

calomel electrode at 30° C. (This temperature was used because of the high ambient temperature.) To measure the wave-height draw a straight line through the curve at -0.6 V. and -1.0 V. and extend to meet the -1.3 V. perpendicular. The vertical distance between this intersection and the wave at -1.7 V. gives the wave-height.

The gamma isomer concentration is read from a standard curve prepared using pure gamma isomer. A convenient range for this curve is 3-15 mgm. gamma isomer, this yielding a straight line, not passing through the origin.

Further aliquots of the alcohol extract are analysed for total benzene hexachloride by normal dehydrohalogenation methods, and the ratio gamma isomer/total benzene hexachloride calculated.

The method of extracting the benzene hexachloride is essentially that of Hitchcock *et al.*, as briefly described by Mackerras⁴, while the empirical method of wave measurement is similar to that of Dragt².

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² Dragt, G., *Anal. Chem.*, **20**, 737 (1948).

³ Ingam, G. B., and Southern, H. K., *Nature*, **161**, 437 (1948).

⁴ Mackerras, I. M., *Austral. Vet. J.*, **23**, 185 (1947).

Isolation from the Lower Respiratory Tract of Chickens of Bacteria administered by Mouth

It is generally assumed that bacteria ingested in food or water do not enter the trachea but that all of them pass down the oesophagus. One interesting observation in this respect was made by White and Minett¹, who noted that when calves were fed milk containing *Mycobacterium tuberculosis* primary lesions of tuberculosis were sometimes found in the lungs, indicating that some of the bacteria administered may have been aspirated into the lungs. More direct evidence that this may occur was obtained as a result of some experiments that were designed to trace the route by which *Salmonella gallinarum* enters the body following its administration to chickens by mouth.

In the initial studies large numbers of *Salm. gallinarum* bacteria were mixed in a commercial mash and fed to chickens which were then killed at different times and selected organs cultured. *Salm. gallinarum* was isolated from the lungs of an unexpectedly high proportion of chickens, in some after an interval of time so short as to preclude them having reached this organ via the intestine.

In view of this, four groups each consisting of fifteen nine-week-old chickens were taken. The first group was fasted for 12 hr. and then fed on a commercial mash into which had been mixed an 18-hr. broth culture of *Salm. gallinarum*, 1 ml. per 10 gm. of mash. The culture contained approximately 50×10^7 bacteria per ml. Water was withheld from the second group for 12 hr. This group was then

provided with drinking water, every 10 ml. of which contained 1 ml. of the broth culture. 1 ml. of the culture was placed carefully into the mouth of the chickens in the third group by means of a pipette. A similar amount of culture was given to the fourth group by means of a pipette introduced via the mouth into the oesophagus, care being taken to avoid touching the epiglottis.

The chickens were killed $\frac{3}{4}$ hr. later by dislocation of the neck, and, at the same time, a pair of strong artery forceps was fixed, through the skin, around the upper portion of the trachea so as to occlude its orifice. A post-mortem examination was then carried out using aseptic precautions. Cultures were made from the mucous membrane of the lower end of the trachea on to deoxycholate-citrate agar² and a portion about 1 in. long of this part of the trachea placed in a tube of selenite-F medium³. Most of the left lung was removed, ground in sterile sand and one loopful of this streaked on to a plate of deoxycholate-citrate agar; the remainder was placed in a tube of selenite-F medium. The selenite-F cultures were incubated at 37° C. for 24 hr. and plated on to deoxycholate-citrate agar. All plates of the latter medium were incubated at 37° C. for 24 hr. and then examined for the presence of *Salm. gallinarum*. The results are shown in Table 1.

Table 1

Method of administering <i>Salm. gallinarum</i>	No. of 15 chickens in which <i>Salm. gallinarum</i> was isolated from		
	Trachea	Lung	Trachea and/or lung
In food	4	10	12
In drinking water	4	5	7
Into mouth by pipette	9	3	10
Into oesophagus by pipette	0	0	0

Salm. gallinarum was not isolated from the lower respiratory tract of any of the fifteen chickens to which it was given by means of a pipette into the oesophagus. Quite different results were obtained when it was administered by the other three methods, *Salm. gallinarum* being recovered from the trachea or lungs of a considerable proportion of these chickens. It is noteworthy that *Salm. gallinarum* was isolated more frequently from the trachea than from the lungs of the chickens to which it was given by pipette into the mouth (9-3); the reverse was true in the case of the chickens fed infected food (4-10).

Salm. gallinarum was only isolated by direct culture in one case, from the trachea of a chicken given this organism by pipette into the mouth. The other isolations were made through selenite-F medium. It is therefore probable that infection via the lower respiratory tract would be of little significance under natural conditions since chickens would not be exposed to such large numbers of bacteria. However, it is a point to be borne in mind when the production of experimental infections is under consideration.

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