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H phase change of *Salmonella thompson* at different temperatures

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SUMMARY

H phase II → phase I conversion of *Salmonella thompson* was studied at different temperatures. All strains were isolated from one outbreak, and a convenient method of phase conversion was sought. In our hands this was best effected at 20° C.

INTRODUCTION

In 1959, an outbreak of salmonella food poisoning occurred in a junior school following a Christmas party. The suspected vehicle of infection was trifle prepared from eggs. Some victims of the incident harboured two serotypes: *S. typhimurium* and *S. thompson* (Harvey, Price, Davis & Morley-Davies, 1961).

All strains isolated were examined for both phase I and phase II H antigens, in case other serotypes might be discovered. We have found this necessary from past experience. No difficulty was encountered in identifying strains of *S. typhimurium*. All cultures of *S. thompson* were in H phase II and when phase change was attempted at 37° C., this was unsuccessful and very prolonged. The method of effecting H phase II → phase I transformation was by the soft agar filled capillary pipette described by Harvey & Price (1961). One such pipette was left, by accident, at room temperature after inoculation. The desired H antigen change of *S. thompson* (1,5 → k) took place within a few days. This fortuitous result encouraged us to study the effect of different temperatures on all phase transformations conducted on strains of *S. thompson* isolated in the outbreak.

Incidents caused by this serotype were not particularly common in our area in 1959 and since regulations for pasteurization of egg products came into being in 1964 they are very seldom seen. We are, therefore, presenting a brief record of a technical facet of an incident occurring many years ago for it seems improbable that we shall have an opportunity of studying this phenomenon again.

MATERIALS

Single colonies were picked from brilliant green MacConkey agars and selected as presumptive *S. thompson* strains by O antigen identification. Purification was necessary as single colonies were sometimes mixtures of *S. typhimurium* and *S. thompson*. Mixed colonies are often found in samples likely to contain multiple serotypes. On one occasion a single colony from a selective agar seeded with

tortoise faeces proved to be a mixture of four different serotypes. When purified, *S. thompson* strains were inoculated to moist agar slopes. These provided the material for study.

METHODS

The technique of H antigen phase change was modified from that described previously (Harvey & Price, 1961). Bijoux bottles containing 0·1 % nutrient agar in 4 ml., amounts were prepared. Davis New Zealand agar was used. The concentration employed was less than that usually recommended for orthodox Craigie tubes. Four drops (4 × 0·02 ml.) of Standards Laboratory polyvalent H phase II serum were added to one or more of these bijoux bottles depending on the number of phase changes to be made. Agar and serum were carefully mixed to avoid bubble formation by rotation of the bottles. A sterile unplugged capillary pipette was then filled with serum-agar mixture to the level of the junction of stem and barrel. After filling, a small amount of dense suspension of culture to be examined was sucked into the terminal 1 cm. of stem. This culture suspension had to be in intimate contact with the mixed agar and serum above it. By manipulation of a teat on the pipette barrel, an air bubble was sucked up under the culture suspension and the stem end was sealed off in a bunsen flame. The teat was removed and the pipette placed in a test tube with cotton wool on the bottom. After all pipettes had been filled, the barrel ends were sealed off in a bunsen flame. Sealing was essential to prevent evaporation as many phase changes were prolonged. It was most simply effected by melting the extreme end of the pipette barrel in a flame, attaching another short piece of glass tubing, pulling out the molten section to a capillary, cooling, cutting and sealing in a bunsen as before.

Two series were studied. In the first, the passage of *S. thompson* through the agar columns was observed at 27 and 37° C. The samples were paired. In the second series the two temperatures investigated were 20 and 27° C. As soon as a strain had reached the upper surface of the agar column, the pipette barrel was opened by scoring with a carborundum cutter and touching the score with a red hot glass rod. The fracture of the barrel occurred at the score. Subculture from the agar surface was made to a small agar slope. This was incubated and the H phase of *S. thompson* identified.

RESULTS

The results are presented in Tables 1 and 2. Two effects of temperature were observed – success or failure to change phase and relative speed of travel of the culture through the column of agar. In the first table, out of 56 paired tests conducted at 27 and 37° C., 38 changed phase at 27° C. only, 8 changed at both temperatures and 0 at 37° C. only. The corresponding figures out of a total of 150 tests at 20 and 27° C. were 19, 130 and 1. Table 2 records the association of temperature with relative speed of travel of the cultures. In the first series, 21 cultures passed more rapidly through the agar at 27° C. than at 37° C., and 31 strains migrated more rapidly at 37 than 27° C. Of the 21 strains, all were in phase I, while only 4 out of the 31 cultures had successfully changed phase. In the 20° C./27° C.

Table 1. Salmonella thompson. *Effect of temperature on H phase II–I change*

Temperature	Successful conversion phase II → I
27° C. only	38
37° C. only	0
27 and 37° C.	8
Total tests performed	56
20° C. only	19
27° C. only	1
20 and 27° C.	130
Total tests performed	150

Table 2. Salmonella thompson. *Effect of temperature on rapidity of passage through agar column*

Passage through agar column first at:				Simultaneous appearance at 27 and 37° C.	
27° C.		37° C.			
Phase II → I	No change	Phase II → I	No change	Phase II → I	No change
21	0	4	27	2	2

Passage through agar column first at:				Simultaneous appearance at 20 and 27° C.	
20° C.		27° C.			
Phase II → I	No change	Phase II → I	No change	Phase II → I	No change
66	0	62	4	18	0

series, 66 appeared first at the top of the agar column at 20° C. and the same number at 27° C. Sixty-six incubated at 20° C. were in phase I and 62 incubated at 27° C. were in phase I. Details of cultures appearing simultaneously at 27° C./37° C. and 20° C./27° C. are given in the right-hand half of Table 2.

DISCUSSION

This paper merely seeks to present a brief record of a phenomenon encountered many years ago. It is now perhaps of academic interest only as outbreaks caused by *S. thompson* are rare in the United Kingdom.

Stocker (1949), in a limited experiment on a strain of *S. typhimurium*, found that alteration of medium and of temperature of incubation caused no detectable alteration of rate of mutation per bacterial generation. Our findings with a strain of *S. thompson* appear to contrast with this.

S. thompson, in the past, has always given difficulty in our hands in the phase II → I conversion and all cultures encountered have been in the non-specific phase. It is possible, where strains of *S. thompson* are found, that 20° C. might be a convenient temperature to effect phase conversion. We might be criticized on the grounds that we only studied a single strain of *S. thompson* in this investigation.

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This is true, but a strain of *S. thompson* isolated from a clinical case of gastro-enteritis in 1972 behaved in exactly the same way. This recent culture changed phase (II → I) at 20° C., but remained in phase II at 37° C. The migration through the pipette was also more rapid at the low temperature than at the high temperature.

We have no information on the behaviour of other serotypes as far as phase change is concerned.

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