# ENQUIRY

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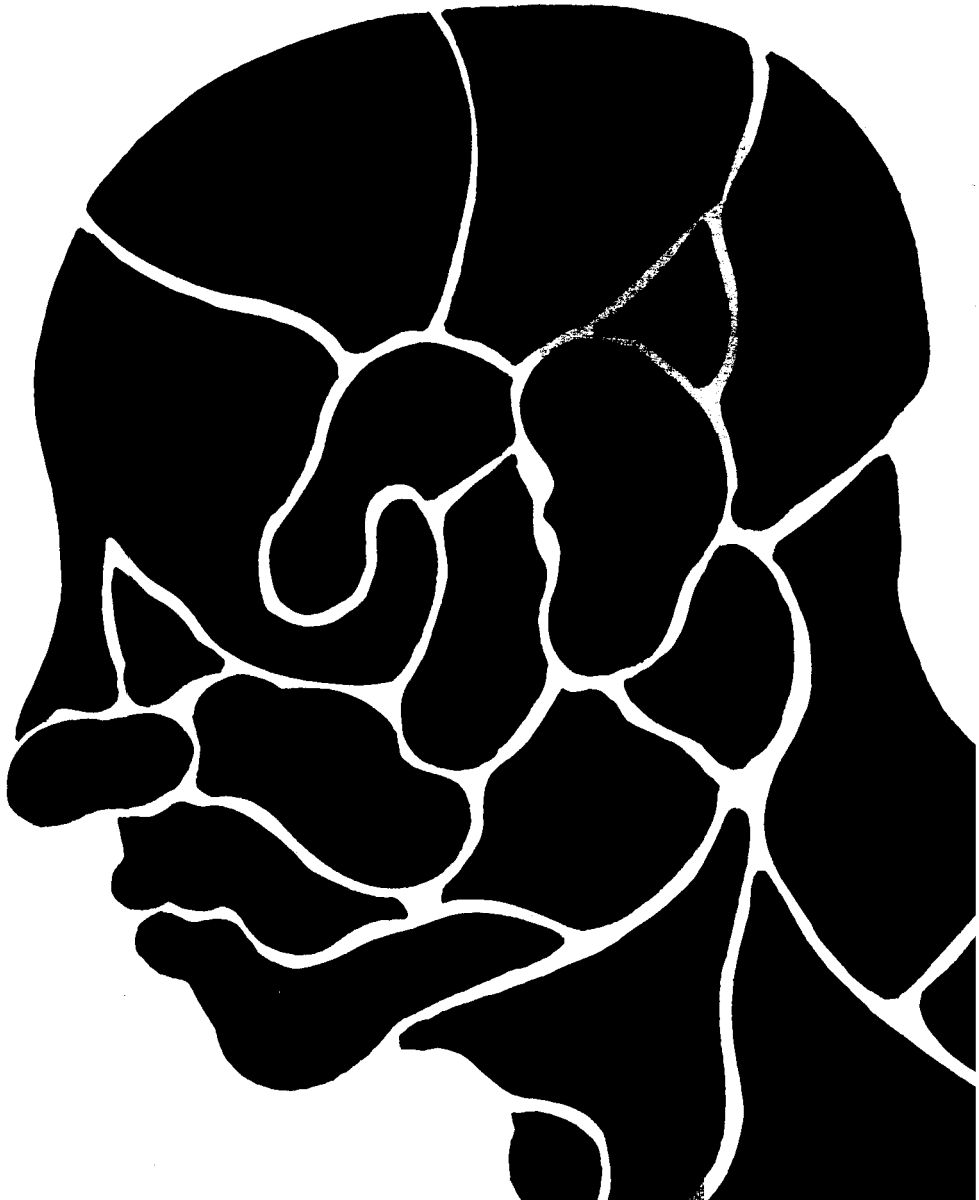

# Contents

# Salmonella, Pigs and Food — Poisoning

PAUL M. NURSE

ESSENTIALLY, this is an outline of a research programme with which I was involved, during a nine month period before going up to University. I was fortunate enough to obtain a position in a research team at Twyford Laboratories, London, which was concerned with the problem of Salmonella (a genus of bacterium) in pigs, and its relationship to food poisoning. A surprising number of stomach upsets, glibly put down to 'over indulgence', is caused by the infiltration of this bacterial genus into the human alimentary canal. One of the commonest sources of infection is poorly cooked pork, which directed the team to a special interest in pigs.

Before I arrived on the scene considerable work had been done into the development of a vaccine to provide immunisation against Salmonella in pigs (preventing the infection at its primary source). This vaccine consisted of a live but attenuated strain of Salmonella, S. dublin, i.e. a strain had been produced by various means of kitchen-like cookery, which though it was live and viable was non-pathogenic. So though the vaccine induced the production of copious supplies of antibodies within the pig, so providing subsequent immunisation, no disease is formed. However, the problem which arose next was in fact to find an efficient method of producing the vaccine, to enable it to be marketed at a reasonable price. This involved considerable book-work to find what conditions are necessary for good bacterial growth, and then experimentation to determine the precise environmental limits.

It was found easy enough to produce a high dry weight of bacteria simply by increasing the nutrient and aeration, but this resulted in very low viability. The best compromise was found by numerous experiments with liquid media, within a sterile flask inoculated with S. dublin and shaken at 37°C in oxygen. The work was then shifted to a fermenter. This was on a larger scale and was a special vessel with many accessories controlling the internal conditions of growth. Use of this eventually gave the best combination of factors for good growth. Perhaps the most confusing factor was that of time. The exact period after growth had finished which was best for highest viability was quite critical. Just after growth (i.e. increase of bacterial dry weight) had finished a phenomenon was observed: the cells continued to divide though their volume was the same, and so the viability was increased. After a certain period, however, cells began to die off again, hence lowering viability. The critical point between these two (which varied according to growth conditions) was particularly difficult to find.

Once the bacteria had been produced they had to be stored in a form that was useful for keeping and eventually dispensing. This was done by freeze-drying in glass vials. The technique of freeze-drying is much used today and is very efficient. The bacteria are cooled down to about -80°C and are then dried by the water subliming off in a vacuum. This leaves a pad of bacterial cells which have a surprisingly high survival rate (90%). On reconstitution in water the cells become viable again.

At this stage in the work the vaccine had been developed and prepared and a method of storage had been found successful. Next it had to be shown that the vaccine worked in a field trial, i.e. that the numbers of Salmonella present dropped substantially after vaccination. For this a good technique had to be found for identifying and isolating Salmonella from animal tissue. The devlopment of such a technique was very difficult and a satisfactory solution was not found while I was there, and in fact has not been discovered even now (January). The method of extraction from animal tissue was quite quickly worked out. By maceration of the tissue (usually lymph-nodes) with a sophisticated food mixer, and then repeated centrifugation and resuspension, a pad of bacteria and shattered cell contents from the tissue was obtained. Then the real problem of identifying the bacteria reared its head.


Most of the older isolating and identifying schemes relied on subjecting the bacteria to a series of adverse environments which tended to be less harmful to the Salmonella than to the other bacteria present. This sort of procedure killed many of the bacteria but left a greater percentage of Salmonella in the survivors. However, it had not been fully realised exactly how many of the Salmonella these techniques destroyed. This we discovered varied considerably from technique to technique but usually it was found that about one bacterium survived from an original inoculum of one thousand. For our field trial this was of course rather unsatisfactory.

To improve on the technique used it was decided to try and cut down on the 'toughness' of the conditions. Inhibitors were reduced or removed, which resulted in a far higher rate of survival of Salmonella. Unfortunately far more of the other bacteria survived too, though this was cut down to a limited extent by incubating the bacteria at 43°C., a temperature 'disliked' by many bacteria but quite suitable for Salmonella. Greater emphasis was placed on developing a nutrient medium which would demonstrate certain of the characteristic metabolic features of Salmonella. One of these - the ability to reduce sulphur - was useful, as a sulphate source (e.g. thiosulphate) when reduced in the presence of lead ions (e.g. lead chloride) would produce a black precipitate of lead sulphide where the Salmonella colony was growing. Another useful property was the ability or otherwise of the bacteria to use certain sugars (i.e. lactose) as a food source. The sugar and a pH indicator are included in the medium, and if the sugar is used acid is produced which changes the colour of the indicator.

With so many different sorts of techniques it was found impossible to combine them on a single nutrient agar plate. To overcome this, use was made of replica plating using a velvet pad which allowed an exact pattern of a colony to be transferred onto another plate. Even so, no truly satisfactory or practical medium was found though I believe that the work is paying off some dividends now.

Another approach which is quite interesting though not entirely practical was the use of fluorescent microscopy. An antibody to a certain bacterium was produced (e.g. S. dublin) and then labelled with a fluorescent dye. On washing a culture of bacteria through with this labelled antibody the latter became attached to the antigen produced by that specific bacterium These all then became attached to a fluorescent dye which under a suitable microscope fluoresces brightly. In theory this enables Salmonella to be picked out from sectioned tissue, but the technicalities involved are quite complex.

It was at this point that I left the team which is why this account has no true beginning or end. However, the work has been very useful to me personally, giving me a good insight into what research is all about. In this respect I am sure that it would be very useful to anyone who gains a university place in science at Christmas to try and get a similar position in a research team.


GAYTONIAN

# Gaytonian

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# contents

# NATURE

Ever since Wordsworth first wandered lonely as a cloud among the daffodils, poets seem to have concentrated on Nature for their inspiration. So it is with some of our contributors, too, but they seem to look at poor old Mother Nature from a rather unusual angle. We have Nature Red in Tooth and Claw, Nature and Realism, and even a hieroglyph.

## Natural History Society

Anyone who was unfortunate enough to enter the Biology Laboratory on a Wednesday afternoon last year would have found a huddle of enthusiasts busy on tasks such as cleaning out rat-cages, or replenishing the grass supply that formed the diet for the swarm of locusts. The most successful projects were probably the keeping of small mammals, and the breeding of budgerigars in the laboratory. Projects undertaken for "A" Level work included keeping a hive of bees, charting the development of a tadpole, and unsuccessfully attempting to install a moth trap on the roof of the school (perhaps the moths were in collaboration with the school authorities). A field trip was arranged to Brent Valley Bird Sanctuary at Ealing.

Talks by visiting speakers are always popular, such as the one by the author, Mr. Richard Fitter, on "Wild Life in Danger". Mr. Brian Tricker of Eton College showed a film, "The Master Mind", consisting of highly amusing shots of some of his boys in unlikely situations, such as tight-rope walking, or going to bed with an apparatus designed to show how much one moves in the night. Mr. J. Maynard Smith, now Dean of the Biology Faculty at the University of Sussex, gave a highly interesting lecture on the Evolution of Altruism, which baffled many of the audience, even though they tried to look intelligent.

Already this year, we have been asked to exhibit in the National Exhibition of the Association of *School* Natural History Societies.

Paul Nurse, Secretary.