CLXXIII. THE EFFECT OF HEAT ON SUGAR SOLUTIONS USED FOR CULTURE MEDIA.

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It is a well-established fact that some types of culture medium are rendered unsuitable for the growth of certain bacteria and fungi if sterilised by autoclaving, and in the literature repeated references to this phenomenon are found. But so far as the author is aware little work has been carried out to determine the nature of the change caused by the heating of the medium.

Pope (private communication) found that cultures of *C. diphtheriae* grown on medium containing Difco proteose peptone, glucose and maltose did not produce good toxin if the medium had been autoclaved. Moreover he found that the heat-labile factor in the medium lay not in the peptone but in the sugar-inorganic salt solution in which the peptone was dissolved.

This suggested that it would be interesting to determine the stability of glucose-maltose-salt mixtures towards heat, under conditions similar to those commonly used in the preparation of culture media.

That sugars are readily decomposed by strong alkalis such as caustic soda is a very well-known fact, the rate of decomposition increasing with the concentration of alkali used. The oxidation of glucose by hydrogen peroxide has been studied by Löb [1911] and by Witzemann [1920] and the oxidation by iodine has been investigated by Kappanna [1928]. All three workers found that phosphates increase the rate of oxidation of the sugar. Kappanna and Löb find the rate of oxidation to be increased by raising the p_H value and Kappanna maintains that the only rôle of the phosphate is in buffering the solution in the neighbourhood of p_H 8 and preventing the fall of p_H due to acid produced from the glucose. Löb, on the other hand, states that when hydrogen peroxide is the oxidisng agent, phosphate exerts a specific accelerating effect when the p_H value is kept constant and Witzemann does not think reaction to be of primary importance—the main factor influencing the rate of oxidation being phosphate concentration.

The following experiments were carried out with a view to determining whether heating in the autoclave caused destruction of maltose and glucose and if so whether phosphates played an important part in the change. It was also considered to be of interest to investigate the relative effects of heating sugar solutions at 100° and under excess pressure in the autoclave.

Methods.

The sugars were estimated by the Shaffer-Hartmann method [1920] modified as described in the previous paper. For maltose and glucose-maltose mixtures the estimation was carried out before and after hydrolysis, and the amounts of each sugar calculated. There is the possibility that some other reducing substance may be produced in the solutions during autoclaving. The presence of such a substance would introduce an error into the calculations. Harden [1911], in discussing the degradation products of glucose, mentions many which are not fermentable by yeast but none which is to any marked extent fermented. Although there is still the possibility that fermentable reducing substances other than sugars do exist, we have been able to find no evidence of this in the literature. It was therefore considered to be of interest to determine the effect of yeast fermentation on the autoclaved solutions and it was found that after such fermentation the reducing power fell to less than 4 % of its value before fermentation. So the solutions contain little or no non-fermentable reducing substances and we have assumed that glucose and maltose are the only reducing substances present. Our calculations are based on this assumption.

Exp. 1. Effect of autoclaving glucose-salt solutions.

The medium was composed as follows:

Glucose	1·5 g.
K_2HPO_4	2·0 g.
Na_2HPO_4	2·0 g.
$MgSO_4, 7H_2O$	0·2 g.
$CaCl_2$	0·1 g.
Glacial acetic acid	10·0 cc.
Cresol red	0·02 g.
NaOH to p_H 8·0	
Distilled water to 500 cc.	

The chemicals used were B.D.H. A.R. chemicals and weighings were accurate to 0·5 %. A portion was reserved as a control, then 100 cc. quantities were placed in bottles and autoclaved at 15 lbs. pressure for various times. The temperature was raised and lowered as rapidly as possible to obviate excessive heating and the period of heating quoted is the actual time when the pressure was 15 lbs. The result is shown in Table I.

Table I. *Showing effect of autoclaving on glucose solutions.*

Time of autoclaving	% glucose present	% of original glucose remaining	p_H
Control: not heated	0·280	100	7·90
5 minutes	0·252	90	7·76
10 ,,	0·236	84	7·64
20 ,,	0·216	77	7·54
40 ,,	0·200	71·5	7·41

Table I shows that in the presence of 0·8 % phosphate, glucose is gradually destroyed with simultaneous lowering of the p_H value.

Exp. 2. Effect of autoclaving maltose solutions.

Exp. 1 was repeated exactly except that 0·6 % maltose was substituted for the glucose.

The results are shown in Table II.

Table II. *Showing the effect of autoclaving maltose solutions.*

Time of autoclaving	% maltose present	% glucose present	Total	% original maltose	% total sugar remaining	p_H
Control: not heated	0·581	0·009	0·590	(100)	(100)	7·96
5 minutes	0·397	0·107	0·504	68·5	85·4	7·46
10 ,,	0·298	0·188	0·486	51·3	82·5	7·37
20 ,,	0·246	0·196	0·442	42·4	75·0	7·16
40 ,,	0·218	0·192	0·400	37·6	68·0	7·04

Table II shows that when autoclaved in the presence of phosphates, the solution becomes gradually poorer in maltose and richer in glucose; in other words hydrolysis of maltose to glucose occurs. The glucose in its turn is slowly destroyed as is shown by the gradual fall in total sugar content. Probably it is oxidised to an acid for the hydrion concentration rises as autoclaving proceeds.

Exp. 3. Effect of autoclaving mixtures of maltose and glucose.

The composition of the medium was the same as for Exps. 1 and 2, except that it contained both 0·6 % maltose and 0·3 % glucose. Portions were autoclaved at 15 lbs. pressure for various times, after which samples were taken, diluted suitably and their sugar contents estimated. The results are shown in Table III.

Table III. *Showing the effect of autoclaving a glucose-maltose solution containing 0·8 % phosphate.*

Time of autoclaving	% maltose present	% glucose present	Total	p_H
Control: not heated	0·619	0·267	0·886	7·68
5 minutes	0·399	0·368	0·767	7·24
10 ,,	0·345	0·384	0·728	7·19
20 ,,	0·342	0·392	0·734	7·08
40 ,,	0·200	0·440	0·640	6·84

Exp. 4.

In view of the work of Löb, Witzemann and Kappanna quoted above it was considered of interest to repeat the experiment on the heating of maltose-glucose mixtures, using different concentrations of phosphates.

Three batches of medium were made up, the composition being as follows:

	A (%)	B (%)	C (%)
Glucose	0·15	0·15	0·15
Maltose	0·3	0·3	0·3
Cresol red	0·002	0·002	0·002
Na_2HPO_4	0·00	0·10	0·20
K_2HPO_4	0·00	0·10	0·20

All three batches were adjusted with NaOH to p_H 8·0 before autoclaving at 15 lbs. pressure.

The results are shown in Table IV.

Table IV. *Showing influence of phosphates on the heat change in sugars.*

	No phosphate A				0·2 % phosphate B				0·4 % phosphate C			
Period of autoclaving	Maltose %	Glucose %	Total %	p_H	Maltose %	Glucose %	Total %	p_H	Maltose %	Glucose %	Total %	p_H
Control: not heated	0·311	0·151	0·462	7·55	0·319	0·141	0·460	7·94	0·313	0·148	0·461	7·82
5 minutes	0·271	0·168	0·439	7·43	0·227	0·181	0·408	7·44	0·213	0·201	0·414	7·66
10 „	0·225	0·201	0·426	7·15	0·147	0·224	0·371	7·17	0·111	0·223	0·334	7·53
20 „	0·253	0·181	0·434	7·22	0·165	0·224	0·389	7·12	0·127	0·228	0·355	7·48

Table IV shows that the hydrolysis of maltose to glucose and the destruction of glucose caused by heating are both accelerated by increasing the concentration of phosphates.

Many workers who realise that autoclaving causes deleterious effects in their culture media, have substituted a period of heating at 100° on three successive days.

In order to determine whether or not sugar solutions could be heated at the lower temperature for long periods without causing changes in the sugar content, the following experiment was devised.

Exp. 5. Effect of boiling mixtures of maltose and glucose.

The medium was composed as follows:

Maltose	1·5 g.
Glucose	0·75 g.
K_2HPO_4	1·0 g.
Na_2HPO_4	1·0 g.
Cresol red	0·02 g.
NaOH to p_H 8·0	
Distilled water to 500 cc.	

The mixture was placed in a flask fitted with an efficient reflux condenser to prevent concentration during boiling and during the whole period of boiling

Table V. *Showing the effect of boiling maltose-glucose mixtures.*

Period of boiling	% maltose present	% glucose	Total
Control: not heated	0·261	0·157	0·418
0·5 hours	0·179	0·196	0·375
1·0 „	0·120	0·215	0·335
2·0 „	0·089	0·217	0·306
4·0 „	0·043	0·217	0·260
6·0 „	Not measurable	0·243	0·243
7·0 „	„	0·246	0·246

a stream of compressed air was bubbled through the solution. Portions were withdrawn after varying periods and analysed for maltose and glucose.

The results are shown in Table V.

The above experiment was repeated, bubbling nitrogen through the liquid instead of compressed air. The results were, within the limits of the experiment, identical with those shown in Table V.

Discussion.

It is shown in Exp. 1, that glucose is slowly destroyed by autoclaving in the presence of phosphates. With 0·8 % phosphate and 40 minutes' autoclaving at 15 lbs. pressure, the concentration of glucose is reduced to about 70 %. Exp. 2 shows that maltose disappears from the solution more rapidly than glucose, since after 40 minutes only 38 % remains. If the change in maltose concentration is entirely due to hydrolysis to glucose, then the loss in total sugar from the solution will be caused solely by the destruction of glucose. The fact that in Exps. 2 and 3 the loss in total sugar during 40 minutes' autoclaving is respectively 32 % and 28 % compared with a loss of 28·5 % of glucose in Exp. 1 for the same heating period, is strong evidence that destruction of glucose accounts for the whole of the loss of sugar from glucose-maltose mixtures.

Exp. 4 clearly shows that the changes caused by heat are accelerated by phosphates. It will be seen, however, that in all experiments the reaction of the solutions becomes less alkaline as autoclaving proceeds. Moreover, the fall in p_H value is greater in the absence of phosphates and becomes progressively less as their concentration increases.

It is possible therefore that the action of phosphates is merely one of buffering the solution at a more alkaline reaction, for most workers are agreed that destruction of glucose is accelerated by increase in hydroxyl ion concentration. The author is of opinion, however, that the change produced by lowering the phosphate concentration is too great to be accounted for by the relatively small lowering of the hydroxyl ion concentration which results. In this connection the observation of Pope (private communication) is of interest. He noted that the deleterious effect caused by heat in medium prepared from Difco peptone and sugars for the production of diphtheria toxin was markedly greater in the presence of phosphates.

When the phosphate concentration was reduced to 0·2 %, toxin production was almost as good in autoclaved medium as in medium sterilised without heat. In medium such as Pope used, any fall in p_H value caused by acid production should be as adequately checked by the peptone as by the phosphates. The effect of phosphate is probably therefore not simply one of maintaining the alkalinity, but is more probably a specific accelerating effect on the destruction of sugars.

Exp. 5 shows the effect of boiling maltose-glucose solutions in air. To compare the effect with that of autoclaving the same solutions, Exp. 5 must

be compared with Exp. 4 B which is its proper control. It will be seen from Exp. 4 B that autoclaving for 20 minutes reduces the concentration of maltose to 52 % and that of the total sugar to 84 % of the amounts originally present. In Exp. 5 after one hour's boiling in air, the concentration of maltose was 46 % of the original amount and that of total sugar 81 %. It therefore appears that as regards the power of destruction of maltose and glucose, one hour at 100° is approximately equivalent to 20 minutes under 15 lbs. excess pressure. If, therefore, the medium to be sterilised contains maltose or glucose it does not appear that steaming for 20 minutes on three successive days would offer any advantage over autoclaving.

Summary.

1. Autoclaving at 15 lbs. pressure causes hydrolysis of maltose to glucose and slow destruction of the glucose with the production of acid.

2. The changes in sugar concentration on autoclaving are accelerated by the addition of phosphates.

3. As regards the power of destruction of maltose and glucose, one hour at 100° is approximately equivalent to 20 minutes under 15 lbs. excess pressure.

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