PENETRATION OF EGGS BY SALMONELLA TYPHI-MURIUM

By G. W. WRIGHT and J. F. FRANK*

In other work conducted by the authors (1), it was shown that sodium hydroxide, in a concentration four times as strong as that which was effective in destroying *Salmonella typhi-murium* in vitro, failed to prevent penetration of the intact egg-shell contaminated with this organism. The reason for this was not established but it was suggested that the failure to prevent penetration might be due to some physical property of the egg-shell which permitted rapid entrance of the organism or protected the organism while it was being dipped in the disinfecting solution. This has led to a study of some of the factors which might affect penetration.

The specific gravity of an egg is commonly used as an index of shell thickness as it has been shown that this property provides a measure of the percentage of the shell present (2). Furthermore, it has been shown by Farnsworth and Nordskog (3) and Taylor and Lerner (4) that a considerable improvement in shell thickness and shell texture is possible by selective breeding. With these points in mind, the following experiment was set up in an attempt to determine whether there was any relationship between the specific gravity of the egg and its penetration by *S. typhi-murium*. A secondary aim was to see whether cooling the egg after infection would facilitate penetration by the organism.

METHODS

Freshly laid eggs were obtained from the Experimental Farm, Nappan, and graded into two lots according to their specific gravity. The grading was done by floating the eggs in salt solutions of two different densities. Eggs with a specific gravity higher than 1.090 were put into the high specific gravity lot, those with a specific gravity lower than 1.070 into the low specific gravity lot, while those with a specific gravity between 1.090 and 1.070 were discarded. The eggs used in these experiments were selected from a day's production of approximately 300 eggs, most of which had a specific gravity in between the levels mentioned above. Immediately after determining the specific gravity, the eggs were washed in tap water. They were then dried, candled to detect any cracks and the cracked eggs were discarded.

Using aseptic technique, a hole one eighth inch in diameter was drilled in the egg-shell over the air sac. The membrane was pierced and the yolk and albumen were sucked out through a sterile Pasteur pipette into a vacuum reservoir. In this and subsequent operations care was taken not to damage the membrane where it was attached to the shell.

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The egg was then filled with tetrathionate broth at 37°C., leaving an air space approximately equal to that found in the normal egg. A rim of molten paraffin was applied around the hole, and the egg sealed with a glass cover slip. Each egg was placed in the incubator until the last egg in the lot had been treated.

When all the eggs had been so prepared, they were infected by dipping the lower two thirds of the egg in a 24 hour broth culture of *S. typhi-murium* a fresh lot of 200 cc. of culture was used for each eight eggs. Eggs from each of the two lots were infected alternately. After all the eggs had been infected, they were stored under the conditions referred to below.

The eggs were examined periodically for evidence of penetration by transferring some of the contents to a tube of tetrathionate broth, incubating, and plating out on MacConkey agar.

Before the experiment was concluded the eggs were candled to ensure that no cracks, which might account for penetration by the test organisms, had occurred during the experiment. The eggs were then autoclaved, the contents re-

TABLE 1

The effect of the specific gravity of eggs on penetration by *Salmonella typhi-murium.*

	Experiment No. 1.			Experiment No. 2.		
Specific Gravity*	Number Penetrated	Number Not Penetrated	Totals	Number Penetrated	Number Not Penetrated	Totals
High.....	7	5	12	6	6	12
Low.....	6	2	8	10	2	12
Totals.....	13	7	20	16	8	24

*High Sp. Gr. = greater than 1.090. Low Sp. Gr. = less than 1.070.

TABLE II

The effect of sudden cooling on the penetration of eggs by *Salmonella typhi-murium.*

	Experiment No. 1.			Experiment No. 2.		
Storage Conditions	Number Penetrated	Number Not Penetrated	Totals	Number Penetrated	Number Not Penetrated	Totals
Store Room or Incubator	5	5	10	9	3	12
Cooled to 4°C., then at 20°C.	8	2	10	7	5	12
Totals.....	13	7	20	16	8	24

moved, and the shell thickness measured at the top (one piece), bottom (one piece), and the middle (4 pieces) of the shell using a microscope and eyepiece micrometer.

Two experiments were conducted using the procedure indicated above. In the first, one lot of eggs was stored in a bacteriological incubator at 37°C., while the other lot was refrigerated at 4°C. for one hour after being infected, then stored in a room whose temperature was approximately 20°C. In the second experiment one lot was stored at approximately 20°C. immediately after being infected, while the second lot was refrigerated for one hour and then stored at 20°C.

RESULTS

The effect of specific gravity on penetration is shown in Table I; the effect of sudden cooling soon after infection is shown in Table II.

In the first experiment, penetration occurred in two eggs by the end of the first day after infection. With the exception of one egg, which did not show penetration until between seven to nine weeks, penetration by the test organism occurred in all the remaining positive eggs by the end of the fifth day.

In the second experiment, penetration occurred in one egg by the end of the first day and all the positive eggs in this experiment were penetrated by the end of the seventh day.

The mean thickness of the egg-shells in the two experiments are shown in Table III. These results confirm the existence of a direct relationship between shell thickness and the specific gravity of an egg.

DISCUSSION

It will be seen from the tables that a slightly greater proportion of eggs was penetrated by *S. typhi-murium* in the low specific gravity group.

In this connection it is interesting to note that Erasmus (5) has shown that different lines of hens laid eggs which showed significant differences in shell thickness and also significant differences in shell porosity between the

TABLE III

The relationship between specific gravity of the egg and thickness of shell.

	*Specific Gravity	Mean Thickness in mm.	Standard Deviation
Experiment No. 1.....	High	0.393	0.020
	Low	0.293	0.030
Experiment No. 2.....	High	0.380	0.026
	Low	0.265	0.032

*High Sp. Gr. = greater than 1.090. Low Sp. Gr. = less than 1.070.

lines, but there was no significant correlation between shell thickness and shell porosity. Therefore, it cannot be assumed that the increased penetration in the low specific gravity eggs is necessarily due to greater shell porosity.

The effect of chilling the eggs after they had been dipped in the *S. typhi-murium* culture acted in opposite directions in the two experiments, so it may be concluded that chilling the eggs has no effect on penetration.

SUMMARY

1) In each of two experiments two groups of eggs, one of high specific gravity and one of low specific gravity, were tested for penetration of the shell by *S. typhi-murium.*

2) An increase in the proportion of eggs penetrated was observed in the low specific gravity groups.

3) No increase in the proportion of eggs penetrated was observed when eggs were chilled after being dipped in *S. typhi-murium* culture.

4) The existence of a direct relationship between shell thickness and the specific gravity of the eggs was confirmed.

RESUME

Au cours d'une étude antérieure sur la désinfection des oeufs contaminés avec *Salmonella typhi-murium,* il a été observé que, même après traitement par l'hydroxyde de sodium, il y a pénétration microbienne dans un certain nombre de cas. Ce fait amène les auteurs à étudier certains facteurs susceptibles d'affecter la pénétration de la coquille de l'oeuf, notamment la gravité spécifique et le refroidissement de la coquille après contamination par le micro-organisme d'épreuve.

Dans une série d'expériences portant sur deux lots d'oeufs, un à gravité spécifique élevée et un autre à gravité spécifique faible, les auteurs notent que la coquille est traversée dans un plus grand nombre d'oeufs à gravité spécifiique faible. D'autre part, ils n'observent pas d'augmentation de la pénétration chez les oeufs refroidis.

L'existence d'une relation directe entre l'épaisseur de la coquille et la gravité spécifique de l'oeuf a été confirmée.

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ON THE PROBABLE PRESENCE OF PARASITIC LIVER CIRRHOSIS IN CANADA

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The aim of the present paper is to discuss the probable presence in Canada of a rare human disease, usually believed to be tropical. The disease, which is basically a parasitic liver cirrhosis, is caused by a nematode, living in the parenchyma of the liver and depositing there its ova. This peculiar helminth, related to the whipworm, was discovered by Bancroft in 1893 (1) in the liver of a rat and named *Trichocephalus hepaticus*. In 1916 Hall (2) created for it the genus *Hepaticola*. In 1931 Baylis (3) re-examined the morphology of *H. hepatica* and showed, that its generic characters coincide with those of the genus *Capillaria*, and that the creation for it of a new genus was not justified. The correct name of Bancroft's *Trichocephalus hepaticus* is therefore *Capillaria hepatica* (Bancroft, 1893).

This peculiar nematode and the disease it causes in its animal hosts remained a parasitological curiosity for over 30 years — until 1924, when the first case of human infection was described by MacArthur on the basis of material furnished by Dive and Lafrenais (4, 5). The patient — a British soldier in India — died in the hospital with the diagnosis "pyaemia." At autopsy the liver was found to contain numerous whitish nodules which proved to be masses of ova of *C. hepatica*. The second case of human capillariasis of the liver was described in 1950 in the U.S.A. by McQuown (6). His patient was a 17-month-old negro girl from a settlement near New Orleans. The child was admitted to the hospital with pneumonitis and died 26 hours after admission. On necropsy the liver was found to extend to the umbilicus. It contained numerous scattered haemorrhagic areas and small (1-3 mm.) greyish-tan nodules. The nodules proved to be granulomas, located in periportal spaces, and containing numerous ova of *C. hepatica*. The third human case, that of Otto *et al.* (7),

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