XLVII. THE HEAT INACTIVATION OF DIPHTHERIA ANTITOXIN.

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AMONGST serum workers there is an impression that the addition of from 1·5 to 2 % of sodium chloride to antitoxic plasma, prior to its being heated, guards against the undue inactivation of the antitoxin, and it is generally accepted that the protective influence of the sodium chloride in this respect is still more marked during the heating of cresylised plasma or serum.

In order to furnish definite evidence on this point a study has been made of the percentage inactivation of antitoxin during the heating of:

- (A) Oxalated antitoxic plasma,
- (B) Plasma (A) to which an addition of 1·5 % of solid sodium chloride was made,
- (C) Oxalated antitoxic plasma containing 0·30 % of cresylic acid,
- (D) Plasma (C) to which an addition of 1·5 % of solid sodium chloride was made,

for stated periods of time at temperatures ranging from 58° to 72°.

Separate volumes of the respective plasmas (A), (B), (C) and (D), each of 25 cc., contained in 30 cc. sealed ampoules, were heated for definite periods of time in water baths regulated to the specified temperatures by means of thermostats.

Determinations were made of the antitoxic values of the respective liquids before and after heating, and, from the results thereby obtained, was calculated the percentage inactivation induced during the heating period.

The data thus furnished from the study of the plasmas (A) and (B), and of (C) and (D), have been included in Tables I and II respectively.

It will be seen that the rate of inactivation rapidly increases with the rise in temperature. Thus (Table I), while the plasma can be kept at 58° for ten hours without suffering any appreciable diminution of its antitoxic value, there is a 10 % loss shown after three hours' heating at 60·5°, or after two hours' heating at 61·5°, or after one hour's heating at 63°. With the progressive rise in temperature the proportional loss of antitoxic value in a given time rapidly increases until at 72° there is a 60 % inactivation of the antitoxin within ten minutes.

A comparison of the results given in Tables I and II shows that the loss of antitoxin during the heating of cresylised plasma is greater than that resulting from the similar treatment of noncresylised plasma. But, contrary to the ideas current amongst serum workers, the evidence obtained in this investigation indicates that the addition of sodium chloride does not appreciably lessen the heat inactivation of antitoxin either in cresylised or in non-cresylised plasma.

Further information as regards the heat-instability of antitoxin was obtained from a similar study of the inactivation of antitoxin in solutions containing only those protein fractions with which the antitoxin is associated in plasma.

The salt-soluble globulin fraction of an unheated antidiphtheritic plasma (potency 450 units per cc. and protein content 7.48 %) was separated and dialysed in the usual way. The residue from dialysis was diluted with distilled water so as to reduce the protein content of the liquid to 7 %.

This solution of antitoxin and its associated proteins, freed in this way from albumin, euglobulin and the other organic and inorganic constituents of the plasma, was submitted to the same treatment as that described above for the cresylised and noncresylised plasmas, (A), (B), (C) and (D).

The results recorded in Table III indicate that, as was anticipated, antitoxin shows a greater stability to heat when in aqueous solution associated with its own particular proteins than when present in the more complex medium of the plasma.

While studying the effect of heat on the inactivation of antitoxin I also made observations on the accompanying increased precipitability of the serum proteins. From the data, thus obtained, was calculated the percentage denaturation of the serum proteins, the results for the respective liquids being also included in Tables I, II and III.

In all cases it was noticed that, during the heating of those sera or plasmas which contained from 1.5 to 2 % of sodium chloride or cresylic acid and phenol in amounts up to 0.6 %, there was an apparently greater conversion of soluble into insoluble protein than occurred during the heating of non-cresylised plasma. However, by means of the refractometer, it was ascertained that the presence of sodium chloride or of cresylic acid and phenol in the plasma had not affected the extent of the heat-denaturation of the proteins, these substances had merely stimulated the coagulation of the particles of denaturated proteins into aggregates of sufficient size to be more readily discerned by the eye.

From the results recorded both here and in my previous papers it has been found that the extent of the denaturation of the proteins is a function of:

- (a) the temperature at which the heating was conducted;
- (b) the reaction of the plasma;
- and (c) the duration of the heating of the plasma.

Table I.

Showing the extent of the denaturation of the serum proteins and the inactivation of the antitoxin during the heating of oxalated antidiphtheritic plasma (A) and of the same plasma to which has been added 1.5 % of sodium chloride (B).

Potency of the unheated plasma = 375 units per cc.

Protein-content of the plasma = 8.45 %

P_H of the unheated plasma, 7.8.

Temperature °C.	Duration of the heating	Plasma (A)		Plasma containing 1.5 % NaCl (B)	
		Percentage denaturation of the serum proteins	Percentage inactivation of the antitoxin	Percentage denaturation of the serum proteins	Percentage inactivation of antitoxin
58	4.5 hours	30.3	nil	30.5	nil
"	10 "	31.0	"	31.2	"
60.5	1 hour	13.5	"	14.0	"
"	2 hours	40.0	"	41.0	"
"	3 "	42.0	10	41.5	less than 10
61.5-62	1.5 "	40.0	—	40.0	—
"	2 "	50.0	8	49.0	less than 8
"	3 "	55.0	slightly greater than 13	55.0	about 20
"	4 "	56.5	—	57.5	—
63	1 hour	52.0	10	53.5	10
"	2 hours	64.0	slightly less than 27	66.0	25
"	3 "	72.0	—	70.0	—
65	10 minutes	40.8	—	42.5	—
"	20 "	57.0	about 27	58.5	about 27
"	30 "	65.0	about 33	66.0	about 33
"	1 hour	practically solid	—	practically solid	—
66	10 minutes	44.5	about 13	45.5	about 10
"	20 "	60.0	slightly less than 33	62.0	slightly less than 33
67.5	10 "	62.5	20	63.5	20
"	20 "	69.0	36	71.0	33
68.5-69	5 "	56.5	—	57.0	—
"	10 "	62.0	33	61.0	33
"	20 "	72.0	slightly less than 46	72.0	slightly less than 46
70	5 "	65.0	slightly less than 33	65.0	33
"	10 "	75.0	53	75.0	slightly greater than 46
"	15 "	almost solid	—	practically solid	—
72	5 "	80.0	60	80.0	60
"	10 "	practically solid	—	practically solid	—

Table II.

Showing the extent of the denaturation of the serum proteins and the inactivation of antitoxin induced during the heating of antidiphtheritic plasma containing 0.30 % of cresylic acid (C), and of the same cresylised plasma to which 1.5 % of sodium chloride has been added (D).

Potency of the original plasma = 375 units per cc.

P_H of the original plasma, 7.4.

Protein-content of the plasma = 8.45 %.

Temperature °C.	Duration of the heating	Cresylised plasma (C)		Cresylised plasma containing 1.5 % of NaCl (D)	
		Percentage denaturation of the serum proteins	Percentage inactivation of the antitoxin	Percentage denaturation of the serum proteins	Percentage inactivation of the antitoxin
58	4 hours	25.0	nil	25.4	nil
"	8 "	26.0	"	26.0	"
60	1 hour	41.6	negligible	40.6	negligible
"	2 "	45.5	10	45.4	10
"	3 "	48.5	17	48.3	17
62	1 "	45.0	17	47.2	13
"	2 hours	55.0	—	57.5	24
"	3 "	57.0	—	57.5	—
63	1 hour	47.2	17	50.0	17
"	2 hours	64.0	—	65.0	—
"	3 "	70.0	—	practically solid	—
64	30 minutes	53.0	15	52.0	17
"	1 hour	63.5	—	66.0	—
"	2 hours	practically solid	—	practically solid	—
65	20 minutes	53.0	40	52.0	40
"	30 "	65.0	—	64.0	—

The extent of the inactivation of the antitoxin seems to be regulated by:

(a) the temperature at which the heating is conducted;

and (b) the time of heating.

The time of heating required to produce the maximum value for the denaturation of the proteins became shorter as the temperature was raised. In this respect the behaviour of antitoxin and of the serum proteins is somewhat analogous. However the heat-inactivation of the antitoxin seems to be mainly a function of the temperature and independent of the extent of the accompanying heat-denaturation of the serum proteins, for, by suitable adjustments, the heat-denaturation of the proteins at a given temperature can be rendered complete without any corresponding change in the coefficient for the heat-inactivation of the antitoxin at that temperature.

These observations lead to considerations as to whether antitoxins should be regarded as separate entities rather than as structural modifications of the serum proteins induced during the process of immunisation. In this connection, it had been my intention to plot curves for the time taken, at each degree of temperature, to reduce the antitoxic value of the liquids to half

Table III.

Showing the extent of the heat-denaturation of pseudo-globulin and of the inactivation of antitoxin in solutions which have been heated at temperatures between 58° and 72°.

P_H of the solution of pseudo-globulin, 7.0.
Potency = 450 units per cc.; protein-content, 7.48 %.

Temperature °C.	Duration of the heating	Percentage denaturation of the pseudo-globulin	Percentage inactivation of antitoxin
58	4 hours	25.0	negligible
"	10 "	26.0	"
61.5	1 hour	23.7	nil
"	2 hours	26.6	negligible
"	3 "	31.6	"
63	30 minutes	31.0	"
"	1 hour	40.0	"
"	2 hours	51.0	"
"	3 "	55.0	"
66	20 minutes	40.0	"
"	40 "	46.0	"
67.5	10 "	50.7	"
"	20 "	61.7	nearly 13
68.5	10 "	57.0	8
"	20 "	67.0	—
70.0	5 "	50	negligible
"	10 "	70	30
"	15 "	practically solid	—
72	8 "	90	greater than 50

value, and to make therefrom a comparison between the heat inactivation of antitoxin and that of certain enzymes. But, unfortunately, owing to the prolonged scarcity of experimental animals, I have been unable to carry my investigations further in this respect.

The work involved in this investigation was, in part, carried out during my war time appointment with the Lister Institute.

SUMMARY.

During the course of the investigation it has been found that:

- (1) The addition of 1.5 to 2 % of sodium chloride to plasma, whether noncresylised or cresylised, prior to its being heated, in no way reduces the extent of the heat-inactivation of the antitoxin.
- (2) The rate of the heat-inactivation of antitoxin is a function of the temperature at which the heating is conducted.
- (3) The extent of the heat-inactivation of the antitoxin in an aqueous solution of the antitoxin bearing proteins is considerably less than that evidenced during the heating of antitoxic plasma.