THE EFFECTS OF ADDED PEPTONE ON THE BACTERICIDAL ACTION OF SOLUTIONS OF FORMALDEHYDE

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When formaldehyde (HCHO) is used as a disinfectant in the presence of peptone the peptone and the bacteria compete for the HCHO[1]. An equilibrium distribution of the HCHO is reached only slowly. The state of combination of HCHO in the presence of 1 per cent peptone

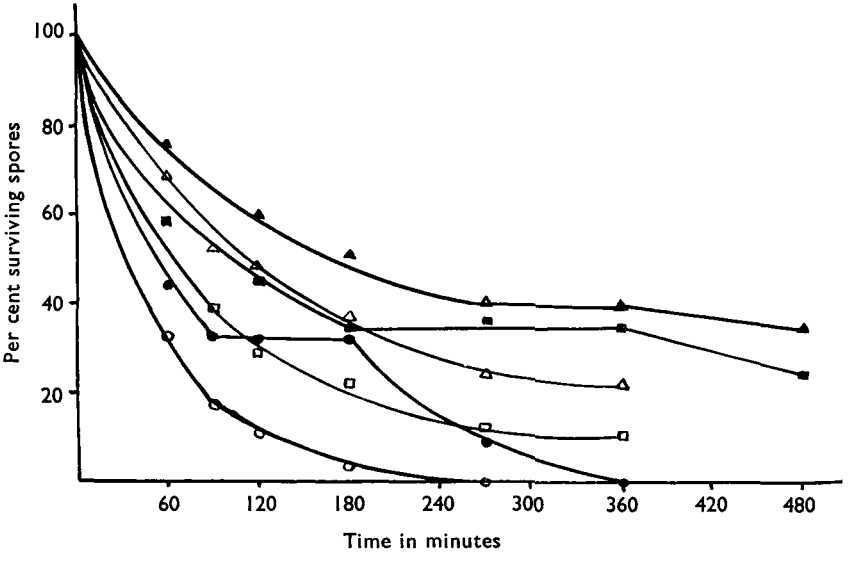

Fig. 1. The influence of the presence of peptone on the survival of *B. subtilis* spores in dilute solutions of HCHO.

A	○	0·1M HCHO at 37°.	D	●	0·1M HCHO + peptone at 37°.
B	□	0·05M HCHO at 37°.	E	■	0·05M HCHO + peptone at 37°.
C	△	0·1M HCHO at 25°.	F	▲	0·1M HCHO + peptone at 25°.

varies with its previous relationship to the peptone. If, in all states of combination with peptone, HCHO were fully available to act as a disinfectant peptone would neither retard nor reduce such action. Estimates of the proportions of HCHO in three different degrees of binding with peptone having been made we now enquired in which of these the HCHO remained bactericidal.

The methods of preparing *B. subtilis* spore suspensions and making roll-tube viable counts have been described[2]. All the solutions containing HCHO were brought to the required temperature and 1 ml. of stock

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spore suspension added to 9 ml. of test solution. After thorough mixing 1 ml. was removed and serial dilutions prepared for roll tube viable counts, from which results the initial count (usually about 20,000/ml.)

TABLE I

EFFECTS OF THE INCREASED BINDING OF HCHO IN FORMOL-PEPTONE ON ITS DISINFECTANT ACTIVITY

Time in minutes	Percentage surviving spores		
	One per cent peptone with added formalin		Formol-peptone
	0·1 M HCHO	0·05 M HCHO	0·1 M HCHO
65	34	55	40
180	34	34	29
245	15	34	26
480	0	24	0

could be calculated. The desired quantity of HCHO was now added and the whole mixed. After suitable time intervals 1 ml. quantities of the mixture were removed for viable counts of the surviving organisms. The results were expressed as the percentage of the spores remaining viable.

Curves A and B of Figure 1 demonstrate the bactericidal effects of 0·1M and 0·05M HCHO respectively in water at 37°. Curve C was obtained

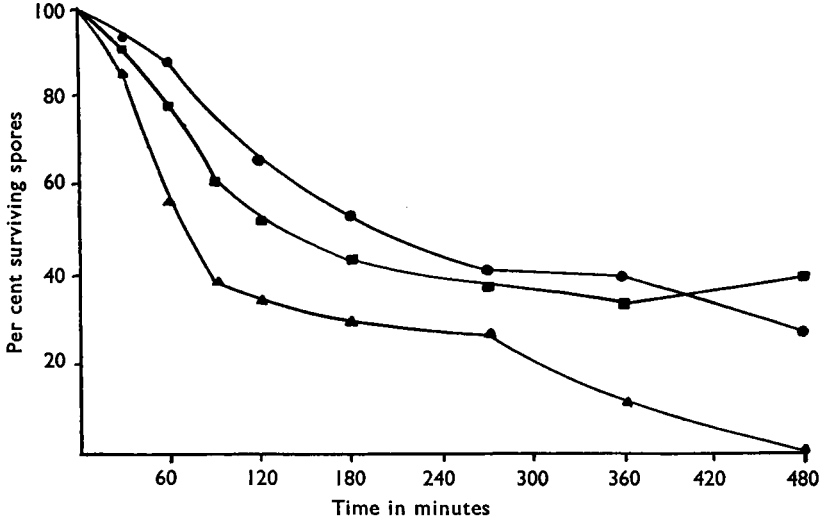

FIG. 2. The survival of *B. subtilis* spores in formol-peptone solutions.
H ▲ ▲ 0·1M HCHO at 37°.
J ● ● 0·1M HCHO at 25°.
K ■ ■ 0·05M HCHO at 37°.

with 0·1M HCHO at 25°. Curves D, E and F represent corresponding experiments in the presence of 1 per cent peptone. Increasing the peptone content from 1 to 5 per cent had no effect. Attempts were made to explain the flat portion of curve D. If the peptone and HCHO were

kept at 37°, for 3 hours before addition of the spores the shape of the curve was unaltered. If the peptone solution and spores were incubated at 37° for 3 hours before the addition of HCHO all the spores were killed in 60 minutes by 0·1M HCHO and in 90 minutes by 0·05M HCHO. Incubation in nutrient solution is known to induce, in a very short time, incipient germination of spores, possibly such germination, even in the

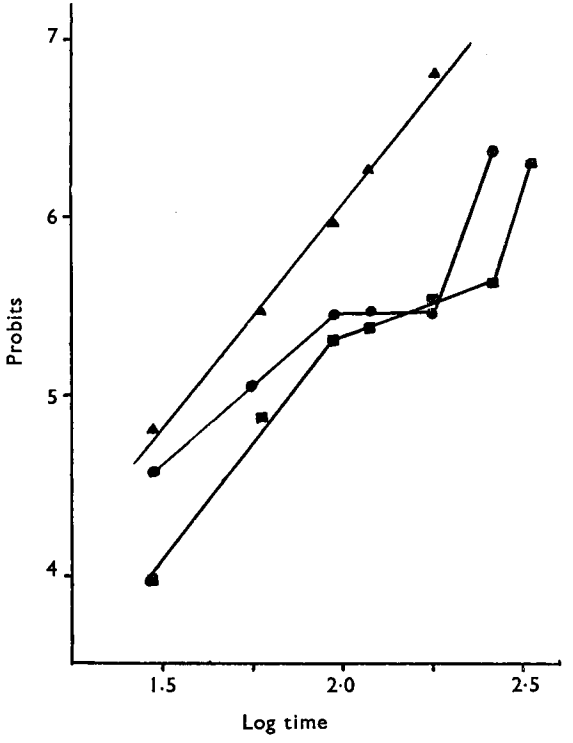

FIG. 3. Probit log-time regression curves of the survival of *B. subtilis* spores.
L ▲ ▲ 0·1M HCHO at 37°.
M ● ● 0·1M HCHO + peptone at 37°.
N ■ ■ 0·1M HCHO from formol-peptone at 37°.

presence of low concentrations of HCHO might explain the broken curves of Figure 1.

In further experiments such quantities of formol-peptones were dissolved in water that the resultant solutions contained 0·1M or 0·05M total HCHO estimated by chromotropic acid. Peptone was added, if necessary, to bring its concentration to 1 per cent. Time survivor curves for spores in such solutions are shown in Figure 2. Figure 3 shows the probit-log time regression curves drawn from the data used to construct curves A and D (Fig. 1) and H (Fig. 2). In Table I the action of 0·1M HCHO derived from formol-peptone is compared with that of 0·1M and 0·05M HCHO added as a dilution of formalin to 1 per cent peptone solution.

The increased binding of the HCHO to the peptone, previously demonstrated in the formol-peptone solutions, has surprisingly little effect on its disinfectant action. Most of the HCHO bound to the peptone sufficiently firmly to resist removal under reduced pressure in the dry state was bactericidal when the formol-peptone was dissolved in water. Even the HCHO bound to the peptone so firmly that it was not transferred to dimedone in solution appeared to possess some, even if a reduced, bactericidal activity.

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REFERENCES

1. Bullock and Rao, *J. Pharm. Pharmacol.*, 1958, **10**, *72 T.*
2. Bullock, Keepe and Rawlins, *ibid.*, 1949, **1**, 878.

DISCUSSION

The Paper and Short Communication were presented together by PROFESSOR K. BULLOCK.

The CHAIRMAN. Could sodium bisulphite combine with bound as well as free formaldehyde?

DR. F. HARTLEY (London). According to Table I in the Short Communication the presence of peptone did not modify the disinfectant properties of formaldehyde to the same extent as previously reported. Was this because moisture was present?

MR. G. SYKES (Nottingham). It was possible to draw a straight line through the black triangles, dots and squares of Figure 1 of the Communication. Only 7 points were recorded from which a curve, a straight line and a curve had been created. Instead of a flat portion, the curve should dip away very quickly, more regularity in the change would be expected and for the curve to become suddenly flat was against the rules of the distribution of resistance in a bacterial population. The N.T.V. of a peptone was not connected with its composition, it was dependent on the acidity or alkalinity at which it was made. Since the summations of F.T.V. and N.T.V. for A, B and C were the same, there was no apparent difference between them according to these estimations.

MR. H. D. C. RAPSON (Betchworth). Even the reaction between formaldehyde and simple amino acids is complex and may involve NH groups as well as NH_2. Formaldehyde may possibly react with hydrogen bonding in a protein and in addition there was the possibility of aldol condensation. Had the Authors considered the technique of vapour phase titration?

DR. A. H. BECKETT (London). Under controlled pH the NH_2 group of proteins was converted to CH_2OH. In work on bacterial surfaces he had found that formaldehyde could block NH_2 groups.

DISCUSSION

DR. J. B. STENLAKE (Glasgow). Acid amide groups react with formaldehyde and primary and secondary amines in Mannich type reactions. Some of the links formed in the Authors' experiments were possibly of this type and the products would be labile. Infra-red techniques might indicate the type of link. The release of water could be accounted for by Mannich type links. Dehydration resulting in azomethane links was also probable. Investigation by end-absorption or ultra-violet techniques might be helpful.

PROFESSOR BULLOCK replied. Sodium bisulphite had not been used. The formal peptones had a higher ratio of formaldehyde than those in previous work. The slightest trace of moisture greatly increases the disinfectant action of the formaldehyde. The curves in Figure 1 were smooth in the absence of peptone, but it was not possible to draw smooth curves when this was present. The flattening of the curve could not be explained by changes in the bacterial population. Dr. Rao considered that the formaldehyde became irreversibly bound and could not be recovered even after boiling with sulphuric acid. Dr. Rao's theory was that the viable count decreased until the available formaldehyde dropped below a certain concentration, then the flat part of the curve was obtained until the spores germinated and these were killed again. Although the difference in A, B and C may have been due to the processes of manufacture, it still might well affect the uptake of formaldehyde. The reactions of formaldehyde were complex and it could react with the SH group. Vapour phase titration had not been tried. The control of pH was extremely important. Dr. Rao had said that the amide group was known to react with formaldehyde.