYPE B ANTIGEN (HB Ag) FROM HUMAN PLASMA BY HEAT COAGULATION

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SUMMARY

The heating of the HB Ag contaminated plasma with a high decrease of the normal, human plasma proteins provided a simple method for the partial purification of the hepatitis type B antigen (HB Ag). Coagulation started at 60°C, as well as the decrease of the protein contents. At 90°C the antigen maintained its immunoreactivity and a 90.2% reduction of the protein contents was observed.

INTRODUCTION

Great difficulty has been encountered in obtaining hepatitis type B antigen in a sufficient pure state for chemical analysis, diagnosis and production of monoespecific antisera. The procedures used presently are rather time-consuming, depend on expensive equipment, and have the limitation of only permitting the processing of small volumes.

Hepatitis type A and type B virus were found to be heat-stable and retain their infectivity after exposures to 56-60°C for 30 minutes by HAVENS [8] and WARD et al. [18].

PURCELL et al. [14] reported decreases of the HB antigenic titer of up to 50%, as detected by immunodiffusion, after heating at 56°C for 30 minutes. This could be caused by the aggregation without degradation since the complement-fixing antigen was not destroyed by overnight incubation at this temperature (GERIN et al. [7]). MILLMAN et al. [13] demonstrated no appreciable loss of antigenic activity after heating the antigen at 56°C for one hour, or after 20 minutes at 59°C. The same authors heated the antigen at 85 and 100°C destroying its detectable immunological activity as revealed by the immunodiffusion test. Later, KRUGMAN et al. [11] evaluated the effect of boiling the serum containing strains MS-1, and MS-2 at 98°C, for one minute over the high heat of an electric burner on the immunogenicity of hepatitis virus type A and B (HAV and HBV). They observed that heating changed the infectivity but not the immunogenicity of the HBV.

In the present study the immunoreactivity of HB Ag contained in whole human plasma under the effect of different temperatures was investigated.

MATERIALS AND METHODS

Serum hepatitis associated antigen (HB Ag)

Plasma from several HB Ag carriers or patients was used as source of the antigen.

Hepatitis type B antibody (HB Ab)

The antiserum used undiluted in all the screening for HB Ag was obtained from an hemophiliac with a high titer of HB Ab.

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Titration of HB Ag

The discontinuous counterimmunoelectrophoresis (DCIE) described by WALLIS & MELNICK [17] was used in the titrations. Glass slides 8.3 by 10.2 cm. (Kodak lantern slide coverglasses) were coated with 15 ml agarose (Seakem, Marine Colloids, Inc., Rockland, Maine, USA) in veronal buffer, 0.015 μ ionic strenght. The wells were punched following a pattern that permitted the obtainment of 21 pairs of wells. The distance between the wells was 5 mm. Serial dilutions of test samples were made in 0.015 μ veronal buffer.

Protein determination

The Lowry test (Folin-Ciocalteau) was used for quantitation of proteins using purified bovine albumin as standard [12].

Protein electrophoresis

Plasma electrophoresis was performed as described by CAWLEY [4]. Experiments were carried out with a Helena electrophoresis apparatus (Helena Laboratories, Beaumont, Texas, USA), model 6902. Each of the 800 ml chambers were filled with 600 ml veronal buffer pH 8.6, ionic strenght 0.075 μ. A constant potential of 200 v was applied and cellulose acetate strips (Millipore phoroslide electrophoresis strips) obtained from Millipore Corporation (Bedford, Massachusets, USA) were soaked in veronal buffer 0.075 μ. Strips with electrophoresed plasma were stained for 5 minutes with Ponceau dye (Ponceau-fixative dye, concentrate, from Millipore Corporation), and washed repeatedly in 5% acetic acid solution. Bands were scanned on a Beckman Microzone Densitometer, model R-110.

Heat coagulation of plasma

A tube containing 25 ml of plasma was suspended in a beaker over a heater-magnetic stirrer unit. The beaker containing water was slowly heated while a magnetic bar gently stirred the plasma. The temperature was constantly double-checked by two thermometers which were immerged in the plasma without touching the bottom of the tube. When the plasma attained each pre-determined temperature level (60, 70, 75, 85, and 90°C), three ml were collected as sample and set aside for assay. The plasma remaining in the tube continued being heated until reaching the next temperature level. All samples, collected at the various temperature levels were centrifuged at 1500 X g for 30 minutes. Generally, 50-60 minutes were necessary to reach 90°C starting from 23-25°C. The supernatants, as well as the unheated control sample, were assayed for HB Ag by DCIE, and for proteins by the Lowry method.

RESULTS

The partial purification of HB Ag which is associated with the alpha fractions of plasma [1, 2] may be easily carried out by the use of precipitants such as polyethylene glycol (PEG 6000) [15], ammonium sulphate and sodium sulphate [16], etc., with a minimum consumption of time, and great reduction of the contaminant human plasma proteins.

On the basis of previous data indicating that the hepatitis type B antigen was not inactivated at certain temperatures [7, 8, 11, 13, 14, 18] the present investigation was undertaken to determine the effect of different temperature levels on the immunoreactivity of the HB Ag.

Results obtained in this study on heat-coagulation of HB Ag contaminated plasma performed with the purpose of obtaining an antigen with low contents of contaminating plasma proteins, are shown in Table I. Coagulation, as well as the decrease of the protein

TABLE I

Heat-coagulation curve of HB Ag contaminated plasma

Temperature (°C)	HB Ag titer	Protein (mg/ml)	Reduction of proteins (%)
Control unheated	1:80	87.5	
60	1:80	87.5	0.0
70	1:80	81.6	6.7
75	1:80	65.6	25.0
85	1:80	14.8	83.0
90	1:80	8.5	90.2

contents started at 60°C. In the supernatant of the centrifuged sample, collected at 70°C, a reduction of 6.7% was observed. The reduction continued, increasing to 25.0, 83.0, and 90.2%, respectively at 75, 85, and 90°C. When tested for HB Ag by DCIE, all the supernatants showed the same titer of the unheated control plasma. Figure 1 shows tracings of the HB Ag purification attained at different temperatures. The reduction of protein attained at 90°C (90.2%) seems to indicate that the plasma heat-coagulation can be a convenient step for the purification of the HB Ag.

The reproducibility of the method was confirmed when several lots of plasma of different qualities were tested side by side. Lots 6 and 9 were heavily contaminated while lots 8 and BS were not. Table II shows that only an excess of bacterial contamination could cause the procedure to be non-effective.

DISCUSSION

Concentration and purification procedures in current use are time consuming and depend on long runs of centrifugation, column chromatography, or electrophoresis [3, 5, 6, 7, 10, 13].

In the present study it was demonstrated that the simple heating of the HB Ag contaminated plasma to 90°C provoked a 90.2% reduction of human plasma proteins in less than 90 minutes. Thus, the use of the procedure before the application of any other method of purification would be expected to yield HB Ag preparations of high purity with the use of rather common equipment. When the time necessary for the completion of the heating procedure is considered, its convenience and its inexpensiveness are outstanding.

Heat treatment at 85 and 100°C as reported by MILLMAN et al. [13] destroyed detectable immunological reactivity of a purified HB Ag resuspended in 0.15 M NaCl, as revealed by the immunodiffusion test. However, heating at 56°C for one hour did not decrease the reactivity of the same antigen. In this study the antigen contained in plasma maintained its immunoreactivity even after heat-treatment at 90°C, as proven by the DCIE test. The success of the purification under these conditions seems to be due to the fact that the process was performed with whole plasma instead of a semi-purified antigen.

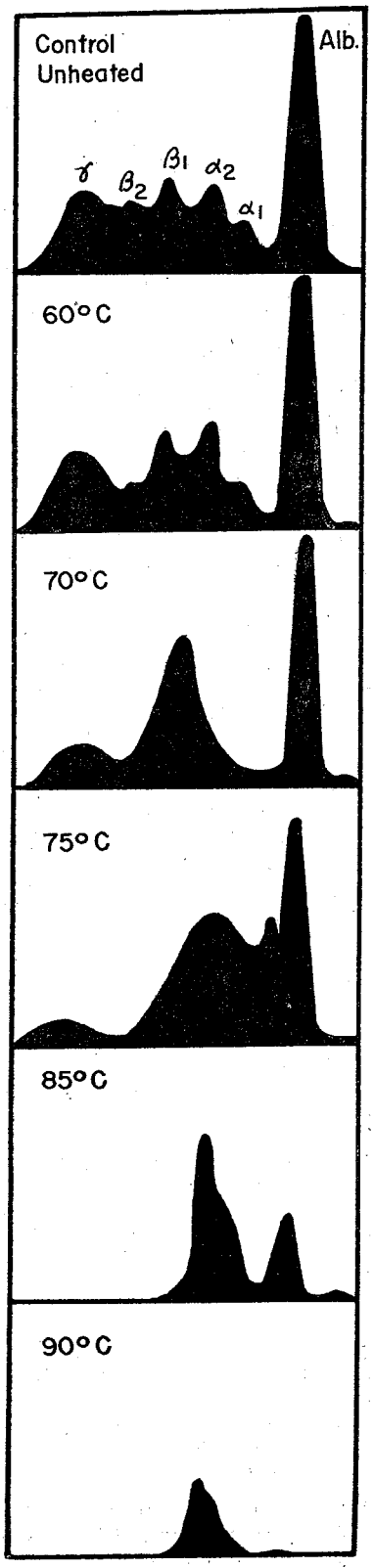

Fig. 1 — Reduction of plasma protein by heat-coagulation.

TABLE II

Purification of HB Ag by heat-coagulation

	Plasma Lot	pH	Volume heated (ml)	Volume of supernate (ml)	HB Ag titer	Proteins (mg/ml)
6	unheated	5.36	—	—	1:80	93.7
	heated		35.0	2.0	1:10	5.0
9	unheated	6.77	—	—	1:80	100.0
	heated		35.0	5.0	0	9.4
8	unheated	6.89	—	—	1:80	93.7
	heated		35.0	8.0	1:80	7.5
BS	unheated	7.68	—	—	1:320	87.5
	heated		35.0	12.0	1:160	6.9

RESUMO

Purificação do antígeno da hepatite tipo B (Ag HB) de plasma humano pela termo-coagulação

Pela termo-coagulação de plasma humano contaminado pelo antígeno da hepatite tipo B (Ag HB) foi possível purificar parcialmente o antígeno, pela remoção das proteínas alheias ao mesmo. A coagulação teve início a 60°C, assim como a redução do teor protêico. A 90°C o antígeno manteve sua reatividade imunológica, e foi observada uma redução de 90,2% do teor protêico.

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