A BACTERIOLOGICAL STUDY OF HOSPITAL DIARRHOEA

WITH SPECIAL REFERENCE TO

CLOSTRIDIUM WELCHII FOOD-POISONING

by

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The consumption of food contaminated with potentially pathogenic organisms is a risk to which mankind in general is subjected. However, no one is more liable to suffer ill effects therefrom than the patient in hospital, whose resistance to infection may be considerably lowered.

A newspaper article [The Bulletin, (Glasgow), Nov. 2, 1955, page 5] accusing hospital managements in general of buying cheap and, by an inference of the writer of the article, dangerously contaminated food, stimulated an inquiry to find out the incidence of potentially pathogenic organisms in the food bought by one particular hospital (The Western Infirmary of Glasgow).

This investigation was also made necessary by the concern of the clinical staff about the incidence of 'hospital diarrhoea'. By hospital diarrhoea the clinicians concerned referred to non-specific forms of gastrointestinal disturbance of different degrees of severity from which specific intestinal pathogens (salmonellas and shigellas) were not isolated. Routine bacteriological laboratories usually examine specimens of faeces for intestinal pathogens of the Salmonella and Shigella genus only, but two other possible bacterial

causes of food-poisoning are widely distributed in hospital environments - namely Clostridium welchii and Staphylococcus aureus. Therefore, I decided to extend the laboratory examination of faeces from hospital patients to include Cl. welchii, especially of the 'nonhaemolytic' food-poisoning variety, and Staph. aureus.

My investigation of 'hospital diarrhoea' included also a bacteriological study of cases of necrotising enterocolitis. Specimens from some such cases were inevitably sent to me because one of the first signs of this disease is often a watery diarrhoea. Because there is a wide range of opinion and great uncertainity about the aetiology of necrotising enterocolitis I carried out a bacteriological examination of all the fatal cases which came to autopsy during the period of this study, in order to determine as far as possible whether any particular bacteria seemed to predominate in individual cases or to appear with regularity in different cases.

The main purposes of this thesis are to report:-

1) the ascertained degree of contamination of hospital food with potentially pathogenic organisms;
2) the fact of relatively frequent contamination of 'cold chicken' cooked in hospital with Clostridium welchii with reference to the role of this food in producing Cl. welchii food-poisoning in hospital;

3) the results of the bacteriological investigation of Cl. welchii food-poisoning outbreaks in hospital;

4) the incidence of Cl. welchii and Staph. aureus in the faeces of hospital patients as ascertained during the present investigation of hospital diarrhoea; and

5) the bacteriological findings in cases of necrotising enterocolitis.

Practically all of the work in this thesis was done without scientific collaborators other than my supervisor, Professor J. W. Howie. When the work involved collaboration with a clinician or a pathologist the details are reported in the text.

REVIEW OF LITERATURE

REVIEW OF THE LITERATURE

BACTERIAL CONTAMINATION OF FOOD

Tanner (1944) compiled a comprehensive account of the microbiology of a wide range of foods, and gave detailed methods for examining the different items. From his review it is evident that food, especially uncooked food, is liable to be contaminated with a wide variety of micro-organisms; according to their numbers and nature, these may render the food unsuitable for human consumption. Despite the laws and regulations governing the inspection, hygiene, manufacture, and storage of foods, however, it is on occasions difficult to interpret the significance of bacterial contamination, and before a final assessment is made it is necessary to consider all the evidence including the source and nature of the food and the methods involved in the treatment and processing of the product before its consumption.

Large numbers of nonpathogenic bacteria may affect the keeping quality and lead to spoilage, but although this may render the food unpalatable, putrefaction does not necessarily give rise to toxic substances. Indeed there are many examples, especially in cheese-making, in which putrefaction is an intrinsic quality of the final product. On the other hand, it is an almost universally accepted standard that pathogenic bacteria

should not be tolerated in foods. Thatcher (1955), however, gave examples in which he considered that this standard should not invariably apply. Organisms such as Clostridium welchii and Staphylococcus aureus are found almost everywhere; so foods cannot escape them. This point was emphasised by Dack (1955) in his review on the significance of enteric bacilli on food. He urged public health authorities to accept it that our environment and non-sterile foods in our habitat contain enteric bacilli regardless of our efforts to eliminate them. Similar arguments were used by Appleman (1955) regarding the origin and significance of faecal bacteria in citrus juices. All aspects considered, however, the condemnation of food is justified when extensive contamination is established.

Bacteria causing food-poisoning

Food may act as a vector in the transmission of many diseases. The role of milk and dairy products in the dissemination of tuberculosis and brucellosis is well recognised. Food-borne outbreaks of dysentery (Savage, 1938), streptococcal infection (Boissard and Fry, 1955), and typhoid and paratyphoid fevers (Thomson, 1953; Newell, 1955; Wilson and MacKenzie, 1955, Couper, Newell and Payne, 1956) have also been reported. Although the term 'bacterial food-poisoning' properly includes such food-borne infections, it is generally restricted to acute

gastro-enteritis following fairly soon after the ingestion of certain species of bacteria or their products in food or drink. An exception to this definition is botulism in which the gastro-intestinal symptoms are a minor component of the disease. Food-poisoning, including that due to chemicals and poisonous plants and animals, was well reviewed by Dack (1956a).

The organisms against which the evidence of their causative role in food-poisoning seems definite are Clostridium botulinum types A, B & E (Meyer, 1956), members of the Salmonella group (Savage, 1956), enterotoxin-producing strains of Staph. aureus (Dack, 1956a), nonhaemolytic heat-resistant strains of Cl. welchii (Hobbs, Smith, Oakley, Warrack and Cruikshank, 1953), certain strains of Streptococcus (Moore, 1955) and Bacillus cereus (Hauge, 1955). Evidence has also been presented which suggests the implication of coliforms, proteus, paracolons and pseudomonas but the aetiological relationship has not been established (Taylor.J, 1955.) It may be that any organism that grows abundantly enough in food may be a potential danger. The symposium held by the Society for Applied Bacteriology (1955) discussed these and many other problems associated with food-poisoning.

Botulism and staphylococcal food-poisoning are examples of food-poisoning in the true sense; both are due to a toxin produced by the growth of the

causal organism in the food. The signs and symptoms are the result of the action of the preformed toxins, and living organisms may be absent when the food is eaten. The other organisms involved in food-poisoning, however, must be present in the food in large numbers when it is eaten. The actual numbers required to produce symptoms in man depend on the species, strain, food vehicle, and the human subject.

A good indication of the role of the different organisms as causes of food-poisoning in Britain is given by the reports of the Public Health Laboratory Service (1954; 1955; 1956). These reports cover general outbreaks, family outbreaks and sporadic cases which were investigated by the P.H.L.S. in the years 1951-1955. From their figures it is apparent that salmonellas, staphylococci and Cl. welchii are most often incriminated.

Foods associated with food-poisoning

The foods involved in bacterial food-poisoning are usually those in which the causative organisms can grow. It is clear that meat and meat products (including poultry) figure most prominently as causal agents (P.H.L.S. 1954; 1955 and 1956). Their importance was also stressed in the World Health Organisation's publication on Meat Hygiene (1956). This book provides a good review of the diseases which may be spread by

meat and its products, and deals thoroughly with preventive measures which should be taken in the processing, examination and condemnation of such products. On p. 236 of the W.H.O. publication Jepson stated that 'although, theoretically, any kind of food may deteriorate or acquire harmful qualities through contamination, infection, or development of toxic substances, practical experience has shown that meat and meat products much more frequently present important problems of food hygiene.'

For these reasons meat and meat products have been incriminated in all types of bacterial food-poisoning except for that caused by B. cereus in which the customary vehicles have usually been starchy foods. Cl. welchii outbreaks particularly have been characteristically associated with meat dishes (McClung, 1945; Hobbs et al, 1953; Beck, Foxell and Turner, 1954; Collee, 1955; Taylor C, 1955; Linzenmeier, 1956; Norval, 1956; Smith and Wallace, 1956; Norval and Collee, 1957). Meat and its products are also important in the transmission of salmonella and staphylococcal food-poisoning. Galton, Lowery and Hardy (1954) in America investigated the presence of salmonella in fresh and smoked pork sausages and found 23% and 12.5% positive respectively, and Miller and Ramsden (1955) described two outbreaks of salmonella infection due to meat pies.

Fresh fish is often blamed but in truth rarely involved in outbreaks of food-poisoning, and in this country fish in all its forms appears to be one of the safest items of diet. Food-poisoning, including type E botulism, caused by fish and fishery products was reviewed by Shewan and Liston (1955). They found that the majority of authenticated cases attributable to fish have been caused by processed products such as fish cakes, pastes and patties contaminated with Cl. botulinum, salmonellas, staphylococci or Cl. welchii.

Salmonella food-poisoning has often been associated with duck and hen eggs. The P.H.L.S. reports indicate that duck eggs have been more frequently the causal agents but outbreaks due to the eating of infected hen eggs have also been reported (Watt, 1945; Jellard, 1956). During the years 1951-1953 Murdock (1954) examined 249 batches of duck eggs and 1,010 batches of hen eggs collected from various parts of Northern Ireland. Salmonellas, usually Salm. typhimurium, were isolated from 48.2% of the batches of duck eggs and 2.1% of the hen eggs. An interesting complication of the infection of eggs was noted in the spread of salmonellas by the exportation of American dried egg (see for example Lundbeck, Platzikowski and Silverstople, 1955). In addition Semple, Davies and Parry (1956) reported the possible dangers involved in the importation of Chinese egg albumen containing salmonellas.

Foods such as custards and creams may thus prove to be a vehicle of transmission of salmonella food-poisoning due to the use of imported egg products in their manufacture. Sweatmeats are also involved on occasions because of the contamination of dairy products. Synthetic creams figure frequently as a vehicle for staphylococcal as well as salmonella food-poisoning (Hobbs and Smith, 1954). In fact in most staphylococcal outbreaks the foods concerned have been milk products (Wilson and Miles, 1955 p.1809). Hobbs (1955) and Anderson and Stone (1955) gave details of outbreaks of staphylococcal food-poisoning in which the causative agents were associated with dried milk. On the other hand Jones, King, Fennel and Stone (1957) investigated staphylococcal food-poisoning due to raw milk and found it to be necessarily uncommon as the chances of milk becoming toxic within 24 hours were practically negligible. Norton and Armstrong (1954) reported a large outbreak due to Salm. typhimurium in which the vehicle of infection was a raw tuberculin-tested milk supply. Outbreaks of disease caused by pathogenic organisms in dairy-products were reviewed by Smith (1956) who considered that cheese as a vehicle for such infections is widely underestimated. Although home-cured vegetables have been responsible for the greatest number of cases of botulism in the United States (Dack, 1956a p.61), fruits and vegetables have seldom been incriminated in food-

poisoning caused by other bacteria.

Mode of infection of food and distribution of food-poisoning organisms in nature

Contamination of food may take place both in its living state - the meat of an infected animal, for example - or during the handling and processing before it is consumed. Hobbs (1954) gave a comprehensive review of the potential reservoirs of food-poisoning in various types of food. For convenience in the present work each of the causal organisms is considered separately.

Cl. botulinum types A and B are widely distributed in soil, both virgin and cultivated. Foods of plant and animal origin are frequently contaminated with soil, and the organisms in this way gain access. The distribution of Cl. botulinum type E, which has usually been associated with food-poisoning due to fish, was reviewed by Pederson (1955). It seems possible that the fish may be contaminated at the source because type E has been isolated from the intestines of sturgeon and perch (Shewan and Liston, 1955).

The species of Salmonella which cause food-poisoning in man commonly produce intestinal and septicaemic diseases in birds, pigs, rats, mice, cats, and dogs. Outbreaks traced to a human carrier have been reported

(Boyer and Tissier, 1949) but on the whole the part played by human carriers in spreading salmonella food-poisoning is slight compared to that attributable to animals. Smith and Buxton (1951) found salmonellas in 16 of 650 turkeys, 2 of 100 geese, 6 of 500 ducks and 5 of 750 chickens. In America, Galton, Smith, McElrath and Hardy (1954) discovered salmonellas in 25 of 100 faecal specimens of pigs in the slaughterhouse whereas Murdock and Gordon (1953) found that the incidence of Salm. dublin in the faeces of 1000 apparently healthy cattle in Northern Ireland was 8.6%. From these figures it is evident that birds, pigs and cattle are frequent reservoirs of salmonellas, and their products eggs, meat. and milk may be infected at their source. Further spread from infected carcasses takes place in the abattoirs and processing stations; this aspect was authoratively discussed in Meat Hygiene (World Health Organisation, 1957), and the spread of organisms during the commercial processing of poultry was reviewed by Walker and Ayres (1956).

The faeces of infected rodents and domestic animals also provide ample opportunity for the contamination of human food with salmonellas. Khalil (1938) isolated salmonellas from 7.3% of wild rats in Liverpool and Ludlam (1954) reported an incidence of 4.4% in rats killed in the Nottingham area. Ludlam (1954) also investigated the incidence of salmonellas in rats in a

butchers' by-products factory. From 1949 to September 1953, 6.4% of 94 rats were positive; in the last three months of 1953 the incidence rose to 40% of 60 rats examined and in the first 4 months of 1954 the incidence was 27.6% of 29 rats. The high incidence of salmonellas coincided with the overloading of the factory with offal from the autumn killings. The author concluded that a large variety of salmonellas was brought into the factory on the offal. Delay in processing the offal led to an increase in the rat population and to the multiplication of salmonellas in the infected material. Thus the rats became infected with salmonellas and conditions favoured the transmission from rat to rat and reinfection of the offal by the rats. These figures serve to show the important role which rodents may play in the contamination of food with salmonellas. Hardy and Galton (1955), in addition to reviewing the potential sources and means of spread in poultry farms, abattoirs, and dried-egg factories, discussed more recent studies on the prevalence of salmonella infections in domestic animals. In 1952, Galton, Scatterday and Hardy found 27.6% of 8157 cultural examinations of dogs, positive for salmonellas.

Some outbreaks of salmonellas food-poisoning have been attributed to non-animal products; one such outbreak, in which dried yeast was the source, was reported by Kunz and Ouchterlony (1955). By eliminating other sources,

the contamination of the yeast was discovered to have been introduced from the air outside the building through a ventilation duct. The authors considered that this outbreak was of interest because it revealed the possibility of air-borne contamination of manufactured products.

In staphylococcal food-poisoning, unlike salmonella food-poisoning, the human carrier is extremely important and contamination of the food during processing and preparation for consumption is responsible for the majority of reported outbreaks. A good account of the epidemiology of staphylococcal food-poisoning is given by Lack (1956a). About 30 to 60% of normal persons carry coagulase-positive Staph. aureus in their anterior nares and a proportion of these people carry the organisms on their hands. In addition, staphylococci are the usual cause of boils and septic lesions. From these sources, therefore, Staph. aureus is inevitably a frequent contaminant of food. Milk may also become infected at its source because of an infection of the cow's udder but staphylococci found in cow's milk are generally, though not always, harmless and not food-poisoning strains. Not all strains of coagulase-positive Staph. aureus are capable of producing food-poisoning; because there is not a convenient or reliable method of testing for enterotoxin production it is difficult to determine which strains are potentially dangerous in foodstuffs. From experience, however, it appears that enterotoxigenic

strains are widely distributed and may readily gain access to food from food handlers and dust.

Cl. welchii is a normal inhabitant of the soil and of human and animal intestines. From these sources it is widely distributed in dust, sewage, and blow-flies and hence to meat, water and milk. The reservoirs of non-haemolytic heat-resistant Cl. welchii, the form of the organism which has hitherto been almost exclusively considered as the cause of Cl. welchii food-poisoning, are not fully known; but a review of the potential sources was given by Hobbs et al (1953). These authors found that 2.2% of 45 healthy children and adults carried heat-resistant Cl. welchii. The faecal carrier rate in pigs was 18.4%, rats and mice 14.6%, and cattle 1.7%; they also isolated heat-resistant strains from blow flies. There is therefore ample opportunity for food to become contaminated from both human and animal sources. Hobbs and her associates also considered that meat may become contaminated by the invasion of intestinal organisms during life but it is still a matter of conjecture whether or not this takes place. Their examination of raw meat revealed that the incidence of heat-resistant Cl. welchii was 20%, 24.1%, 14.3% and 0% in samples of pork, beef, veal and lamb respectively but it is difficult to assess whether this contamination took place before or after death.

Streptococcus faecalis, strains of which have been

incriminated in outbreaks of food-poisoning (Sherman, Gunsalas and Bellamy 1944), is found in human and animal faeces; and B. cereus is reported to be the commonest aerobic spore-bearer in the soil (Wilson and Miles; 1955, p.965). Food may therefore be contaminated with these organisms during its preparation for consumption. Their true significance in food-poisoning remains to be assessed.

The survival and development of food-poisoning organisms on food

The survival and development of food-poisoning organisms on food depends on such things as the nature of the food, methods employed in its preparation for consumption, methods of storage, and the nature of the contaminating organism. Engley (1956) discussed briefly the methods by which food may become contaminated and considered the subsequent survival and multiplication of the organisms in the food. He abstracted from an extensive bibliography the survival times of individual species in a variety of foods. This would have been a valuable piece of work if the author had shown or even critically discussed the significance of any part of this accumulated evidence on food hygiene.

The chemical and physical nature of a particular food influence both the survival and subsequent growth of contaminating organisms. The low-acid and medium-acid

products are most favourable to the growth of food-posioning organisms. Winter, Wieser and Lewis (1953) and Wieser, Winter and Lewis (1954) studied the growth of salmonellas and Staph. aureus in chicken salad. They found that the acidity, caused by the vinegar in the salad dressing, retarded the growth of both species of organism although Staph. aureus grew readily on dried chicken held at from 21 to 27°C. Certain inhibitory substances may also be present in natural raw foods. For example Ayres and Taylor (1956) discovered that with the exception of Serratia marcescens, organisms responsible for the spoilage of eggs and Salm. pullorum did not become established quickly in egg albumen due to the presence of inhibitory factors. Once the organisms reached the yolk, however, they reproduced rapidly.

The survival of organisms in food depends to a large extent on whether or not the food is heated or cooked after contamination. Sporing organisms such as Cl. botulinum, Cl. welchii, and B. cereus are more likely to survive heating and cooking than salmonellas and staphylococci. The nature of the food affects the ease with which it is sterilised by heat. Solid foods such as large pieces of meat are not uniformly heated and the penetration of heat may not be sufficient at the centre to produce a high enough temperature for a sufficient period of time to kill contaminating organisms.

The pH of the food and other undetermined factors also affect the sterilisation. Osborne, Straka and Lineweaver (1954) studied the heat resistance of 18 strains of salmonellas in liquid whole egg, egg yolk, and egg white. They found that the heat-resistance was greater near pH 5.5 than above pH 7. These workers also stressed the difference in heat-resistance of individual strains of salmonellas; strains of the serotype Salm. seftenberg were particularly marked in heat-resistance. Similar results were reported by Anellis, Lubas and Rayman (1954). Hussemann and Bryske (1954) made a study of the effect of heat on the survival of Salm. typhimurium in chicken muscle. They observed that this organism appeared to survive higher temperatures in chicken muscle than that reported for its destruction in other products. However, they considered the conditions of the experiments were stringent and that it might not be possible to make direct application of their results to more natural situations.

Cl. botulinum and enterotoxigenic strains of Staph. aureus grow in food and therein produce their harmful toxins. These toxins are more heat-stable than the vegetative organisms, and so if heating food is to make it safe the temperature must be high enough to destroy the toxins.

The growth of organisms which survive cooking and are inoculated after cooking depends on suitable conditions

for the individual organism. Cl. botulinum and Cl. welchii are anaerobic organisms; cooked meat and such foods provide admirable media for the proliferation of anaerobic organisms because of their low oxygen content, high content of reducing substances, and absence of non-sporing competitors. If food-poisoning organisms are present on a suitable foodstuff subsequent growth and formation of any toxins will take place only if the food is left for long enough at a favourable temperature. Room temperature, especially that of a warm kitchen, is ideal for the growth of all species of food-poisoning organisms and this may be enhanced if the food is cooked and left to cool slowly. The temperatures attained in refrigerated foods are usually adequate to prevent the growth of food-poisoning organisms.

Some foods such as dairy products and sweetmeats are prepared and eaten without further cooking. The temperatures at which such foods are kept are important as they are so frequently contaminated with salmonellas and staphylococci. Pullinger and Kemp (1938) found that salmonellas grew well in both raw milk and commercial grades of heat-treated milks and survived for at least 2 months when incorporated in salted and unsalted butters. Such foods constitute a great hazard when stored at temperatures suitable for bacterial growth.

Control of bacterial food-poisoning

Commercial food-preservation makes use of a number of methods to reduce bacterial contamination and to ensure the preservation of food. These methods include (1) addition of chemical substances, (2) low temperatures, (3) high temperatures e.g. canning, (4) fermentation and (5) abstraction of moisture. These procedures are reviewed by Tanner (1944) and Baumgartner(1946).

The hygienic precautions necessary to prevent food-poisoning, however, concern the whole course of the food from its source - the carcase of the slaughtered animal, for example - to the final preparation for consumption. Murrell (1955) provided an excellent review of the methods of food-handling which would reduce the possibility of food-poisoning and spoilage.

Initially a thorough system of meat inspection is essential and the meat of animals that are ill or "emergency-slaughtered" should as a rule be condemned. The measures taken to ensure hygienic treatment of meat and the cleanliness of premises where made-up meat is prepared are dealt with thoroughly by the report of the World Health Organization (1957). Even careful veterinary inspection before and after slaughter, however, will not detect the healthy animal carrier, and raw meat and raw milk must be regarded as potentially infective for man and should not be eaten without thorough cooking.

Similarly salmonellas in eggs survive light cooking, and as duck eggs are frequently contaminated they should be boiled for at least 15 minutes (P.H.L.S. 1954, p.38).

High standards of hygiene must be observed in commercial catering establishments. Hobbs and Kluth (1950) and Wilson and Carter (1955) surveyed the kitchens of catering establishments and discussed measures which should be taken to improve the conditions. Attention must be paid to good sanitation, the use of easily cleaned equipment, prevention of and protection from dust, handling methods which prevent the carry over of material from previous batches, installation of fly screens and control of rodents. Strict control of hygiene by food handlers must be observed and known human carriers of infection excluded.

Cooking does not necessarily sterilize food. All food in which bacteria can grow readily - such as meat, fish, milk, cream, and custard dishes - should be kept cold till cooking. If the food is not to be eaten immediately after cooking it should be cooled quickly and kept preferably in a refrigerator. Food-poisoning organisms such as Cl. welchii and Staph. aureus are ubiquitous and thus are frequent contaminators of food. Stringent regulations are therefore necessary to ensure adequate heating and subsequent refrigeration of the food.

Recent advances have been made into the use of antibiotics for the preservation of meat and fish. The problems involved in their use have been discussed by Ingram and Barnes (1955) and Ingram, Barnes and Shewan (1956). Although it appears that the use of antibiotics could extend the storage life of some foods, the medico-legal aspects are dangerous and these authors considered that their use is undesirable.

'HOSPITAL DIARRHOEA'

The aetiology of diarrhoeal diseases is widely varied and in many cases unknown. Hardy (1956) discussed this in relation to the difficulties involved in public-health control and stressed the important role which diarrhoeal diseases play in the illnesses of man. Diarrhoea which affects hospital patients is no exception; the causal agent is very often not diagnosed and the danger incurred by an attack of diarrhoea in a patient whose general condition may be low and whose powers of recovery may be impaired is often serious.

Due to the high risk of cross-infection within a closed community with many highly susceptible members, outbreaks caused by salmonellas and shigellas are well known hazards of hospital patients. Diarrhoea caused by these organisms, however, is invariably diagnosed because faeces are routinely examined for salmonellas and shigellas.

On the other hand, diarrhoeas from which no specific intestinal pathogen is isolated are usually termed 'hospital diarrhoea' or some other non-specific term and no further attempt is made to diagnose them. Many factors such as mental stress, the effect of the drugs used in treatment, and the direct effects of surgery may contribute to the signs and symptoms of gastro-intestinal disturbance in a patient, but these are out-with the scope of the present review. However, the bacteriological examination of faeces does not always include techniques for the isolation of Cl. welchii and Staph. aureus and consequently diarrhoea caused by these organisms may often be included in the term hospital diarrhoea. These two organisms are widely distributed in hospital environments; their possible role as causative agents of diarrhoea is therefore discussed.

The role of Cl. welchii

As early as 1895, Klein reported the isolation of Cl. welchii from the stools of patients suffering from diarrhoea in two outbreaks involving 59 and 144 cases. It is only in recent years, however, that more definite evidence for their being involved in bacterial food-poisoning was presented. Before Hobbs et al (1953) published their excellent review of Cl. welchii food-poisoning, outbreaks were reported relatively infrequently (Knox and Macdonald, 1943; McClung, 1945). Since 1953,

however, more attention has been drawn to Cl. welchii as a causal agent of food-poisoning and many outbreaks have been recorded in the literature (Beck et al, 1954; Collee, 1954 and 1955, Taylor.C,1955; Linzenmeier, 1956; Norval and Orr, 1956; Smith and Wallace, 1956; Dickie and Smith, 1957; Norval and Collee, 1957; McNicol and McKillop, 1958). From these accounts it is evident that an outbreak of Cl. welchii food-poisoning follows a typical pattern.

About 8 to 22 hours after eating contaminated food, the affected persons develop acute abdominal pain and diarrhoea; nausea and vomiting are rare; and pyrexia, shivering, headache and other signs of infection are seldom present. The illness is usually of short duration and the patient recovers by the next day. The medium of infection is almost invariably a cold or warmed-up meat dish (see p. 8) made from meat which has been boiled, steamed, or stewed for 2-3 hours on the day before it is required and allowed to cool slowly overnight. When eaten the meat is usually appetising, and a bad smell or unpleasant taste is not observed.

There is some indication that individuals differ in their susceptibility to Cl. welchii food-poisoning; all at risk are not usually affected. Likewise all strains of Cl. welchii are not equally capable of producing food-poisoning; this is clear from the different results reported by different workers when they attempted to

produce Cl. welchii food-poisoning experimentally. McClung (1945) reported that a single volunteer showed typical symptoms after eating a sample known to be contaminated with a strain of Cl. welchii isolated from an outbreak of food-poisoning. Similarly, Hobbs et al (1953) observed mild food-poisoning similar to that seen in natural epidemics in 2of3 volunteers after the ingestion of a culture of heat-resistant Cl. welchii isolated from meat involved in an outbreak. On the other hand, Dack, Sugiyama, Owens and Kirsner (1954) examined the potentialities of 4 of McClung's strains isolated from food-poisoning by feeding the organisms or their products of growth or both together to human volunteers. They reported that, under the experimental conditions employed, the organisms were incapable of producing food-poisoning symptoms. Dische and Elek (1957) carried out further feeding experiments to elucidate the position and found that three heat-resistant strains tested gave different results. One produced food-poisoning symptoms in most of the volunteers, the second gave indefinite results, and the third appeared to be inactive. They suggested that virulence may be lost in subculture and that this may account for the negative findings of some workers with human volunteers. From the results of their experiments Dische and Elek (1957) also postulated that Cl. welchii food-poisoning is due to a mild transient infection; they found that bacterium-free culture filtrates

and cultures which had been heated to 100°C did not produce symptoms whereas live cultures and suspensions did.

Since the description of Hobbs and her colleagues (1953) of the causal organism as a non-haemolytic heat-resistant variety of Cl. welchii type A, subsequent reports have incriminated similar organisms. These organisms may be isolated from the food and faeces of the affected people. Before 1953, however, details of the haemolysis and heat-resistance of strains isolated in outbreaks were often omitted. In addition Hobbs et al (1953) reported two outbreaks in which the causal organism was a haemolytic Cl. welchii.

In addition to the role of Cl. welchii in food-poisoning, the possibility of an endogenous infection must be considered (MacLennan, 1956). The presence of haemolytic Cl. welchii in stools with reference to its possible implications as the cause of diarrhoea was reviewed as early as 1911 by Kendall and Smith. More recently Howie, Duncan and Mackie (1953) reported a high incidence of haemolytic Cl. welchii in the stomach after partial gastrectomy and Duncan, Goudie, Mackie and Howie (1954) isolated such organisms in large numbers from 5 cases of post-gastrectomy diarrhoea. These results suggested that indigenous Cl. welchii type A may sometimes act as a human intestinal pathogen.

The role of Staph. aureus

Staph. aureus has been recognised as a causative agent in food-poisoning for many years. Dack (1956a) in his authorative review of staphylococcus food-poisoning quoted a reference from as early as 1884 which indicated that round spherical bodies were the cause of food-poisoning after eating cheese. Similar discoveries were made on a number of occasions and the role of staphylococci was finally accepted after 1930 when Dack, Cary, Woolpert and Wiggers reported successful experiments with human volunteers. Since that date, outbreaks of staphylococcal food-poisoning have been reported frequently and Dack (1956a) considered that in America it was probably the commonest of all food-poisoning.

Staphylococcal food-poisoning is caused by the ingestion of preformed toxin and the conditions necessary for an outbreak are as follows: contamination with enterotoxin-producing staphylococci of a suitable food in which the organism can grow and the keeping of this food for a sufficient time at a temperature compatible with growth and toxin production. The symptoms appear 1 to 6 hours after ingesting the food containing the enterotoxin, the incubation period being influenced by the amount of enterotoxin ingested and the susceptibility of the individual. The signs and symptoms are usually

nausea, vomiting, abdominal cramp and diarrhoea, accompanied by marked prostration. Headache, muscular cramp, sweating and either a high or a subnormal temperature may be present in the more severe cases. Recovery is usually rapid in from 1 to 3 days, depending on the severity of the attack. Dairy products are frequently the vehicle of infection (see p. 10) but meats such as ham and tongue and made-up meat dishes are also frequently incriminated. The proof of the causative role of staphylococci in food-poisoning rests upon experiments with human volunteers; this aspect was well discussed by Dack (1956a). Similarly it is now generally accepted that the capability of individual strains to produce enterotoxin can only be demonstrated conclusively by the use of human volunteers. No convenient laboratory test for the detection of enterotoxin exists and the use of laboratory animals has proved of no value. The susceptibility of kittens to intraperitoneal injection was claimed to be diagnostic but further work has determined this to be of doubtful significance.

Evans and Niven (1950) studied a number of strains of staphylococci and found that, without exception, the enterotoxigenic strains comprised an extremely homogeneous group. All enterotoxigenic strains produced coagulase but not all coagulase-positive strains produced enterotoxin.

On the other hand, all coagulase-negative strains studied by these workers failed to produce enterotoxin. More recently, bacteriophage-typing has shown that all strains of Staph. aureus associated with food-poisoning belong to 'phage group III (Williams, Rippon and Dowsett, 1953; Anderson and Williams, 1956).

In addition to their role as a causative agent of food-poisoning, staphylococci, are also implicated in diarrhoeas, usually after antibiotic therapy. The source of such staphylococci may either be the patient, if he is a nasal or faecal carrier, or from external reservoirs in the hospital. Antibiotic-resistant staphylococci are widespread in hospital environments (Hinton and Orr, 1957) and have therefore easy access to patients. During the course of antibiotic treatment such strains are selectively propagated and have thus been reported to cause severe enteritis (Fowler, 1955; Dearing and Heilman, 1953; Dearing,1956). Cook, Elliott, Elliot-Smith, Frisby and Gardner (1957) considered that Staph. aureus was the chief cause of post-operative diarrhoea in the Radcliffe Infirmary at Oxford. Staph. aureus has also been associated with necrotising entero-colitis; this is reviewed fully below.

FAECAL CARRIAGE OF CL. WELCHII AND STAPH. AUREUS

Haemolytic Cl. welchii are normal inhabitants of the human bowel and as such no great importance has been placed on their presence in faeces and no figures relating to their actual incidence appear in the literature. The relatively recent incrimination of nonhaemolytic heat-resistant Cl. welchii as causal agents of food-poisoning, however, has led to a few reports on the faecal incidence of these organisms. Hobbs et al (1953) studied different groups of people and reported that the faecal incidence of heat-resistant Cl. welchii in the individual groups was, 2.2% in 43 healthy adults and children, 2.1% in 142 miscellaneous cases of diarrhoea, 3.8% in 80 people involved in an outbreak of sonne dysentery, 7.7% in 65 cases of food-poisoning other than Cl. welchii and 15.1% in 53 old people in hospital (with and without diarrhoea). The figures reported by Dische and Elek (1957) are somewhat higher than the respective figures quoted by Dr. Hobbs and her colleagues. Dische and Elek examined the stools of 50 healthy people (hospital staff and their families) and 85 unselected hospital patients with and without diarrhoea. The proportion of positives varied from 12% in patients with salmonella, shigella and E. coli infections to 20% in the healthy, with an

overall average of 15.6%. These authors found a similar incidence in cases of experimental food-poisoning (97%) to that reported by Hobbs et al in cases of naturally occurring food-poisoning (89.9%).

More attention has been drawn to the carrier rate of Staph. aureus in faeces because Staph. aureus has long been recognised as a dangerous organism in hospital environments. The risk of cross-infection and wound sepsis has stimulated investigations into all possible reservoirs of Staph. aureus. Nasal carriers are important sources but Brodie, Kerr and Sommerville (1956) considered that faecal carriage plays a more important part in communicating staphylococcal disease than hitherto realised. These authors found that the faecal carrier rate of serotype ac/- increased sevenfold from 5 to 35% after the admission of patients to hospital. Matthias, Shooter and Williams (1957) confirmed the findings of Brodie et al (1956) on the frequency of Staph. aureus in the stools. They found that 23% of 142 patients who had stools examined within 7 days of admission to hospital were positive for Staph. aureus and noted that there was some indication that the frequency of stool carriage increased with the time the patient had been in hospital although no actual figures were recorded to support this view. Moustardier, Dulong de Rosnay and Tournerie (1957) reported that 30% of 63 normal stools of hospital patients yielded

staphylococci whereas 37% of 73 diarrhoeal stools were positive. Similarly Neuman, Meitert, Bocirnea, Friedman and Dediu (1957) determined that 1.5% of 64 patients with various illnesses not involving the alimentary tract yielded staphylococci compared with 12.5% of 96 patients suffering from food-poisoning (not thought to be due to Staph. aureus) and other diarrhoeal diseases. Oeding and Austrarheim (1954) examined the intestinal contents of patients who died from various causes (not necrotising enterocolitis) and found incidences of Staph. aureus of 26% and 15% respectively in groups of patients who, before death, had and had not been given antibiotic treatment. From these reports it is apparent that different workers have found different incidences of Staph. aureus in the faeces; the reason for this is not known.

NECROTISING (PSEUDOMEMBRANOUS) ENTEROCOLITIS

Necrotising enterocolitis has been recognised by pathologists for over 50 years. Yet it is still not a well-defined entity and is often confused with staphylococcal enterocolitis in which there is no formation of necrotic tissue. It is characterised clinically by the sudden onset of acute peripheral, circulatory failure and profound shock which do not

respond to vigorous treatment. Abdominal pain and distension, diarrhoea, hyperpyrexia and anuria may be present but are not constant findings. The importance of this condition as a post-operative complication has been stressed by many workers including Prohaska, Govostis and Tabenhaus (1954) and Bruce (1955) but it has also been associated with a variety of non-surgical conditions of which heart disease is the commonest (Kleckner, Bargen and Baggenstoss 1952).

The outstanding pathological feature of necrotising enterocolitis is necrosis of the mucosal surface of the bowel wall, producing in some instances a distinct pseudomembrane. The pathological changes are distinct but not unique and can be produced in the gastro-intestinal tract by a number of causes, for example bacillary dysentery, chemical poisons (mercury, arsenic), and uraemia (Cappell, 1951). Clinically the diagnosis is presumptive unless a membrane is passed. To confirm the diagnosis it is therefore necessary to consider both the clinical and pathological features. One of the few recorded recoveries was reported by Williams (1954), the diagnosis being made after the patient vomited a large membrane cast.

The aetiology of pseudomembranous enterocolitis is by no means certain and various mechanisms have been postulated. From a study of 40 cases Penner and Bernheim (1939) concluded that the vasomotor

disturbances associated with shock were responsible for the lesions. Many authors, however, tend to regard shock as a consequence rather than the cause of the bowel lesions. Local vasomotor disturbances were considered by McKay, Hardaway, Wahle and Hall (1955) to cause the mucosal necrosis and these authors succeeded in producing widespread mucosal necrosis in the mucosal capillaries of the gastrointestinal tracts of dogs by introducing quantities of human blood into the aorta in the region of the mesenteric arteries. What relation the lesions produced in the animals bears to the condition of necrotising enterocolitis in man is purely speculative.

Necrotising enterocolitis has come into much greater prominence in recent years since the advent of the antibiotic era and the apparent increase has therefore been attributed to the use of antibiotics. However, Kleckner et al (1952) observed that there had been no increase in the incidence of acute, fatal pseudomembranous enterocolitis in the files of the Mayo clinic since the introduction of antibiotics and therapeutic agents in the treatment of intestinal disorders. Similarly Prohaska et al (1954) from their own experience doubted whether the preoperative use of antibiotics was a causal influence. Newman (1956), on the other hand, considered that antibiotics through

their antibacterial properties predispose the onset of pseudomembranous enterocolitis although many aetiological agents may lead to this condition.

Many of those who attribute the incidence of necrotising enterocolitis to the use of antibiotics believe that a super-infection of resistant bacteria is an essential part of the disease, although Helmer (1954) speculates that it may be an allergic reaction to antibiotics. Many accounts have been written incriminating Staph. aureus but it must be remembered that in numerous cases of staphylococcal enteritis following antibiotic treatment, the morbid-anatomical changes are not those of necrotising enterocolitis (see for example Fowler, 1955; Dearing and Heilman, 1953; Dearing, 1956). Great confusion has arisen from calling such cases necrotising enterocolitis.

Cases of true necrotising enterocolitis in which staphylococci were isolated have also been reported by many workers (Brown, Winston and Sommers 1953; Terplan, Paine, Sheffer, Egan and Lansky 1953; Todd and Hopps 1954; Williams, 1954; Corridan, 1956), hence the recent tendency to use the term 'staphylococcal pseudomembranous enterocolitis'. Surgalla and Dack (1955) reviewed 32 fatal cases of enteritis after antibiotic therapy and isolated enterotoxin-producing strains of Staph. aureus from 30. A pseudomembrane was present in some of the cases and the authors suggested that staphylococci may

actually proliferate and produce their enterotoxin within the gut. At a later date Dack (1956b) used the term 'pseudomembranous enterocolitis' to represent the more severe cases of 'micrococcic enterocolitis'.

On the other hand, Dearing and Heilman (1953) and Dearing (1956) recorded cases of pseudomembranous enterocolitis in which no staphylococci were present in the bowel and inferred that pseudomembranous enterocolitis and staphylococcal enterocolitis are two essentially unrelated conditions although they may occur together. Earlier reports on the failure to isolate Staph. aureus cannot be regarded as conclusive as they may very well have been overlooked through failure to employ appropriate techniques for their isolation.

A spontaneous outbreak of 'staphylococcal enterocolitis' in chinchillas which had been fed on pellets containing a small quantity of chlortetracycline was described by Wood, Bennet and Yardley (1956). These authors considered that the condition was similar to that of necrotising enterocolitis in man but the evidence presented was not altogether convincing.

A variety of Cl. welchii (Type F) has also been reported as the causative factor of necrotising enterocolitis. In 1946, Beckerman and Laas described several cases of a fulminating and frequently fatal variety of primary necrotising enterocolitis in

Hamburg. Filamentous rod-shaped organisms and chains of cocci were sometimes seen in the deep parts of the necrosis. The authors considered that the lesions were caused by something acting from within the lumen of the bowel. A similar condition was reported by Jeckeln (1947; 1948) from Lübeck and was named "Darmbrand". Hormann (1947), Fick (1947) and Fick and Wolken (1949) described probably the same condition from Hamburg as 'necrotic jejunitis'. The outbreaks appeared to be due to an infection associated with eating contaminated food of which meat and fish products were the commonest. Schutz (1948), Willich (1949) and Zeissler and Rassfeld-Sternberg (1949) performed further bacteriological tests on organisms isolated from cases of necrotising enterocolitis in Hamburg and found them to resemble Cl. welchii. They produced a large amount of β -toxin which was presumed to have caused the necrotic lesions in the gut and this variety was named Cl. welchii type F (Oakley, 1949). Feeding this strain orally to guinea-pigs was unsuccessful but when young cultures were injected directly into the lumen of the small intestine a condition indistinguishable from that in man was produced (Zeissler and Rassfeld-Sternberg, 1949). Outbreaks of this condition appear to have been confined to Northern Germany and there has been no evidence incriminating Cl. welchii type F as the cause of necrotising enterocolitis elsewhere. Paterson and

Rosenbaum (1952) in America, however, reported the isolation of Cl. welchii from the ulcers and heart blood of a fatal case of necrotising enterocolitis. Unfortunately no detailed study of the organism was made and this may have been no more than an incidental post-mortem finding.

From a review of the literature it is therefore evident that the aetiology of necrotising enterocolitis is by no means established. In all probability there is more than one possible cause leading to the same condition. The nomenclature used by different workers leaves much to be desired and it seems advisable to term the condition necrotising enterocolitis as opposed to pseudomembranous enterocolitis because a pseudomembrane is not always present whereas necrotic tissue is a constant finding. Likewise the term staphylococcal pseudomembranous or necrotising enterocolitis has aetiological implications which have not yet been convincingly proved and it would therefore seem better to omit such an inference.

M A T E R I A L S A N D M E T H O D S

MATERIALS AND METHODS

Source of Food Specimens

During a period of two and a half years 300 samples of food were collected from the kitchens of the Western Infirmary of Glasgow. The items of food were selected to include (a) a variety of uncooked foods purchased by the hospital, (b) samples of the same foods after they were cooked and prepared for serving in the hospital kitchens, and (c) foods which were bought already cooked, and served with a minimal amount of further preparation. Specimens were taken to the laboratory in sterile containers and examined the same day. Note was made if the food specimen, at the time of collection, had a putrid appearance or bad smell. A smear was made and stained by Gram's method only if the food appeared bad to the unaided senses.

Examination of Food Specimens

For the work reported in this thesis, the examination of food was undertaken as a qualitative rather than a quantitative survey of bacterial contamination. Nevertheless, in order to assess the potential danger of the food under investigation, it was necessary to determine roughly the degree of contamination. This was done by direct plating of each sample on to solid media. No difficulty was involved in carrying this out when

fluid specimens were examined, but the majority of food samples investigated were of a solid nature, such as meat or fish; for these the following method of examination was used.

A portion of about 15 gm. of the food was placed in a sterile petri dish and finely shredded with a sterile scalpel; about 10 ml. of nutrient broth was added and the specimen allowed to stand for a few minutes to allow dispersal of the contaminating organisms into the broth. A loopful of the resulting fluid was inoculated directly on to two horse-blood agar plates and on to MacConkey's medium; and a quantity of the shredded food, sufficient to cover two scalpel blades, was inoculated into each of the following enrichment and selective media:

1 x 50 ml. of Robertson's cooked meat broth

1 x 50 ml. of selenite F medium

1 x 10 ml. of Robertson's cooked meat broth + 10%NaCl

1 x 25 ml. of nutrient broth

The nutrient broth was dispensed in universal containers with screw-capped tops and was boiled for 15 minutes before incubation at 37°C. One blood-agar plate was incubated anaerobically at 37°C. in a MacIntosh and Fildes' jar and all other media were incubated aerobically at 37°C.

Source of Faecal Specimens

At intervals during the same period as that in which the hospital food was surveyed, 347 specimens of faeces from 272 patients in the Western Infirmary of Glasgow were examined. This survey was undertaken in order to ascertain the faecal incidence of Cl. welchii and Staph. aureus and to determine if any particular bacteria were responsible for hospital diarrhoea. 200 of the specimens were collected from 170 patients suffering from diarrhoea and 147 from 102 patients who showed no sign of a gastrointestinal disturbance. The diarrhoeal specimens were stools, from patients within the hospital, which were sent for investigation to the routine bacteriological laboratory. The normal stools were collected, by the co-operation of the nursing staff in two of the medical units in the hospital, from the patients in these units.

Examination of Faecal Specimens

The investigation of faecal specimens was extended to include all the organisms for which an examination was made in the hospital food and the same range of media was therefore inoculated. A smear of all diarrhoeal specimens was made and stained by Gram's method. The diarrhoeal specimens were generally fluid enough to be inoculated directly on to solid media but when the stool was hard, as was frequently the case with

normal stools, a portion about the size of a pea was emulsified in about 1 ml. of sterile nutrient broth to provide a suitable inoculum. Each specimen was inoculated on to 2 horse-blood agar plates, MacConkey's medium and each of the following enrichment and selective media:

3 x 5 ml. of Robertson's cooked meat broth

1 x 10 ml. of selenite F medium

1 x 10 ml. of Robertson's cooked meat broth + 10% NaCl

The Robertson's cooked meat broth was dispensed, for convenience, in bijoux (5 ml.) bottles with screw-capped tops. One bottle was boiled for 15 minutes before incubation, one for 1 hour and the third was incubated without heating. All media was incubated aerobically at 37°C with the exception of one blood-agar plate which was incubated anaerobically at 37°C.

Isolation and Identification of Cl. welchii

Strains of haemolytic and nonhaemolytic Cl. welchii were isolated directly from the anaerobic blood-agar plates and from the Robertson's cooked-meat broth after subsequent plating on to blood agar (incubated anaerobically). During the course of the investigation it was decided to incorporate neomycin at a concentration of 100 µgm./ml. into the horse-blood agar. This is a modification of

the selective medium for Cl. welchii described by Lowbury and Lilly (1955). Over a period of 6 months a series of inoculations upon blood agar with and without neomycin showed no difference in the proportion of isolations of Cl. welchii, but when neomycin was incorporated the contamination with aerobic organisms was very much reduced, and this was a great help towards the isolation of Cl. welchii in pure culture. This was especially advantageous when the specimen was contaminated with proteus.

The boiling of faecal specimens for 1 hour is the generally accepted method for isolating heat-resistant strains of Cl. welchii (Hobbs et al, 1953). In the work described in this thesis, a portion of each faecal specimen was boiled for 15 minutes and another for 1 hour in order to note if any difference in results was obtained. Although it is well recognised that, probably because the spores have germinated after cooking, specimens of cooked food suspected of causing food-poisoning do not usually yield growths of Cl. welchii after having been heated at 100°C, this is not always the case as illustrated by Norval and Collee (1957). In addition, many specimens of food in this investigation were received uncooked. Unheated as well as heated specimens of faeces and food were therefore always cultured. In examining them for heat-resistant Cl. welchii, the specimens of food were boiled for 15 minutes in

nutrient broth. This boiled broth was found to be an adequate medium for the growth of Cl. welchii, and the addition of meat and fat with the food specimens enhanced the anaerobic conditions. After over-night incubation the heated cultures were examined for the presence of heat-resistant Cl. welchii by plating on to horse-blood agar (incubated anaerobically).

All colonies suspected of being Cl. welchii were inoculated into Robertson's cooked-meat broth and , after being tested for purity, the identity of the strains was verified by testing for the production of welchii α-toxin. It is of interest to note that during 3 years observations not more than 10 organisms which were suspected on initial isolation to be strains of Cl. welchii proved to be negative on further testing.

Test for the Production of α-toxin

The examination for welchii α-toxin was made by means of lecitho-vitellin (L.V.), a filtered preparation of 1 egg yolk in 250 ml. saline (Macfarlane, Oakley and Anderson 1941). Two tubes were prepared each containing 0.5 ml. L.V. and 0.5 ml. nutrient broth. Three drops of an aqueous solution of Cl. welchii antitoxin containing 40 units/ml. were added to one only of the 2 tubes; both were then inoculated with 1 drop of an overnight broth culture of the suspected organism and incubated

anaerobically at 37°C for 18 hours. A positive result was recorded when there was opalescence of the L.V. in the tube without antitoxin and no opalescence in the control tube containing antitoxin.

Serological typing of many of the strains of Cl. welchii was carried out by Dr. Betty Hobbs of the Food Hygiene Laboratory of the Public Health Laboratory Service at Colindale. In addition, I prepared antisera to 3 strains of Cl. welchii isolated during outbreaks of food-poisoning. The sera were made as follows:-

Bacterial suspensions were prepared according to the methods described by Henderson (1940). The cultures were grown overnight at 37°C in glucose broth (steamed and cooled before inoculation). The organisms were washed twice and resuspended in distilled water to 10 x 5 on Brown's opacity scale; these suspensions were checked for purity and treated with 0.4% formalin for 2 days.

One of the antisera was prepared by the intravenous inoculation of a rabbit with successively increasing doses (from 0.1 ml. to 1 ml.) of bacterial suspension at intervals of 2-3 days as recommended by Dr. Hobbs. Subsequently, because of the death of 2 rabbits immediately after their final injection due to massive pulmonary emboli containing Gram-positive bacilli, the last two ingestions of 1 ml. were given 0.5 ml. intravenously

and 0.5 ml. intramuscularly. The resulting antisera were of a low titre, 1/100 as compared with 1/800 by the previous method, but this method ensured the survival of the rabbits.

Tube agglutinations were made with a dense living suspension of test organisms prepared by resuspending the sediment of a 10 ml. overnight glucose broth culture in about 1 ml. of distilled water. One drop of the bacterial suspension was added to 0.5 ml. quantities of doubling dilutions of antisera. The tubes were immersed in a 43°C waterbath for 4 hr. and thereafter placed in the refrigerator for a further 18 hours. Readings were made after another 2 hours at room temperature.

Isolation and Identification of Staph. Aureus

The blood-agar plates which were inoculated directly from the specimens were incubated aerobically and examined for staphylococci. As control, plates containing nutrient agar + 6% NaCl were also inoculated during a period of some 6 months in order to see if the growth of Staph. aureus was masked by abundant growth of other organisms, especially in the faecal specimens. There was no evidence to support this view; if Staph. aureus was present in faeces in significant numbers the organism was easily identified on the blood agar. Subsequently nutrient agar containing

6% salt was used only for plating from the enrichment media when the specimen was contaminated with a proteus.

The Robertson's meat broth + 10% salt was plated on to blood agar and further strains of staphylococci were thus isolated. Neither pigment nor haemolysin production was regarded as significant and all suspected colonies were inoculated into nutrient broth and incubated overnight at 37°C. As the production of coagulase was taken as the criterion of a potentially pathogenic Staph. aureus these cultures were tested accordingly and all coagulase-negative organisms discarded.

Coagulase Test

An aliquot (0.1 ml.) of an overnight broth culture was added to 0.5 ml. of a 1 in 10 dilution of fresh citrated human plasma and incubated for 3 to 4 hours at 37°C. A known positive and a known negative were set up with each test. The tubes which were negative after 3 to 4 hours were incubated at 37°C overnight and if still negative were discarded. Further references to Staph. aureus or staphylococci in this thesis denote coagulase-positive Staph. aureus.

The Staph. aureus cultures were phage-typed by Dr. Morag Timbury of the Western Infirmary of Glasgow. The antibiotic sensitivities were determined by using

Evan's Sentests (single strength) on blood-agar plates inoculated by flooding them with an overnight broth culture of the staphylococcus under investigation.

The Isolation and Identification of Specific Intestinal Pathogens

The MacConkey's medium and the Selenite F were examined by the usual routine methods for salmonellas and shigellas. Plates were inspected for the presence of pale colonies and suspected pathogens were inoculated into peptone water. The cultures were examined for the production of urease and indole and the fermentation of glucose, lactose, sucrose, manitol and dulcitol was tested. Final identification was made by specific antisera.

A check on the isolation of salmonellas and shigellas from diarrhoeal stools was made by consulting the findings of the routine bacteriological laboratory and in no case was a member of either genus revealed in one examination and not in the other.

Such further details of materials and methods as appear necessary are given within this thesis in the appropriate sections describing the observations and experiments.

OBSERVATIONS

AND

EXPERIMENTS

OBSERVATIONS AND EXPERIMENTS

PART I

AN INVESTIGATION OF THE CONTAMINATION OF UNCOOKED AND COOKED FOOD FROM THE HOSPITAL KITCHENS WITH POTENTIALLY PATHOGENIC ORGANISMS

300 samples of food from the hospital kitchens were examined for the presence of potentially pathogenic organisms. Note was also made of the degree of bacterial contamination on direct plates and the nature of the predominating organisms isolated on direct and enrichment cultures. It is not convenient or necessary to name every organism isolated from every specimen, but a description of every item examined and the significant bacteriological findings are listed in Appendix I.

The items of food in this survey were divided into three main catagories:-

a) uncooked food as it was purchased by the hospital;

b) cooked food which was prepared for serving in the hospital kitchens; and

c) cooked food bought by the hospital and served

to the patients with a minimum of further preparation.

The results of each group are discussed separately under the appropriate heading.

a) Uncooked food purchased by the hospital

In this group 89 specimens were examined; the incidence of Cl. welchii and Staph. aureus are shown in table 1. Only one specimen, an uncooked sausage, contained a salmonella. This proved to be Salm. enteritidis which was present in small numbers, being isolated only after enrichment culture. No shigellas were isolated.

On direct culture many samples of uncooked food were contaminated with fairly large numbers of saprophytic organisms such as anthracoids, coliforms and micrococci. From only one sample, uncooked chicken, was a potentially pathogenic organism - namely Cl. welchii - isolated on direct plate; and as this isolate was one colony of a haemolytic strain, it cannot be regarded as significant.

After enrichment culture 65 (73%) of the samples of uncooked food were found to be contaminated with haemolytic and 16 (17.9%) with nonhaemolytic Cl. welchii. This organism was isolated from every sample of sausage examined, but this is not surprising in view of both the method of manufacture and the ingredients of sausages. However, it was noted with interest that

the 7 samples of chicken examined contained Cl. welchii, and that one of the two strains of heat-resistant Cl. welchii isolated was found in this item of food, the other being isolated from a sausage. Unfortunately the number of samples of chicken examined was rather small due to the necessity of cutting into a whole bird to procure a specimen for investigation.

Staphylococci were present in 15 samples (16.8%) in this group but due to the ubiquitous nature of both Cl. welchii and Staph. aureus their presence in small numbers on uncooked food could not be regarded as an indication of uncleanliness. After arrival at the kitchens the foods were placed in a refrigerator thus preventing the further multiplication of these organisms before the foods were cooked.

The results of this investigation revealed that the organisms present should as a rule be killed by adequate cooking. Chicken and sausage, however, were singled out for special attention due to the relatively high proportion of samples of each of these foods which contained Cl. welchii and the isolation of a heat-resistant strain from one sample of each.

b) Cooked food, prepared for serving in the hospital kitchens

In selecting samples of food for this group I

attempted to include the same items which were examined in group (a), and wherever possible portions of the same food were examined both before and after cooking. This is shown in Appendix I where both samples are given the same number. This group also included cold roast meats and other cold meats which were prepared in the kitchens, and samples of fish cakes and savoury croquettes. Both cooked and uncooked samples of the last two foods were included as they were prepared from already cooked materials and then recooked. The total number of samples in this group was 173 and the results are presented in table 11.

No salmonellas or shigellas were isolated and an examination of a cooked sausage from the batch in which an uncooked sausage contained Salm. enteritidis proved negative. The gross contamination with saprophytes was found to be very much reduced after the food was cooked and ready for serving and, as a rule, contamination was greater in foods such as cold meats, which required further handling after cooking. Similarly the incidence of Cl. welchii and Staph. aureus was higher in such items. No foods which were served after cooking without further preparation were contaminated with staphylococci and only 10 of 63 contained Cl. welchii. The cooking must therefore have been adequate to destroy the majority of organisms and the contamination of cold meats with small numbers of Cl. welchii and

TABLE II

Contamination of food, cooked in the hospital kitchens, with Cl. welchii and Staph. aureus

Nature of food	No. of samples examined	No. containing					
		Cl. welchii					Staph. aureus
		haemolytic		nonhaemolytic		heat-resistant	
Fish	6		1E		0	0	0
Liver	1		0		0	0	0
Steak & Mince	15		1E		1E	0	0
Tripe	6		1E		0	0	0
Sausage	25		4E		1E	0	0
Sausage gravy	1	1D	1E	1D	1E	0	0
Black Pudding	2		2E		0	0	0
Ulster Fry	2		0		0	0	0
Chicken	46	8D	21E	4D	11E	1	9E

TABLE II (continued)

Nature of food	No. of samples examined	No. containing					
		Cl. welchii					Staph. aureus
		haemolytic		nonhaemolytic		heat-resistant	
Chicken broth	3	0		0		0	0
Cold roast meats	15	4E		2E		0	2E
Other cold meats	37	3E		3E		0	7E
Fish-cake (uncooked)	1	1E		0		0	1E
Fish-cake (cooked)	1	0		0		0	0
Croquette (uncooked)	8	4E		4E		0	1E
Croquette (cooked)	4	0		0		0	0
Total	173	9D (5.2%)	43E (24.8%)	5D (2.8%)	23E (13.3%)	1 (0.6%)	20E (11.5%)

D = isolated on direct culture; E = isolated after enrichment culture

Staph. aureus was probably due to surface contamination.

The samples of uncooked fish-cake and savoury croquette which were examined contained a fairly large number of saprophytes and in 7 instances a small number of Cl. welchii and Staph. aureus. These organisms were presumably inoculated during the preparation of the foods. After recooking, however, only small numbers of micrococci and anthracoids were isolated.

The incidence of Cl. welchii in sausages was very much lower after cooking and no sample of sausage, either stewed or fried, was found to contain heat-resistant Cl. welchii. One sample (F 95), however, was brought to my attention by the kitchen staff on account of its unpleasant smell. The sausages in this batch were stewed and allowed to cool overnight as a preliminary to making 'toad-in-the-hole'. While this dish was being prepared the sausages were noted to have a foul smell. This was discounted by some as due to an unusually large amount of seasoning but I was nevertheless called upon to give an opinion. When a smear of the gravy was stained by Gram's method and examined, a large number of Gram-positive bacilli, typical of Cl. welchii, were seen and this was confirmed the next day by a heavy growth of haemolytic and non-haemolytic Cl. welchii on the direct plates from the

sausage gravy. Haemolytic and nonhaemolytic strains were also isolated from a sample of the sausages but only after enrichment culture. No heat-resistant strains were isolated. On no other occasion were sausages found to be grossly contaminated after cooking but this example served to show their potential danger. Fortunately in this case the contamination was discovered in time to prevent any harmful effects.

The most outstanding finding of this examination, however, was the frequent contamination of cold chicken with Cl. welchii (24 of 46 samples). Both haemolytic, nonhaemolytic and, on one occasion, slightly heat-resistant Cl. welchii were isolated. As Staph. aureus was also present in small numbers in 9 of 46 specimens, it was regarded at first as a high rate of contamination due to the large amount of handling involved in carving chickens. Early in the investigation, however, it was noted that some samples of cold chicken contained a fairly large number of Cl. welchii on direct plates and an incident among the laboratory staff, reported on p.85 , indicated that this was a potential source of Cl. welchii food-poisoning. This incident also indicated that Cl. welchii food-poisoning was not necessarily caused only by heat-resistant strains but could also be caused by haemolytic non-heat-resistant strains. During general discussions on the topic of food-poisoning in hospital, the medical and nursing

staff of the hospital volunteered the information that cold chicken on the patients' menu was regarded with some apprehension; and the reason for these fears was made apparent by the bacteriology of the food. In all, 10 of the 46 samples of cold chicken examined showed growth of Cl. welchii on direct culture. None of the three samples of chicken broth sampled contained Cl. welchii. This was presumably due to the fact that the broth was sampled the same day as the birds were cooked whereas the chicken samples were received the next day, after carving.

Despite the frequency of contamination with Cl. welchii, only on two occasions did samples of chicken appear bad to the unaided senses. Both these samples were brought to my attention by members of the kitchen staff. One (F 136) was reported to have 'gone bad' and the specimen received for bacteriological examination was reheated. A direct smear showed a fair number of Gram-positive cocci but anthracoids were the only organisms isolated even after enrichment culture. From the direct microscopic examination the reason for deterioration did not appear to be Cl. welchii but it is unfortunate that a sample of the chicken taken before reheating was not available for examination.

The second sample of 'bad' chicken (F 150) was served to the patients and returned uneaten due to the unpleasant smell. When smeared and stained by Gram's

method a large number of Gram-positive bacilli, typical of Cl. welchii were seen. A large number of haemolytic Cl. welchii grew both on direct and enrichment culture but no nonhaemolytic or heat-resistant strains were isolated. Further reports of slimy but not bad smelling chicken were received from the wards from time to time but this was not observed personally. Usually the cold chicken, although it contained on occasions large numbers of Cl. welchii, neither appeared putrid or smelled bad, thus giving no indication from its external appearance of the bacterial contamination. No other item of food, apart from the one specimen of sausage noted above, appeared to have 'gone bad'. With the exception of cold chicken, therefore, the items of cooked food were relatively free of potentially pathogenic organisms when served to the patients.

c) Cooked foods, bought by the hospital

This group contained 33 specimens and comprised cold meats, pies, and sausage rolls. The cold meats required slicing only, after being purchased, and the mutton pies and sausage rolls were heated before serving. The results are given in table III.

Bacterial contamination of the samples in this group was minimal. In the community as a whole, pies and sausage rolls are frequently incriminated as the

TABLE III

Contamination of cooked food, purchased by the hospital, with Cl.welchii and Staph.aureus

Nature of food	No. of samples examined	No. containing			
		Cl. welchii			Staph.aureus
		haem-olytic	nonhaem-olytic	heat resistant	
Mutton pie	7	0	0	0	0
Sausage roll	5	0	0	0	0
Cold meats	26	1E	1E	0	3E
Total	38	1E (2.6%)	1E (2.6%)	0	3E (7.8%)

E = isolated after enrichment culture

vehicles of food-poisoning organisms. In this survey, therefore, it is of interest to note that these particular foods were of a very high standard of bacterial cleanliness. No growth was observed on direct plates from any of the 12 specimens examined, and anthracoids and micrococci were the only organisms isolated in enrichment media. Slight contamination with Staph. aureus and Cl. welchii in addition to saprophytic organisms was observed in the 26 samples of cold meats but adequate refrigeration once more prevented any further growth. These organisms were most likely surface contaminants acquired during slicing.

Conclusions

The samples of items of uncooked food, which were purchased by the hospital, were found to be contaminated by fairly large numbers of saprophytic organisms but only minimal numbers of potentially pathogenic organisms such as Cl. welchii and Staph. aureus were present. This contamination was greatly reduced during the process of cooking, and cold chicken was the only item of food which on bacterial examination was considered to be frequently in a dangerous condition when prepared for serving, the offending organisms being Cl. welchii. The appearance and smell of this food did not, as a rule, give any indication of its bacterial content.

Samples of foods which were bought by the hospital

already cooked showed only a slight degree of bacterial contamination and this was most probably on the surface.

P A R T II

CHARACTERISTICS OF CL. WELCHII AND STAPH. AUREUS ISOLATED FROM FOOD

On primary isolation, note was made of the morphological and colonial appearances of the strains of Cl. welchii and Staph. aureus isolated from foodstuffs. These observations are listed below along with further investigations which were made on a few of the strains.

Clostridium welchii

The morphology of all strains of Cl. welchii isolated from foods was typical. No spores were seen on smears of any strain either on primary isolation or in cultures on blood agar or Robertson's meat medium.

On solid media the colonies of most strains were smooth and round with an entire edge but occasionally rough colonies with an irregular edge and radial striations were observed. The difference in colonial form was unrelated to the production of haemolysis on horse blood. Both haemolytic and nonhaemolytic strains were found in foods; only 3 heat-resistant strains were isolated and all three were nonhaemolytic.

The haemolytic strains showed a well-defined zone of β-haemolysis on horse-blood agar due to the production of Θ-toxin. The nonhaemolytic strains usually had no effect on horse blood on primary isolation, but a slight zone of incomplete α-haemolysis sometimes developed round the colonies after a few days.

When tested with lecitho-vitellin all strains gave a positive reaction for the production of toxin (lecithinase). This was specifically inhibited by type-A antitoxin. One of the originally heat-resistant strains of Cl. welchii (from F 77A in Appendix I) retained its heat-resistance in culture, surviving 1 hour's boiling. Cultures of the other two heat-resistant strains were killed by 5 minutes' boiling.

6 strains of Cl. welchii isolated from cold chicken were serotyped by Dr. Betty Hobbs of the Food Hygiene Laboratory of the Public Health Laboratory Service at Colindale. These strains were not heat-resistant; three were haemolytic and 3 nonhaemolytic. One of the haemolytic strains was untypable with the available sera; the other two were type 6. Two of the nonhaemolytic strains proved to be untypable; the remaining one was type 1. On occasions, more than one strain of Cl. welchii was isolated from one individual sample of food.

Staphylococcus aureus

All strains of Staph. aureus isolated from foods were morphologically typical. The colonies of individual strains differed slightly in size and markedly in haemolysis on horse-blood agar and pigment production. The staphylococci showed different degrees of β-haemolysis on horse-blood agar or were completely nonhaemolytic. Some strains produced a marked golden pigmentation and others remained remarkably white even after prolonged standing at room temperature. All gave a positive coagulase test in 3-4 hours at 37°C.

25 of the 38 strains of Staph. aureus isolated from food were 'phage-typed. The numbers in each 'phage group are given in table IV. 15 (60%) of the strains belonged to either group I or II and 10 (40%) were in group III or were untypable. No individual sample of food was found to harbour more than one strain of staphylococcus.

Conclusions

In the present investigations, most of the strains of Cl. welchii isolated from food were non-heat-resistant. This is not necessarily an indication that they were harmless especially when isolated from cooked foods.

The staphylococci were of a wide range of 'phage-

TABLE IV

Distribution of Staph. aureus isolated from food in 'phage groups

No. examined - 25		
'phage group	No.	%
I	7	28
II	8	32
III	6	24
N.T.	4	16

N.T. = untypable

types but the majority belonged to groups I or II, suggesting that they were most probably surface contaminants from food-handlers. The presence on food of even small numbers of group III or untypable strains is undesirable because the majority of the enterotoxigenic strains of Staph. aureus are of this type, and it is considered that refrigeration of the foods from which they were isolated in this series may have prevented the foods from becoming toxic.

P A R T III

FURTHER OBSERVATIONS ON THE CONTAMINATION OF CHICKEN WITH CL. WELCHII

Due to the relatively frequent contamination of cold chicken with Cl. welchii further observations were made regarding the cooking and storing of the fowls. Because one of seven samples of uncooked fowl from the hospital kitchens contained heat-resistant Cl. welchii it was necessary to determine if the cooking procedure would allow the survival of such strains and if the methods of storing after cooking would permit their subsequent growth. In addition, many samples of cooked chicken were found to be heavily contaminated with haemolytic or non-haemolytic Cl. welchii which were not heat-resistant; for various reasons and from evidence presented in other parts of this thesis I regarded these as equally important as possible causes of food-poisoning. The possibility of contamination with Cl. welchii after the birds were cooked was therefore investigated.

A Comparison of the Growth of Cl. welchii in Chicken-Extract Broth and in Meat-Extract Broth

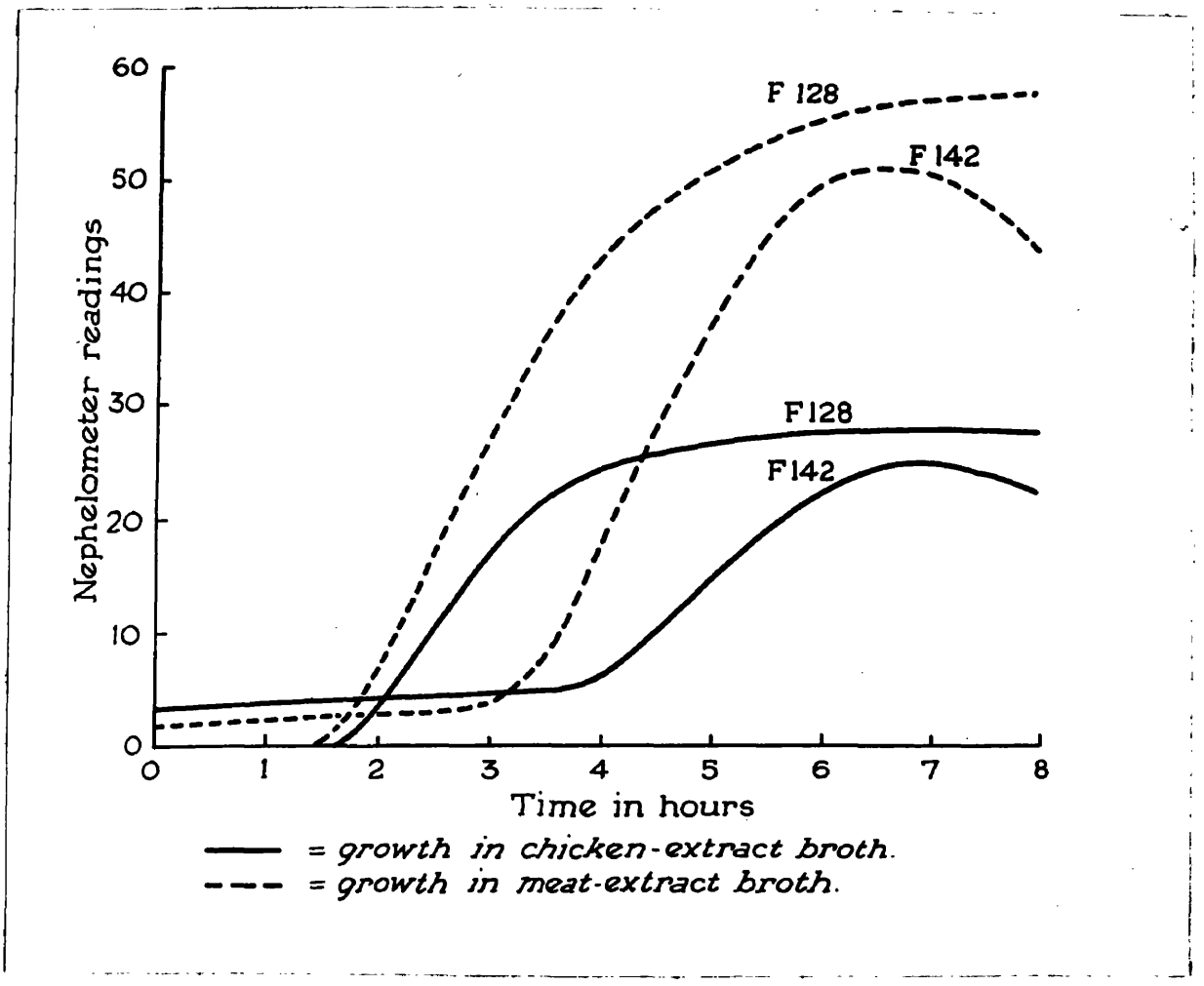
It was first thought advisable to determine if the high rate of contamination of cold chicken as compared with

other meats examined from the hospital kitchens was primarily due to nutrients in chicken which favoured the growth of Cl. welchii. To investigate this, I studied the growth of two strains of non-haemolytic Cl. welchii (isolated from cold chicken samples F 128 and F 142 in Appendix I) in chicken-extract and meat-extract broths.

100 ml. quantities of the media, dispensed in screw-capped bottles, were steamed for 1 hour and cooled before inoculation. Each strain was inoculated into 1 bottle of chicken-extract broth and 1 bottle of meat-extract broth and incubated at 37°C; the inoculum of strain F 128 was 0.5 ml. of an over-night Robertson's meat-broth culture and that of strain F 142, 0.5 ml. of an eight-day culture. 5 ml. aliquots were withdrawn at intervals over 8 hours and the growth determined by measuring the opacity of the aliquots on a nephelometer. The nephelometer readings were assumed to be directly proportional to the number of organisms per ml. and growth curves were traced by plotting these readings against time (fig. I).

Both the strains examined grew better in meat-extract than in chicken-extract broth. F 142 had a longer lag phase due to the age of the inoculum but little difference was found in the maximum growths of the two strains when grown in the same medium.

Figure I



Growth curves of 2 strains of Cl.welchii in chicken-extract broth and in meat-extract broth.

On the basis of this experiment, chicken-extract broth did not appear to be an especially favourable medium for the growth of Cl. welchii.

Comparison of the Cooked Chicken Received from the Main and Special-Diet Kitchens

The chicken samples examined in part 1 were received from two separate kitchens in the hospital, the main kitchen which served the staff and the majority of the patients, and the special-diet kitchen which supplied meals for patients on special diet only. Of the 46 samples examined, 31 came from the main kitchen and 15 from the special-diet kitchen. The contamination with Cl. welchii of the chicken from the respective kitchens is presented in table V.

On comparing the results from the two kitchens there was a striking difference in the number of samples containing Cl. welchii. No samples of chicken from the special-diet kitchen grew Cl. welchii on direct culture whereas 8 (25.8%) and 4 (12.9%) from the main kitchen grew haemolytic and nonhaemolytic strains respectively and in some instances these organisms were in almost pure culture in large numbers. The numbers from which Cl. welchii were isolated in enrichment culture showed a similar difference. The methods of cooking and preparing the fowls in both

TABLE V

Contamination of cold chicken from main and diet kitchens with Cl. welchii

Kitchen	No. of samples examined	No. containing Cl. welchii		
		haemolytic	nonhaemolytic	heat-resistant
Main	31	8D 19E (25.8%) (61.3%)	4D 9E (12.9%) (29%)	0
Special diet	15	2E (13.3%)	2E (13.3%)	1 (6.6%)

D = isolated in direct culture

E = isolated after enrichment culture

kitchens were therefore investigated.

Methods of Cooking Fowls in the Hospital Kitchens

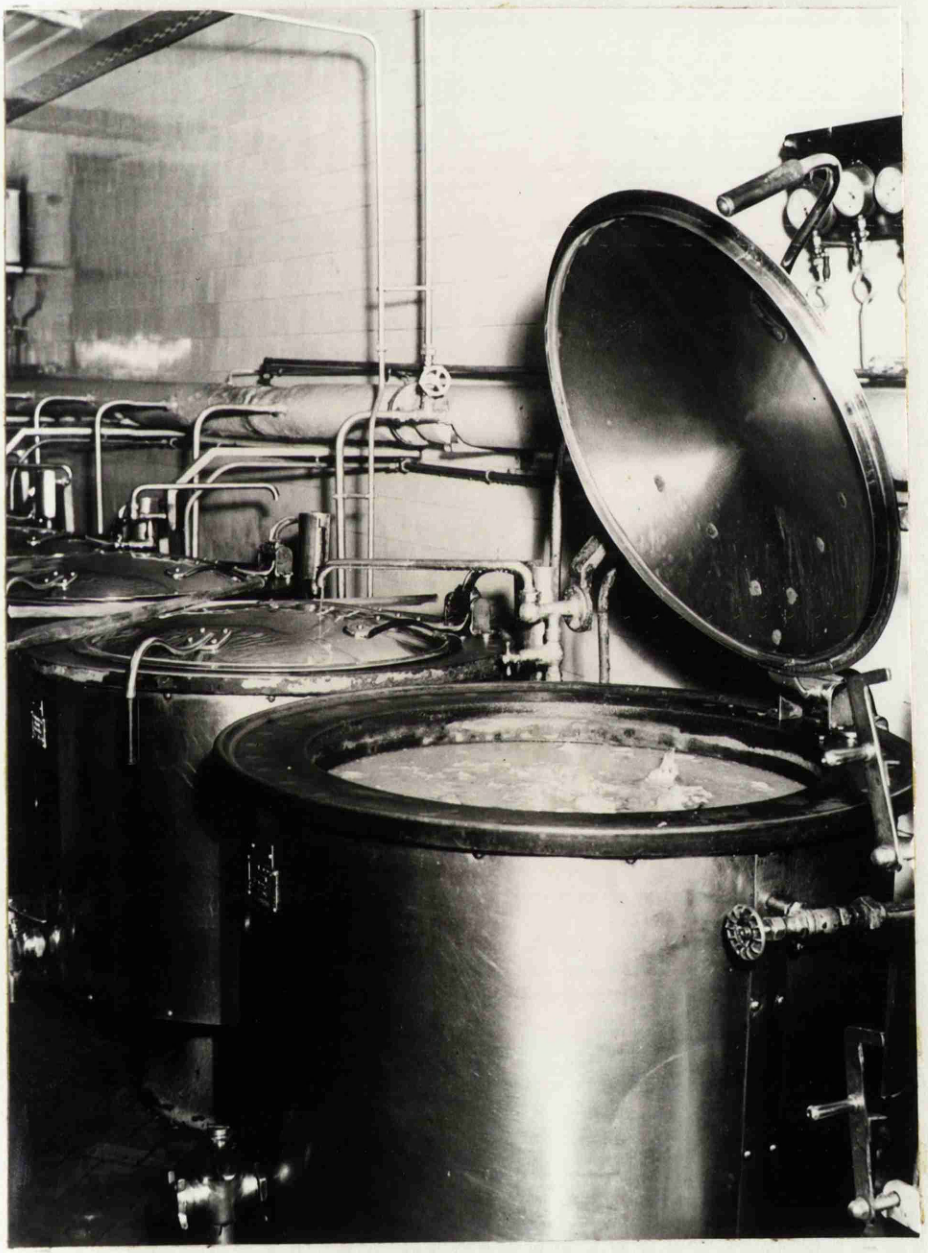
Main kitchen:- The fowls in batches of 22 were cooked in water in a large steam-jacketed boiler (fig. II) for 2½ to 3 hours depending on the age of the birds. As the lid was not constantly closed no pressure was maintained. In order to determine if the birds were cooked the firmness of the legs was tested by hand.

Special-diet kitchen.- The cooking of the fowls in the special-diet kitchen took place in a steam oven, (fig. III), 2 or 3 birds being cooked at one time. The birds were placed in water in pie-dishes which stood on shelves in the oven above the source of steam; a chimney allowing the outflow of excess steam prevented any raising of pressure. The birds were tested for tenderness after 2-3 hours by pulling a leg or testing the firmness with a fork.

The actual cooking procedures in both kitchens were essentially the same and the chances of survival of heat-resistant Cl. welchii after cooking therefore appeared to be similar. This was further investigated by determining the actual temperatures attained in the flesh of the fowls during cooking.

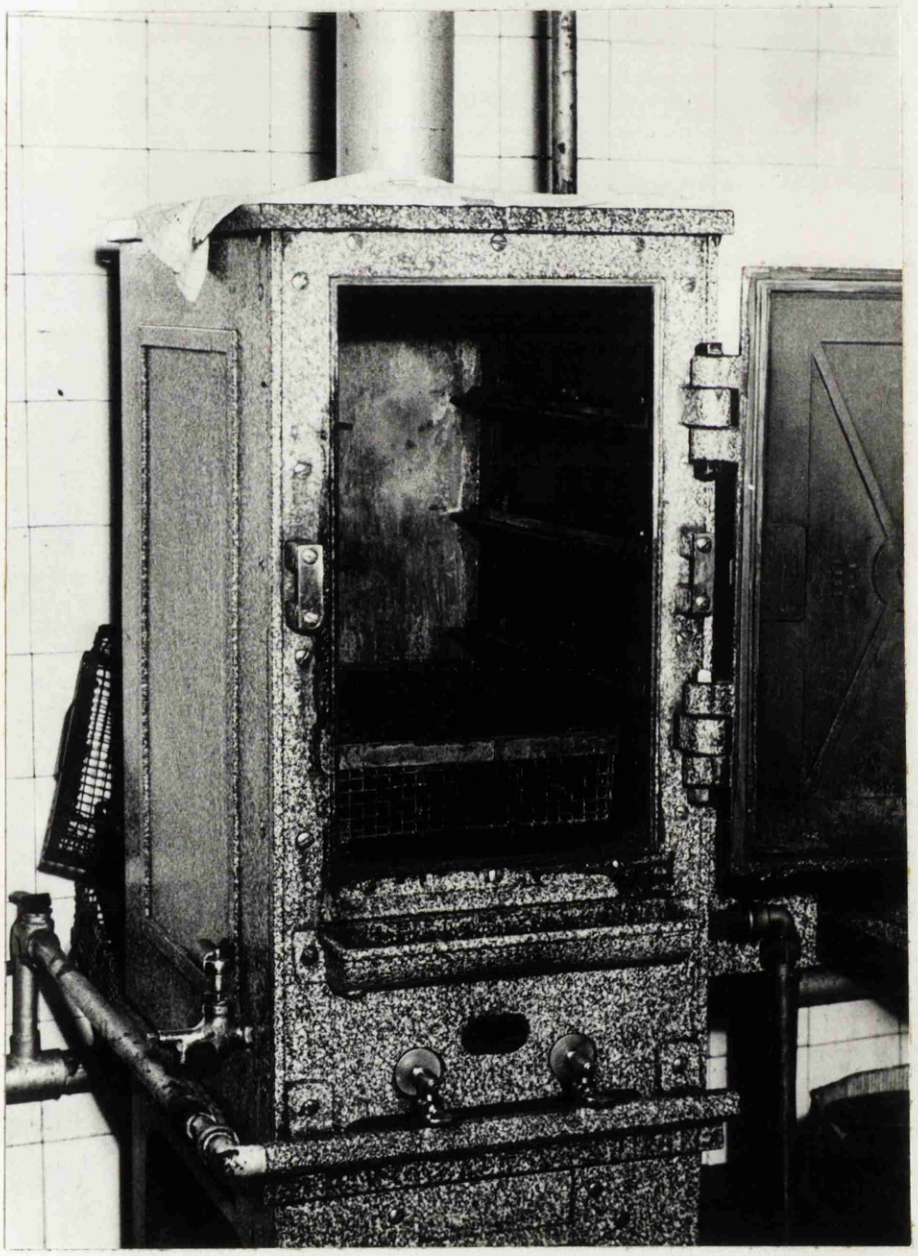
Using a copper-constantan thermocouple attached to

Figure II



Steam-jacketed boilers in which chickens were cooked in the main kitchen. x $\frac{1}{13}$ approx.

Figure III

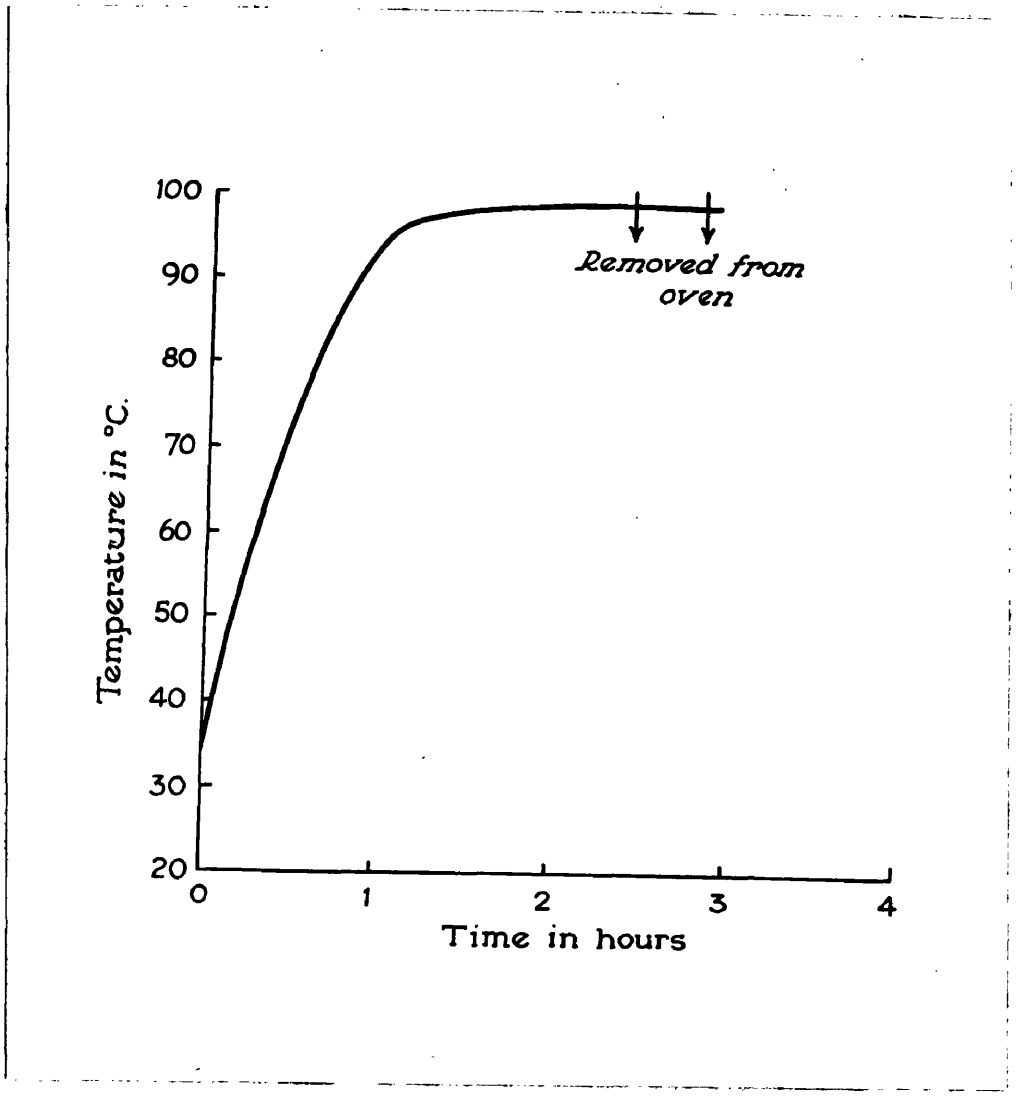


Steam oven in which chickens were cooked in the special-diet kitchen. x $\frac{1}{8}$ approx.

a galvanometer, readings were taken during the cooking of the fowls by immersing one end of the thermocouple in the flesh of a fowl and the other in ice. The thermocouple was immersed deep in the flesh of the fowls just above the leg. The galvanometer readings were converted to temperatures by means of a straight-line graph kindly prepared, for the thermocouple and resistance used, by Dr. M. M. Bluhm of the Regional Physics Department of the Western Regional Hospital Board.

In all, 5 recordings were taken, 4 in the special-diet kitchen and one in the main kitchen. The difficulties encountered in attaching the thermocouple to the fowls in the large steam-boiler in the main kitchen were numerous; and as the results of one observation were comparable to those recorded in the special-diet kitchen, the further tests were made in the special-diet kitchen. The temperature in the flesh of the fowl being cooked rose to approximately 97°C in from 55 to 65 minutes and remained at between 97°C and 100°C until the cooking was completed; the duration of the period in the neighbourhood of 100°C was 80, 85, 90, 100 (main kitchen) and 115 minutes in the 5 recordings made. On no occasion was a temperature of over 100°C attained. Fig. IV shows the average temperatures observed in the flesh of the birds during cooking.

Figure IV



Average temperatures attained in fowls during cooking.

Heat-resistant spores of Cl. welchii are reported to survive up to 5 hours at 100°C. (Zeissler and Rassfled-Sternberg, 1949; Hobbs et al., 1953). The cooking of the fowls in neither kitchen was therefore adequate to kill such strains or even less heat-resistant strains, which are more commonly encountered, if they were present in the fowls before cooking. One of 7 samples of uncooked chicken from the kitchens contained a heat-resistant Cl. welchii and this was thus established as an actual hazard. The proliferation of such strains, which would survive boiling as described, and of any strains which might gain access after cooking, would depend upon the suitability of conditions during the cooling and storing of the cooked chickens. These conditions were therefore investigated.

Methods of Cooling and Storing Cooked Chicken in the Hospital Kitchens

Main kitchen.- It proved difficult to find out the procedure actually followed after the cooking of the birds in the main kitchen and many conflicting accounts were given by different members of the kitchen staff. Eventually, however, it was possible to confirm that the fowls were removed from the steamer in which they were cooked and placed with the broth in a large metal container (fig. V) which was left uncovered outside

Figure V

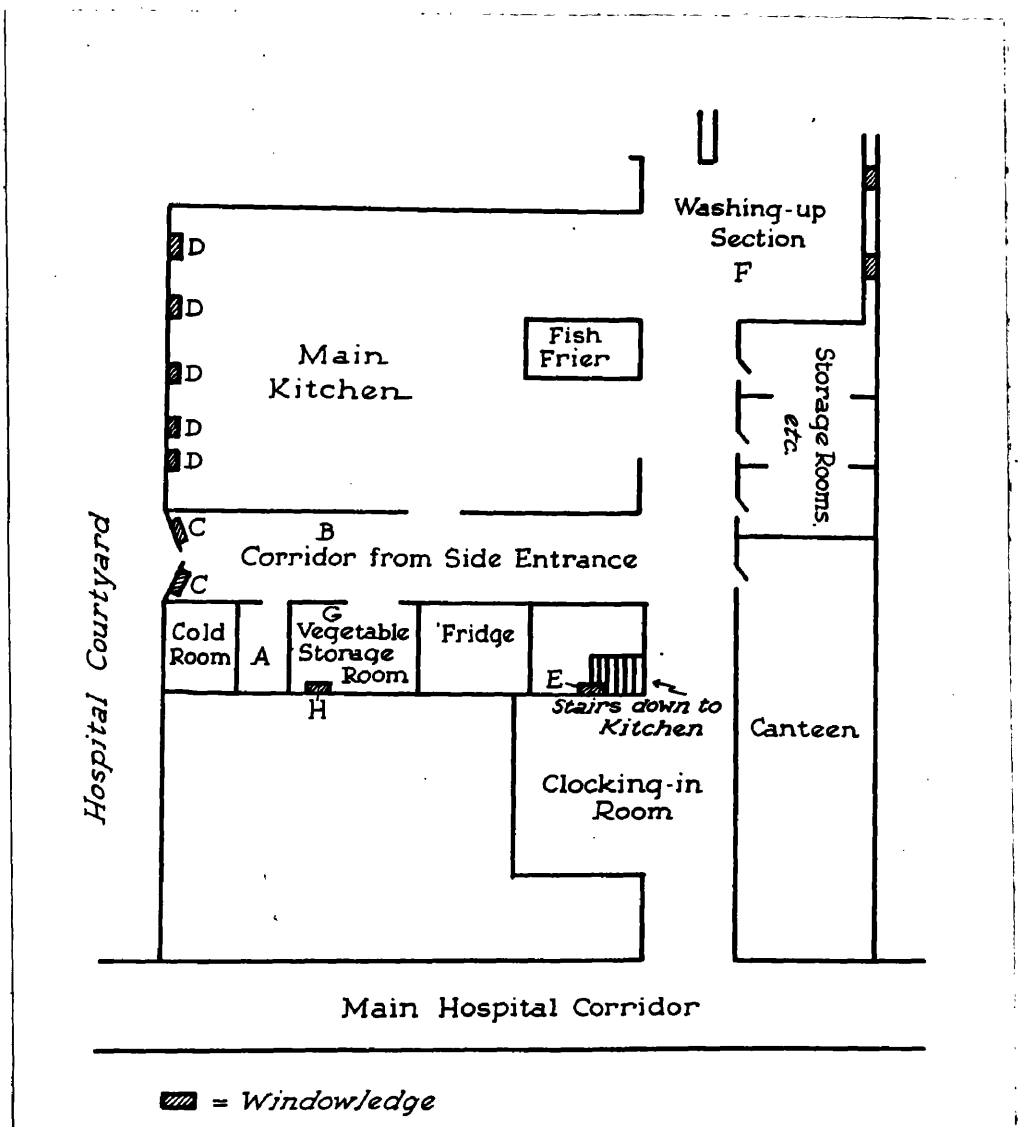
Cooked chickens cooling in their broth in the one large container. This figure illustrates the method of cooling in the main kitchen. x $\frac{1}{6}$ approx.

the cold room overnight (see A in plan of main kitchen, fig. VI). Occasionally the cooked fowls were also observed in the corridor from the side entrance (fig. VI) during at least a part of the cooling period. The next morning the chickens were removed from the broth, carved, and distributed to the wards for lunch. As the birds were often cooked around mid-day this entailed a lapse of almost 24 hours before serving, and during about 20 hours of this period the birds were immersed in broth, some 22 at a time in one container.

Special-diet kitchen.- After cooking, the fowls in the special-diet kitchen were removed from the dishes in which they were cooked and placed on shallow trays (fig. VII); the broth was poured separately into a jug. The fowls and the broth were left, in their respective containers, on a side bench to cool and, some 3-4 hours later, were put in the refrigerator where they remained until required.

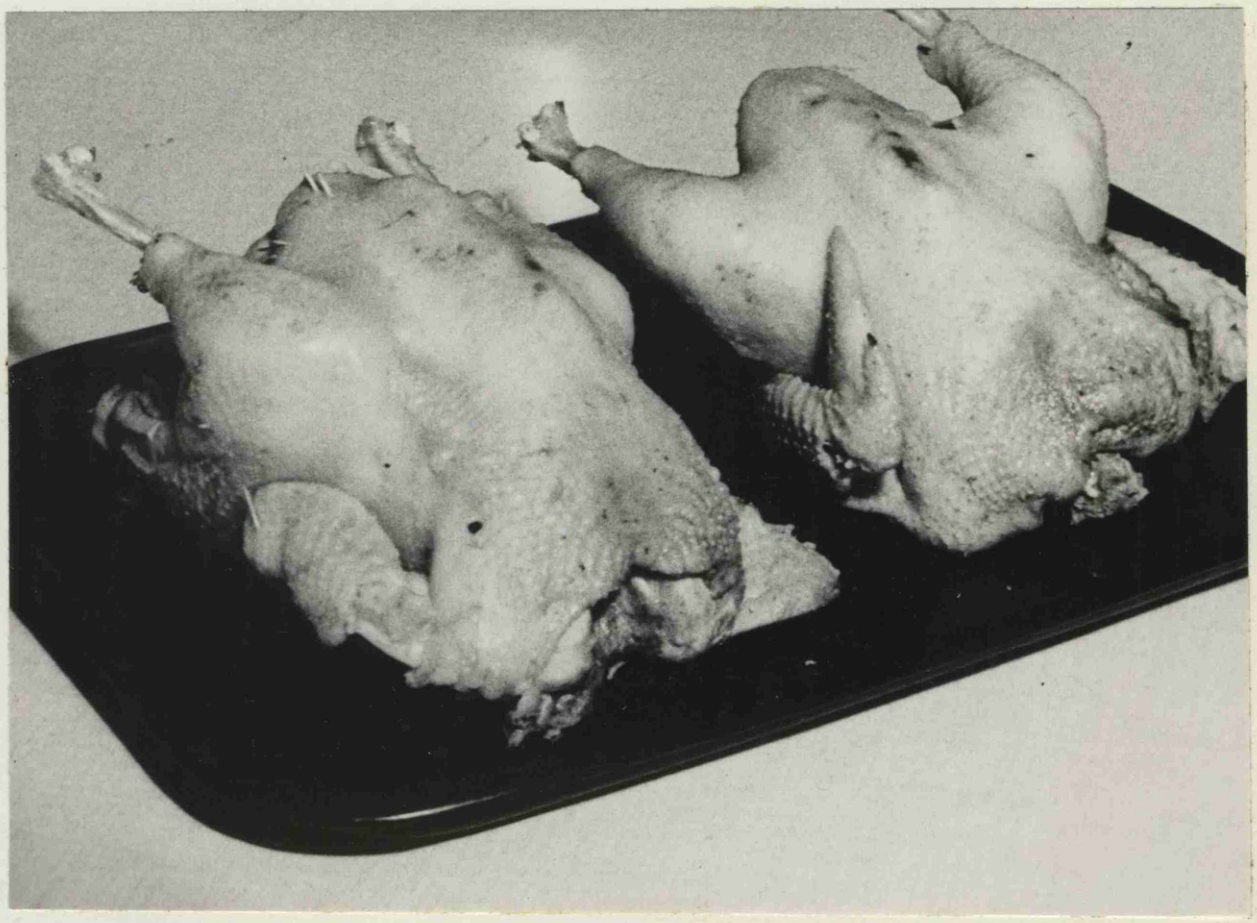
The temperatures during the cooling of the birds were ascertained by the same method as detailed above. The thermocouple was again immersed in the flesh of the birds just above the leg. The cooling temperatures were determined either on birds on shallow trays or birds at the top of the pot in the main kitchen. It was not possible to take temperatures of birds

Figure VI



Plan of the main kitchen.

Figure VII



Cooked chickens cooling on shallow trays. This figure illustrates the method of cooling in the special-diet kitchen. x $\frac{1}{3}$ approx.

deep in the pot as the risk of contamination caused by the handling would have been too great.

The cooling temperatures of 5 fowls cooked on different days are shown in table VI. The actual temperatures recorded differed in each observation presumably because the fowls on each occasion were placed in different sites, some of which were rather cooler than others. The comparative rates of cooling, however, were similar. The temperature fell to approximately 50°C in a matter of 1 to 2 hours and thereafter the rate of cooling was much reduced. This indicated that, if left unrefrigerated, the cooked birds would remain within the dangerous range of 50-60°C for long enough to allow considerable growth of Cl. welchii.

The chickens in the special-diet kitchen were placed on shallow trays and removed to the refrigerator after from 3-4 hours' cooling, thus the conditions for the proliferation of Cl. welchii were suitable for only a short time before refrigeration and presumably for a short time after refrigeration. This prevented any Cl. welchii, which either survived cooking or were inoculated after cooking, multiplying to a dangerous extent, a fact borne out by the observation that none of the 15 samples of cold chicken from the special-diet kitchen was grossly contaminated with Cl. welchii. The isolation of Cl. welchii in heat-

TABLE VI

Cooling temperatures of cooked fowls

Time after removal from oven (hours)	Temperature °C.				
½	53	74	75	76	60
1	35	69	69	71	50.5
1½	30	55.5	58	53	40.5
2	28	50.5	50.5	40.5	39
2½	*35	43.5	40.5	35	27.5
3	27	43	38.5	-	-
3½	25	42	34	-	-

*Thermocouple became dislodged and was pushed further into flesh.

resistant (i.e. sporing) form from one bird (F 118 in Appendix I) from the special-diet kitchen both before and after cooking could be taken as an indication that, in this particular instance, the organism did not germinate after cooking although it survived. The evidence is all the clearer because non-heat-resistant Cl. welchii were not isolated in direct or enrichment media from the cooked fowl, and because the heat-resistant strain isolated showed no heat-resistance once in culture.

On the other hand, in the main kitchen, where the rate of contamination with Cl. welchii was high, the time during which the chickens remained at a temperature suitable for the growth of Cl. welchii was very much prolonged due to the habit of storing all 22 cooked birds unrefrigerated in the one large container. Presumably the temperature of the birds deep in the pot remained at a dangerous level for considerably longer than those at the top because the rate of cooling would be much slower. The actual flesh of the birds was particularly suitable for the growth of Cl. welchii because the surrounding broth cooled more quickly. The broth did not constitute a hazard to the patients as it was always adequately boiled before serving.

On no occasion during the past 3 years was there a general outbreak of Cl. welchii food-poisoning in

the hospital indicating a general contamination of all birds in the one batch with the same strain or strains. Rather the food-poisoning strains seemed to be restricted to individual birds because the outbreaks were on a small scale and contamination of different birds of a single batch cooked in one pot with different strains was indicated (p. 90). Presumably the birds initially acted as separate culture media and the temperature fell below a suitable level before the strains contaminating the individual birds had a chance to grow throughout the whole batch to a dangerous extent. In addition, the birds in the centre of the pot were more liable to become extensively contaminated as they cooled more slowly. Thus perhaps only one or a few birds out of 22 would be dangerously contaminated.

The contamination of the cold chicken in the main kitchen could not be regarded as exclusively due to the presence of heat-resistant strains which survived cooking. Although it was difficult to assess if the nonhaemolytic strains isolated were merely vegatative forms of initially heat-resistant organisms, large numbers of haemolytic Cl. welchii, which must have been inoculated after cooking, were frequently present. The conditions of storage in the main kitchen were equally favourable to the proliferation of strains which gained access before and after cooking; and

the arguments already stated regarding the restriction of the growth to individual fowls would likewise apply to the spread of contaminants acquired after cooking.

The high rate of contamination of the chickens from the main kitchen as compared to those prepared in the special-diet kitchen was thus ascertained to be caused by the methods of storing the cooked chicken before serving. Investigations were therefore made into the access of heat-resistant Cl. welchii to the flesh of the fowls before cooking and the contamination, not necessarily with heat-resistant strains, after cooking. The fact that cold chicken was the item of food from the main kitchen with the highest rate of contamination was proved to be due to the fact that it was the only item which was persistantly left unrefrigerated in large quantities in broth for long periods before serving.

Incidence of Cl. welchii in Fowl Viscera

All seven samples of uncooked chicken from the hospital kitchens and a further 5 samples from other sources (pp 79-80) contained Cl. welchii, in one case a heat-resistant strain. The significance of the non-heat-resistant strains with regard to the ultimate contamination of the cooked chicken was not important as they would be killed by the cooking. However, in investigating the contamination of the birds with heat-

resistant Cl. welchii, the non-heat-resistant strains were also considered as their source was most likely the same.

It is the practice of most poulterers to store fowls uneviscerated until they are sold. The contamination of the fowl flesh can therefore originate from the viscera during storage or from sources such as the surface of the birds and the hands of poulterers during evisceration and dressing. References in favour of either method are found in the literature. McCulloch (1945) speculated that resistant, by inference heat-resistant, strains of Cl. welchii may be normal inhabitants of the intestines of turkeys and that these organisms may penetrate the thin intestinal wall thus resulting in the presence of these organisms in the meat during refrigeration in an undrawn condition. Whittle (1956) recommended in a note on broiler production that the birds be thoroughly starved, bled when killed, and stored uneviscerated to prevent deterioration during storage; and Gunderson, McFadden and Kyle (1954) recommended eviscerating as soon as possible to prevent high bacterial loads. On the other hand Tanner (1944) quoted Pennington (1911) and Brown (1907) as having found a much higher bacterial contamination rate in poultry after evisceration than before evisceration, the explanation being that contamination took place during the process of

evisceration. Similarly Walker and Ayres (1956) found that during processing there was an increase in the numbers of organisms present on the surface of commercially dressed poultry attributable to the distribution, by washing and handling, of organisms on the feet, feathers, and intestinal contents, and concluded that washing and handling the birds served to disperse the organisms carried into the processing plant on the birds. To clarify this situation an examination of fowl viscera was undertaken. The viscera were kindly supplied by Thomas Anderson & Sons of West Nile Street, Glasgow, from whom the hospital kitchens purchased their fowls. 34 samples were examined for Cl. welchii, Staph. aureus and Salmonella. No staphylococci, salmonellas, or heat-resistant Cl. welchii were isolated.

In all 34, the contents of the large intestine were examined, in 9 the contents of the small intestine, and in 25 the liver. The liver was examined to give an indication of the spread of the gut flora to other organs. The results are shown in table VII.

23 (67.6%) of the contents of large intestine contained haemolytic Cl. welchii, 9 (26.5%) in fairly large numbers as shown by direct culture. The number from which Cl. welchii was isolated in the liver was very much smaller, being only 5 (20%). In some cases the contamination in the bowel was considerable

TABLE VII

The incidence of Cl. welchii in fowl viscera

	No. of samples examined	No. containing Cl. welchii			
		haemolytic		nonhaemolytic	
Large intestine	34	9D (26.5%)	23E (67.6%)	1D (2.9%)	1E (2.9%)
Small intestine	9	0	3E (33.3%)	0	0
Liver	25	0	5E (20%)	0	0

D = isolated in direct culture

E = isolated in enrichment culture

and yet Cl. welchii was not found in the liver. It is unlikely, therefore, that spread of gut flora before evisceration caused the presence of Cl. welchii in all of the 12 samples of uncooked chicken examined although it most probably accounted for some. The contamination of the fowl flesh would therefore appear to arise from a variety of sources including:- (1) intestinal contents before evisceration, (2) intestinal contents during evisceration (due to rupture of bowel wall), (3) the hands of the poulterers during evisceration and (4) water or airborne contamination after evisceration. The results indicate, however, that the contamination, whether endogenous or exogenous, usually takes place during and after evisceration.

Contamination of the Dust in the Main Hospital Kitchen with Cl. welchii and Staph. aureus

The cooked chicken in the main kitchen was frequently contaminated with Cl. welchii which apparently gained access after cooking. The most obvious and most probably the main source of such organisms was the dust in the kitchen because the fowls were exposed to the dust during the transfer from the cooking pot to the cooling container and during the cooling period. As part of this investigation, therefore, the kitchen dust was examined under my

direct supervision by two junior members of the bacteriology department, Mr. T. M. Joys and Dr. Shiela McCulloch.

Samples of dust were taken from various parts of the kitchen by rotating sterile broth-soaked swabs over the appropriate sites. These swabs were examined for the presence of Cl. welchii and Staph. aureus. No direct cultures were made and the results represent isolation in enrichment culture. No heat-resistant Cl. welchii were isolated from any samples of dust.

58 swabs from 7 sites on the floor and window ledges of the main kitchen were examined. The ascertained contamination with Cl. welchii and Staph. aureus is shown in table VIII, the actual sites being marked in the plan of the main kitchen (fig. VI). Both haemolytic and nonhaemolytic Cl. welchii were isolated from all sites on more than one occasion. Haemolytic Cl. welchii were isolated from 52 (89.6%) of the samples and nonhaemolytic from 47 (81%). Cl. welchii was therefore widely distributed over the floors and window-ledges of the kitchen.

Staph. aureus was present in 14 (24.1%) of the 58 swabs but was mainly found on samples taken from the floor (12 of the 14). 11 of the 14 staphylococci isolated were sensitive to all antibiotics tested, the remaining 3 being resistant to penicillin only.

TABLE VIII

Contamination of kitchen dust with Cl. welchii and Staph. aureus

Position in kitchen from which sample was collected	No. of samples examined	No. containing		
		Cl. welchii		Staph. aureus
		haem-olytic	nonhaem-olytic	
B	10	10 (100%)	7 (70%)	6 (60%)
C	10	8 (80%)	9 (90%)	0
D	11	10 (91%)	9 (82%)	0
E	9	7 (77%)	7 (77%)	1 (11%)
F	10	9 (90%)	9 (90%)	4 (40%)
G	3	3 (100%)	2 (66.6%)	2 (66.6%)
H	5	5 (100%)	4 (80%)	1 (20%)
Total	58	52 (89.6%)	47 (81%)	14 (24.1%)

B = floor of corridor from side entrance
C = window-ledge of door at side entrance
D = window-ledge of main kitchen
E = window-ledge of stairs down to kitchen
F = floor of washing-up section
G = floor of vegetable storage room
H = window-ledge of vegetable storage room

'Phage-typing of the 14 strains showed a distribution of 'phage-groups similar to that found in food. 4 (28.6%) belonged to group I, and 4 to group II. The remaining 6 (42.8%) were equally divided between group III and untypable strains.

These observations thus showed that there was ample opportunity for the food in the main kitchen to become contaminated with haemolytic and nonhaemolytic strains of Cl. welchii and the method of storing the cooked chickens provided suitable conditions for their contamination with this heavily infected dust. Contamination with Staph. aureus was much less likely.

Eight swabs were taken from the floor and ledges in the special-diet kitchen in order to determine if the dust was similarly contaminated with Cl. welchii. All 8 samples yielded haemolytic or nonhaemolytic Cl. welchii and 2, both from the floor, contained Staph. aureus. These observations supported the view that contamination of the cold chicken from the main kitchen was due to the method of cooling and storing; the cooking procedures in both kitchens were essentially the same and there was an equal chance of the chicken in the two kitchens becoming infected with Cl. welchii from the dust.

Experimental Cooking of Pieces of Fowl

Various experiments were performed by cooking

pieces of fowl to reproduce in the laboratory some part of what actually took place in the kitchens - all with a view to discovering methods for reducing the risk of Cl. welchii food-poisoning among patients.

Firstly, four pieces of fowl, purchased from different poulterers, were cooked in a Koch's steam steriliser in a manner similar to that performed in the hospital kitchens. During cooking, temperatures were recorded by attaching a thermocouple in order to ascertain that similar temperatures to those recorded in the fowls in the kitchens were attained. After removal from the steamer the fowl pieces were kept sterile. One part of each was placed in the refrigerator and one part left at room temperature, the broth being divided equally between the two parts. A portion was removed at daily intervals up to 6 days for bacteriological examination.

Before cooking all four contained non-heat-resistant Cl. welchii but no heat-resistant strains. Neither at room temperature nor in the refrigerator did any of the pieces become contaminated with Cl. welchii, although by the 6th day 3 portions kept at room temperature showed a fair contamination with aerobic spore-bearing bacilli and micrococci. The 4 pieces kept in the refrigerator showed only a slight contamination with aerobic organisms by the 6th day, the organisms being isolated in enrichment culture only.

These contaminants probably gained entry on the removal of the portions for examination.

This experiment proved that the temperatures attained in cooking fowls were adequate to kill non-heat-resistant Cl. welchii. Protection of the pieces of fowl from contaminants even without refrigeration during the period soon after cooking when conditions were suitable for the growth of Cl. welchii appeared to prevent subsequent gross contamination with this organism. This was concluded by noting that the organisms which grew in the fowl pieces due to the contamination which took place while removing portions for examination after the pieces of fowl were cold, were exclusively aerobic organisms; moreover this operation was conducted in a laboratory where the atmosphere was proved to be heavily infected with Cl. welchii.

Further experiments were performed on one fowl divided into 6 portions, each of which was treated in a different manner. Before the experiments, the flesh of the fowl contained haemolytic Cl. welchii. Four of the pieces were placed in water in one pan and the remaining 2 were inoculated, deeply into the flesh, with 0.1 ml. of a 6-day culture of a nonhaemolytic strain of Cl. welchii which was known to be resistant to 1 hours' boiling. These two inoculated portions were then put into a second pan. The pans were placed

in a Koch's steam steriliser for 2 hr. 40 min. after which each portion of fowl was transferred to a sterile beaker with a lid. The broth was equally divided among the six portions so that the chicken was about $\frac{1}{4}$ immersed in broth with the upper $\frac{3}{4}$ protruding. One of the 2 portions which were inoculated before cooking with the heat-resistant Cl. welchii was placed in the refrigerator and the other left at room temperature. The broth of another two portions was inoculated with one drop of a 6-day culture of a haemolytic strain of Cl. welchii; one of these portions was placed in the refrigerator and one left at room temperature. The remaining 2 portions were uncovered and left overnight in the hospital kitchens, one in the main kitchen and one in the special-diet kitchen. The next morning they were covered and returned to the laboratory and left at room temperature. The broth and the flesh of each of the 6 pieces was examined 24 and 48 hours after cooking. The results were as follows:-

The broth and the flesh of the 2 portions which were inoculated before cooking with a heat-resistant (1 hour at 100°C) strain of Cl. welchii, yielded no growth whatsoever after either 24 or 48 hours. The Cl. welchii must therefore have been killed by the cooking which was 100°C for 2 hr. 40 min. This indicated that to survive cooking a contaminating strain of Cl. welchii must have a fairly high degree of heat

resistance and it proved that non-heat-resistant strains would certainly be killed by the cooking.

The 2 portions whose broth was inoculated with a haemolytic Cl. welchii after cooking gave markedly different results according to whether the sample was kept in the refrigerator or at room temperature. The broth of the portion in the refrigerator yielded 10 colonies of the strain of Cl. welchii on direct plating after 24 hours and 8 colonies after 48 hours, the strain being identified by slide-agglutination tests with a homologous antiserum. The flesh contained no Cl. welchii after 24 hours and yielded only 1 colony on direct plating after 48 hours. The Cl. welchii, therefore, did not multiply in the refrigerated portion. On the other hand, both the broth and the flesh of the sample at room temperature yielded a heavy growth of the Cl. welchii after both 24 and 48 hours. The Cl. welchii had invaded the chicken flesh and multiplied extensively overnight. The broth of this portion was noticeably cloudy after 2 days and the part of the flesh which was immersed in the broth was pink. Even after 5 days, however, it still did not smell badly, and both the appearance and the smell were quite appetising. This experiment proved that even when inoculated with large numbers of Cl. welchii, subsequent growth of the organisms could be prevented by adequate refrigeration if the fowls were stored

individually.

The portion of fowl which was left in the main kitchen was unfortunately covered up by a member of the kitchen staff and did not therefore give a true indication of the contamination due to air-borne contaminants. The broth remained sterile after 48 hours and the flesh gave a slight growth of anthracoids. The portion in the special-diet kitchen remained uncovered for 16 hours. The broth of this portion yielded no growth after 24 hours and a slight growth of anthracoids after 48 hours, whereas the sample of flesh removed after 24 hours gave a very slight growth of micrococci and a slight growth of haemolytic Cl. welchii. The sample of flesh removed after 48 hours, however, contained only micrococci and anthracoids. The distribution of the Cl. welchii was therefore uneven and this indicated that, on a smaller scale, the distribution of the Cl. welchii in the one piece of fowl was similar to that which took place when many chickens were cooled in one pot. The temperature presumably fell below a suitable level before the whole piece was equally contaminated. These observations also suggested that a higher risk of contamination with Cl. welchii was not of itself likely to be the cause of the higher rate of contamination actually observed in the cold chicken from the main kitchen as compared with that from the special-diet kitchen because

contamination with Cl. welchii did take place in the atmosphere of the special-diet kitchen. The relevant difference doubtless lay in the method of cooling.

Conclusions

Nutrient broth made from an extract of chicken was not found to contain nutrients particularly favourable to the growth of Cl. welchii. A comparison of the cold chicken served by the main and special-diet kitchens of the hospital showed a very much higher rate of contamination with Cl. welchii in the samples from the main kitchen; this was proved to be due to the method of storing the cooked fowls in the main kitchen. In addition to favouring the growth of heat-resistant Cl. welchii which survived cooking, the storing of the chickens unrefrigerated and uncovered provided ample opportunity for their contamination after cooking with kitchen dust which was heavily laden with Cl. welchii. Immediate refrigeration of cooked fowl was proved to prevent the growth of such organisms to a harmful extent if the birds were stored singly.

P A R T IV

OUTBREAKS OF FOOD POISONING INVESTIGATED

During the present investigation, 4 outbreaks of suspected food-poisoning within the hospital were reported. In all 4, cold chicken was the suspected food and in 3 heat-resistant Cl. welchii was isolated from the faeces of the patients involved. Another 3 incidents of suspected Cl. welchii food-poisoning are described below although they did not involve hospital patients; one involved members of the Bacteriology staff, one a member of staff's family, and the third members of the hospital kitchen staff. No outbreaks of either Staph. aureus or Salmonella food-poisoning were reported. It is freely admitted by nurses and doctors to members of the laboratory staff that numerous outbreaks of diarrhoea, mostly - but not all - of a relatively mild character, are associated in this hospital with the issue of cold chicken. These events are regarded as almost 'normal' and are not reported officially 'in order to avoid fuss'.

Outbreak 1

This incident was the first to confirm the suspicions aroused by the investigation of the

hospital food that cold chicken was a potential source of food-poisoning. On the afternoon of 24.11.55 a sample of cold chicken (F 123 in Appendix I) was collected from the hospital kitchens. After a portion required for examination was removed and the appropriate media inoculated, the remainder of the chicken sample was divided into 4 portions and eaten by 4 members of the laboratory staff. Of these 4 people, 2 had gastro-intestinal disturbances of different severities within 12 hours of ingesting the chicken; 2 had no ill effects. One of the affected members suffered mild abdominal discomfort some 12 hours after eating the chicken and his bowel movements on the morning of 25.11.55 were somewhat looser than normal. The other had severe abdominal pain accompanied by diarrhoea commencing 8 hours after the ingestion of the chicken and continuing throughout the next day. Both had completely recovered by 28.11.55. The signs and symptoms of both these people were in accordance with those of Cl. welchii food-poisoning and the only item of food they had eaten in common was the chicken from the hospital kitchens. No specimens of faeces were made available.

On examining the cultures inoculated from the sample of chicken a small number of micrococci and a fairly heavy growth of haemolytic Cl. welchii were noted on the direct media. Staph. aureus was isolated

after enrichment culture but no specific intestinal pathogens were found.

The symptoms of the affected people indicated that Cl. welchii was probably the causal organism of this incident and although Staph. aureus was isolated from the chicken in small numbers, they were presumably incidental contaminants as Staph. aureus food-poisoning presents a completely different clinical picture from that recorded. This incident was noted with interest as the causative organism appeared to be a haemolytic strain of Cl. welchii.

Outbreak 2

On 19.1.56 an incident was reported in one of the wards in the hospital; it involved 3 patients and a ward orderly. The previous day (18.1.56) the 3 patients had eaten a lunchtime meal consisting of cold chicken, the remains of which were ingested by the ward orderly. All four people had abdominal pain and diarrhoea on 19.1.56 and had recovered by 20.1.56. No specimens of stool or food were available for bacteriological examination.

Due to insufficient details no definite conclusions can be drawn from this incident but the clinical features indicate that it was a small outbreak of Cl. welchii food-poisoning caused by the ingestion of cold chicken.

Outbreak 3

2 out of 6 patients in one ward who ate chicken for lunch (12 noon) on 2.2.56 suffered symptoms typical of Cl. welchii food-poisoning 10 hours later. Diarrhoea continued through 3.2.56 and terminated by 4.2.56. The faeces of both the affected patients, collected on 3.2.56, yielded nonhaemolytic heat-resistant Cl. welchii. Other strains of haemolytic and nonhaemolytic Cl. welchii were isolated after direct and enrichment cultures. A further sample of stool from one of the patients on 4.2.56 contained only haemolytic Cl. welchii. No other intestinal pathogens were found in the faecal specimens. No sample of chicken was available for examination.

The heat-resistant organisms isolated from the faeces did not retain their heat resistance in culture, being killed by 5 minutes at 100°C. All the cultures of Cl. welchii isolated were sent to Dr. Betty Hobbs of the Food Hygiene Laboratory of the Public Health Laboratory Service at Colindale for serelogical typing. They proved to be a variety of strains most of which were untypable by her sera. From the faeces of both patients, however, a nonhaemolytic heat-resistant strain serotype 3 was isolated and Dr. Hobbs suggested that this strain most likely caused the symptoms in the two patients as it is commonly found in meat dishes suspected of causing Cl. welchii food poisoning.

Outbreak 4

This outbreak was reported on 31.8.56 and involved 3 patients in one ward. These patients had eaten chicken on 30.8.56 and suffered abdominal discomfort and diarrhoea some 8-12 hours afterwards. The diarrhoea continued through 31.8.56; all had recovered by 1.9.56.

Specimens of faeces from the three patients were received on 31.8.56; heat-resistant nonhaemolytic Cl. welchii was isolated from all three. Haemolytic Cl. welchii was present in the faeces of only one patient and nonhaemolytic strains were found in direct or enrichment culture in the specimens from the three patients. No other pathogenic organisms were isolated.

A sample of the chicken which the patients had eaten was not available from the ward but a sample was collected on 30.8.56 from the hospital kitchens. As 22 birds are cooked at the one time and distributed to the wards after carving it was doubtful whether the sample received from the kitchen was a part of the same bird from which the chicken eaten by the patients in this particular ward was taken. The chicken received from the kitchen was found, however, to be contaminated by a fair number of haemolytic and nonhaemolytic Cl. welchii on direct culture.

The strains of Cl. welchii from both the patients' faeces and the chicken sample were serotyped by Dr.

Betty Hobbs. All three patients were proved to harbour a heat-resistant nonhaemolytic strain of serotype 4 which was presumably the cause of the outbreak. The chicken contained a haemolytic type 6, a nonhaemolytic type 1 and a nonhaemolytic untypable strain. As suspected, therefore, the chicken samples was not the one responsible for the patients' symptoms. The results indicated, however, that in one batch of chicken cooked simultaneously one bird harboured 3 different strains of Cl. welchii while another, responsible for the outbreak in the wards, presumably contained a further strain. This evidence supports the view of multiple contamination of the one batch of chickens with the possibility of different strains of Cl. welchii colonising and proliferating in the flesh of the individual birds.

Outbreak 5

This outbreak was well documented due to the clinical co-operation of Dr. M. McNicol, previously a senior registrar in the Western Infirmary of Glasgow, and a report has been accepted for publication (McNicol and McKillop, 1958). Relevant extracts from the manuscript are given below.

"Around 8 p.m. on 6.8.57 nine patients in a medical unit of 39 beds developed a brief illness characterised by lower abdominal colic and disproportionately slight

general upset. By the next morning all had recovered. No specific therapy was given. Diarrhoea was confined to those taking light diet, among whom nine of fifteen were affected; the patients who ate special or ordinary diets were not affected. The maid attached to the ward (case no. 16) also developed severe diarrhoea on the same evening. The only item of food common to the patients and the ward maid was cold chicken which was served at noon to those on light diet only. The maid had eaten the chicken left over from the patients' lunch and no samples of this food were therefore available for examination". "On the morning of 7.8.57 when the diarrhoea had almost settled, rectal swabs were taken from all those (16) who had eaten the chicken".

"Nonhaemolytic heat-resistant Cl. welchii was isolated from eleven and Staph. aureus, in small numbers only, from seven of the sixteen rectal swabs examined. Both haemolytic and nonhaemolytic strains of Cl. welchii were isolated from a large proportion of the cultures (12 of the 16). Of the ten people with diarrhoea eight had nonhaemolytic heat-resistant Cl. welchii in their stools".

This investigation stimulated reports of diarrhoea from another 2 units in the hospital and specimens of stool were received from a further 6 patients (cases 17-22) who had also eaten chicken for lunch

on 6.8.57 and suffered symptoms similar to those of the patients reported above. Nonhaemolytic heat-resistant Cl. welchii was isolated from all 6 and Staph. aureus from 3. Therefore, from the total of 22 patients, 16 of whom had diarrhoea, 17 had non-haemolytic heat-resistant Cl. welchii in their stools and 10 Staph. aureus; and from 14 of the 16 with diarrhoea heat-resistant Cl. welchii was isolated. These results are presented in table IX.

Serological typing of all the nonhaemolytic strains of Cl. welchii, including the known heat-resistant strains, was carried out by Dr. Betty Hobbs. Of the 53 strains examined 46 were untypable by the available sera. Untypable nonhaemolytic strains were isolated from 18 of the 22 examined and nonhaemolytic heat-resistant untypable strains from 15, 14 of whom had diarrhoea. From 2 of the three who carried heat-resistant Cl. welchii without having diarrhoea the organisms isolated were of different serological types: type 5 and type 8. It is possible that these two typable strains may have been unconnected with the diarrhoea.

Further serological investigations were undertaken to see if the untypable strains, especially the heat-resistant strains, were identical. An antiserum (A) to one of the nonhaemolytic heat-resistant strains was prepared by me and tested against all the non-

TABLE IX

Occurence of nonhaemolytic heat-resistant Cl. welchii and Staph. aureus in the faeces of the patients involved in Outbreak 5

Case No.	Symptoms of diarrhoea	Heat-resistant Cl. welchii	Staph. aureus
1	-	-	-
2	-	-	-
3	-	+	-
4	-	+	+
5	-	-	-
6	-	+	+
7	+	+	+
8	+	+	-
9	+	+	+
10	+	+	-
11	+	+	-
12	+	-	-
13	+	-	+
14	+	+	+
15	+	+	+
16	+	+	-
17	+	+	+
18	+	+	-
19	+	+	+
20	+	+	-
21	+	+	-
22	+	+	-
Total	16	17	10

haemolytic strains isolated in the outbreak. Of the 46 strains examined, antiserum A agglutinated to titre 20 strains, all of which were heat-resistant. 13 patients, 12 of whom had diarrhoea, harboured this strain. The heat-resistant strains from 2 of the patients with diarrhoea were untypable both with Dr. Hobbs's sera and antiserum A. Cross-agglutination reactions were observed with strains of types 5 and 8 but no agglutination took place with type 6.

The staphylococci isolated from 10 of the 22 specimens proved to be of a wide variety of strains (table X) and were present in small numbers. Due to the diversity of types it is unlikely that the staphylococci were responsible for the diarrhoea and were most probably present as a result of it. The reasons for this explanation are discussed more fully on p 101.

The results indicate that 13 of the 22 patients examined in this outbreak were infected with one particular strain of nonhaemolytic heat-resistant Cl. welchii. 12 of these patients had typical symptoms of Cl. welchii food-poisoning and the only item of diet in common to all 22 was cold chicken.

Outbreak 6

On the evening of 22.9.57 while visiting an elderly lady, who lived alone, the son of a member

TABLE X

Antibiotic sensitivity and bacteriophage type of Staph. aureus isolated during outbreak 5

Case	Antibiotic sensitivity					'Phage type
	P	C	T	St	E	
4	R	S	S	S	S	6w/7/75
6	R	S	R	R	S	53(1000 x R.T.D.)
7	S	S	S	S	S	N.T.
9	S	S	S	S	S	7
13	R	S	S	S	S	47/53/75/77
14	R	S	R	S	S	7/53/75 (1000 x R.T.D.)
15	S	S	S	S	S	N.T.
17	S	S	S	S	S	29w/80
19	R	S	R	S	S	7w(1000 x R.T.D.)
21	R	S	S	S	S	6/42E/47/53/75 (1000 x R.T.D.)

P = penicillin
C = chloramphenicol
T = tetracycline
St = streptomycin
E = erythromycin

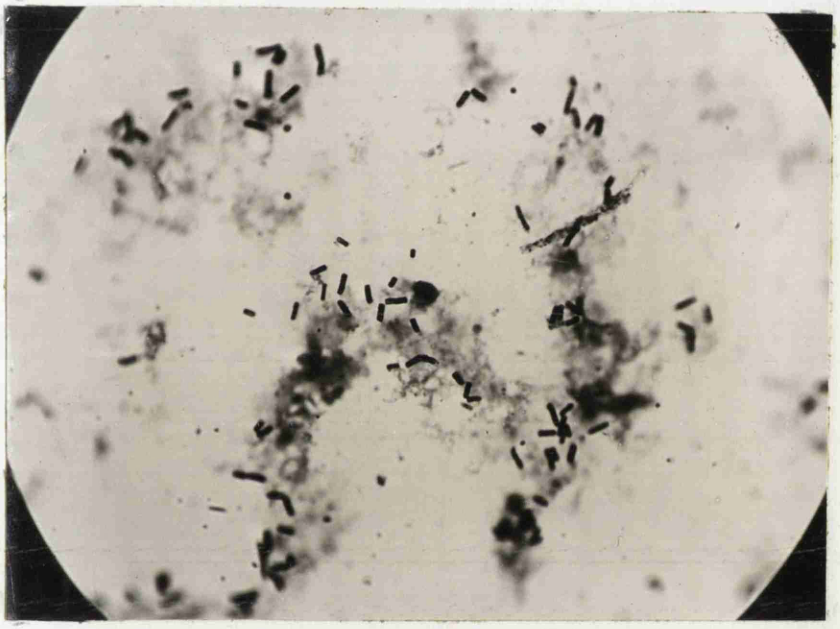
N. T. = non-typable
S = sensitive
R = resistant

of staff ate a meal of cold chicken. Some 8 hours later he had abdominal colic and diarrhoea which persisted to some extent throughout 23.9.57. By the next day he had completely recovered and two specimens of stool were received, one on 23.9.57 and the second on 24.9.57. Another member of the family procured a sample of the chicken and the chicken broth on 23.9.57.

Details of the cooking of the chicken were available. It was boiled on the evening of 20.9.57 and after cooking it was removed from the pan to cool. Leeks and rice were added to the broth and the chicken subsequently returned to the broth and the lid of the pan replaced. The kitchen where the pan was kept was fairly warm and portions of the chicken and broth were removed at intervals to provide a meal. When the samples were taken on 23.9.57 some difficulty was encountered in trying to put a stopper in the tube containing the broth because of the production of gas.

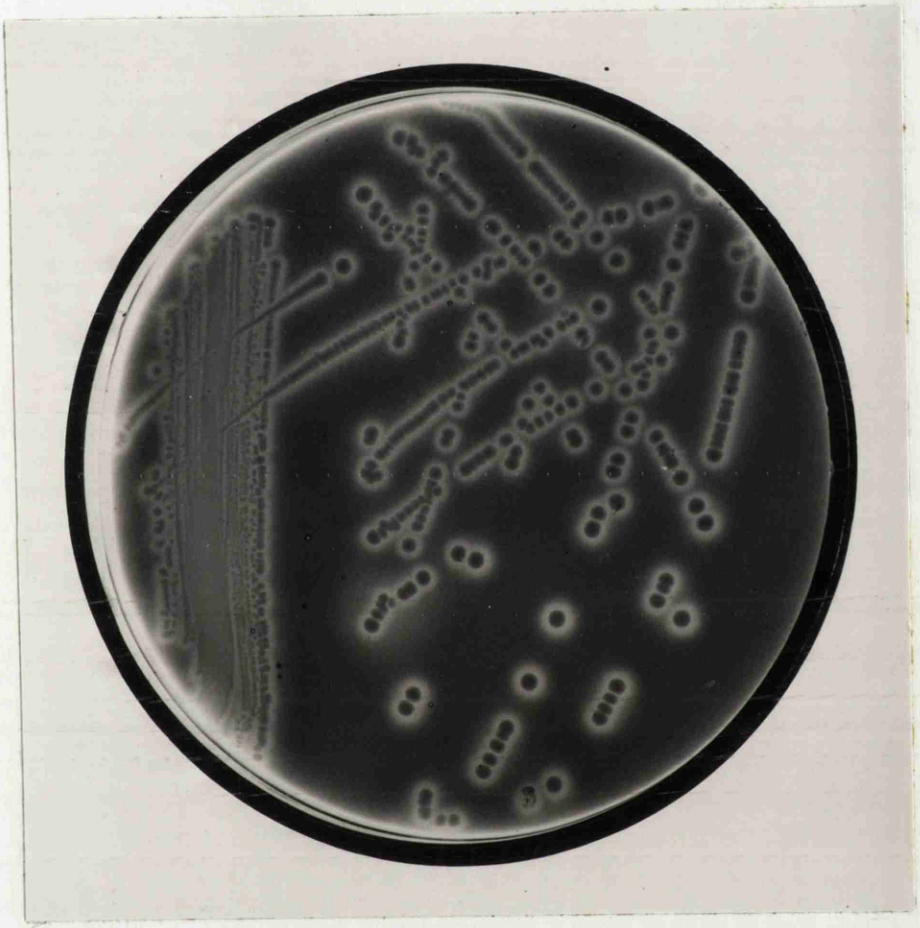
A direct smear of both the broth and the chicken showed numerous Gram-positive bacilli typical of Cl. welchii (fig. VIII). Culture of both these specimens yielded a heavy growth of haemolytic Cl. welchii (fig. IX) and a slight growth of anthracoids and micrococci. Both specimens of faeces gave a heavy growth of haemolytic Cl. welchii (fig. X) and a fair growth of coliforms. No other pathogenic organisms were found.

Figure VIII



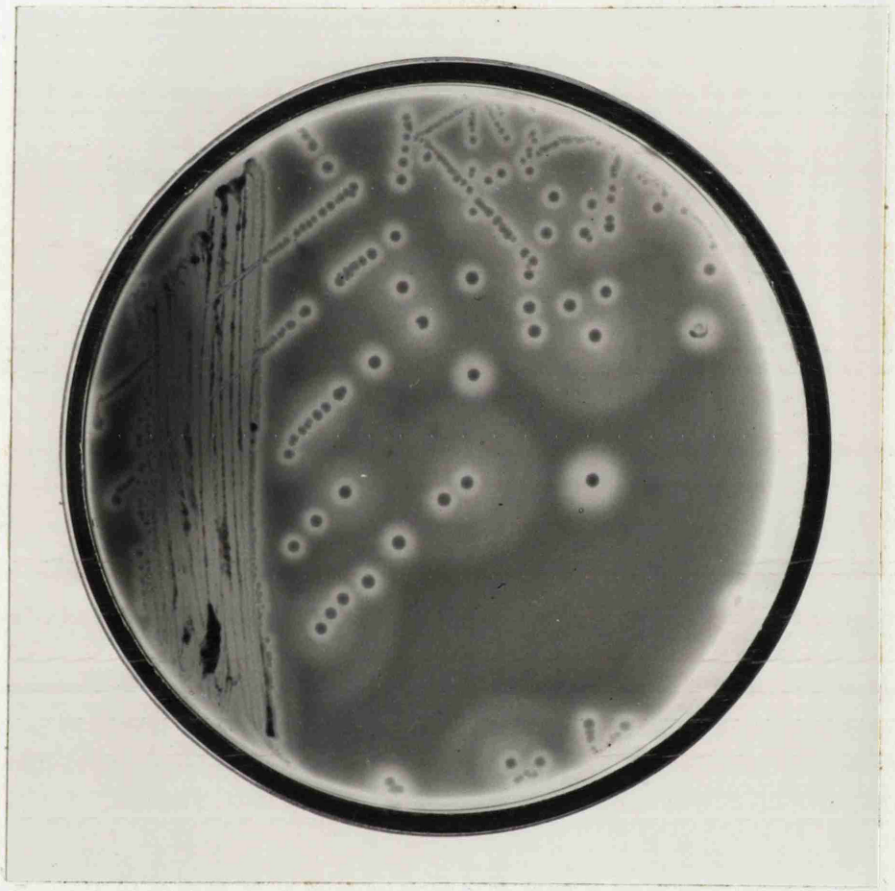
Film of the chicken involved in outbreak 6. Numerous bacilli typical of Cl.welchii can be seen. Gram's method x 600.

Figure IX



Horse-blood-agar plate (incubated anaerobically) inoculated directly from the chicken involved in outbreak 6, showing a heavy growth of haemolytic Cl.welchii. (actual size).

Figure X



Horse-blood-agar plate (incubated anaerobically) inoculated directly from the faeces of the affected person in outbreak 6, showing a heavy growth of haemolytic Cl.welchii. (actual size).

Antisera were prepared to one of the strains isolated from the chicken broth and one of the strains isolated from faeces. Both antisera agglutinated to titre all the strains of Cl. welchii from the chicken and the faeces of the affected person. This is, therefore, considered to be a case of Cl. welchii food-poisoning due to the ingestion of cold chicken contaminated after cooking with a haemolytic strain of Cl. welchii.

Outbreak 7

A chance observation uncovered a first-hand account of a further incident whose details, although no scientific investigations could be made, are considered significant in view of previous findings. Some members of the kitchen staff, after eating chicken sandwiches some 8 hours previously, suffered abdominal discomfort and diarrhoea. By the next day all had recovered and after discussing their disturbances, decided that the chicken was the causal factor as it was the only item of diet they had eaten in common.

On reviewing the clinical picture, this appeared to be another outbreak of Cl. welchii food-poisoning and the implication yet again of cold chicken cannot be dismissed.

Conclusions

The association between *Cl. welchii* food-poisoning and cold chicken was too frequently noted to be a chance observation. Other incidents have doubtless happened in the hospital and failed to be reported. This opinion is supported by noting the bad reputation which the cold chicken cooked in the hospital has with the hospital staff, and this view is also strengthened by outbreak no. 5 above where the industrious investigation by a clinician in one ward stimulated the reports from another two units of further cases which would otherwise have been unreported.

The evidence of these outbreaks indicates that strains of *Cl. welchii* other than heat-resistant strains can be responsible for food-poisoning. In two incidents haemolytic strains were implicated.

P A R T V

AN INVESTIGATION OF THE INCIDENCE OF CL. WELCHII AND OF STAPH. AUREUS IN THE FAECES OF HOSPITAL PATIENTS

347 faecal specimens from 272 hospital patients were examined for the presence of haemolytic, non-haemolytic, and nonhaemolytic heat-resistant Cl. welchii. 312 of the specimens from 255 of the patients were investigated for the presence of Staph. aureus and all were examined for salmonellas and shigellas. An account of the findings for each specimen is presented in Appendix II.

The incidence of Cl. welchii and of Staph. aureus in all specimens is shown in tables XI and XII respectively. As these figures include, on occasions, more than one specimen from each individual patient, the results calculated on the basis of the presence of these organisms at any time in each individual patient are also quoted. In comparing the two sets of results, little difference is noted. This is presumably because the numbers are sufficiently high to accomodate the inclusion of multiple specimens from patients who consistently carried either organism. For accuracy, however, the figures for each patient as opposed to each specimen are accepted as the true incidence of

TABLE XI

The incidence of Cl. welchii
in the faeces of hospital patients

No. examined	Cl. welchii isolated		
	haemolytic	nonhaemolytic	heat-resistant
347 specimens	108D 212E (31.1%) (61%)	33D 65E (9.5%) (18.7%)	46 (13.2%)
272 patients	91D 171E (33.4%)(62.8%)	30D 60E (11%) (22%)	35 (12.8%)

D = isolated on direct culture

E = isolated after enrichment culture

TABLE XII

The incidence of Staph. aureus
in the faeces of hospital patients

No. examined	Staph. aureus isolated (direct and enrichment culture)
312 specimens	68 (21.7%)
255 patients	56 (21.9%)

Cl. welchii and Staph. aureus.

No salmonellas were isolated and Shigella sonnei was isolated from only 3 patients. Haemolytic Cl. welchii was found in 62.8% and nonhaemolytic in 22%. These organisms were present in fairly large numbers in approximately half of the patients from whom they were isolated, the figures for their isolation on direct culture being 33.4% and 11% respectively. As Cl. welchii is a normal inhabitant of the human bowel these numbers are not considered to be unduly high.

The incidence of nonhaemolytic heat-resistant Cl. welchii was 12.8% after 15 minutes or more at 100°C. From 9 specimens heat-resistant Cl. welchii were isolated after 15 minutes and not after one hour's boiling and from 2 after 1 hour's and not 15 minutes' boiling. As the heat-resistant organisms were isolated on two occasions only after 1 hour's boiling, the inclusion of the two periods of boiling served as a double check on each specimen. Nevertheless from the overall results 15 minutes at 100°C gave more positive results than the more usual hour and would, therefore, appear to be superior.

The faecal incidence of Staph. aureus was 21.9% in the 255 patients examined and this organism was isolated from all but five patients after enrichment culture only.

A Comparison of the Incidences of Cl. welchii and Staph. aureus in Patients with and without Diarrhoea

The 272 patients examined included 170 with diarrhoea and 102 without diarrhoea. The patients in the diarrhoeal group were suffering from gastro-intestinal disturbances of different aetiology, some of which are shown in Appendix II, but none was suspected of having Cl. welchii or Staph. aureus food-poisoning. The normal group comprises patients who showed no apparent signs of a gastro-intestinal disturbance. The incidence of Cl. welchii and of Staph. aureus in both groups is presented in tables XIII and XIV respectively.

Shigella sonnei was isolated from three patients in the diarrhoeal group and no salmonellas were isolated in either group.

The incidence of Cl. welchii in patients with and without diarrhoea was of the same order. The number of patients with diarrhoea from whom haemolytic Cl. welchii was isolated was slightly lower than the number without diarrhoea from whom it was isolated (60% and 67.6% respectively); the position was reversed in the case of nonhaemolytic strains (24.7% in the diarrhoeal group and 17.6% in the normal group). 11.1% of the patients with diarrhoea harboured heat-resistant strains in their faeces as compared with 15.6% of the patients without diarrhoea. The overall

TABLE XIII

A comparison of the incidence of Cl. welchii in the faeces of hospital patients with and without diarrhoea

No. examined	Diarrhoea	Cl. welchii isolated				
		haemolytic		nonhaemolytic		heat-resistant
170	+	50D (29.4%)	102E (60%)	21D (12.3%)	42E (24.7%)	19 (11.1%)
102	-	41D (40.1%)	69E (67.6%)	9D (8.8%)	18E (17.6%)	16 (15.6%)

D = isolated on direct culture

E = isolated after enrichment culture

TABLE XIV

A comparison of the incidence of Staph. aureus in the faeces of hospital patients with and without diarrhoea

No. examined	Diarrhoea	Staph. aureus isolated (direct and enrichment culture)
170	+	47 (27.6%)
85	-	9 (10.5%)

results indicate that these differences are not significant.

On the other hand, a striking difference was observed in the incidences of Staph. aureus between the two groups. Whereas this organism was isolated from 27.6% of the patients with diarrhoea it was isolated from only 10.5% of the patients in the normal group. In all the normal and the majority of the diarrhoeal stools from which they were isolated, the staphylococci were present in small numbers only. Specimens from five patients in the diarrhoeal group yielded Staph. aureus on direct culture. In two of these five there were only 1 or 2 colonies of staphylococci on the direct plates and the faecal flora was normal. They can be regarded, therefore, as being present in only insignificant numbers in these patients. However, in the remaining 3 patients Staph. aureus was found to be the predominating organism, giving an almost confluent growth on direct plating on to blood agar and being apparent in the direct film. The specimens of all other diarrhoeal patients when smeared and stained by Gram's method showed a normal bowel flora, as they did on direct plates, but in the three patients harbouring large numbers of Staph. aureus clumps of Gram-positive cocci were obvious on the film. All three patients were undergoing antibiotic therapy and this was considered likely to be the cause of the overgrowth of staphylococci.

On withdrawal of the antibiotic the numbers of staphylococci in two patients fell considerably and the third died of a staphylococcal pneumonia.

As the numbers of staphylococci in the faeces of the patients in the diarrhoeal group were, with the exception of the three cases mentioned above, of the same order as found in the faeces of normal patients, the diarrhoea cannot be attributed to the presence of Staph. aureus. On the other hand, due to the increased rate at which material passes through the bowel after the onset of diarrhoea, some organisms which under normal circumstances would be outgrown by others or killed by the gastric juice and intestinal secretions may survive and be present in the faeces. I consider, therefore that the frequent isolation of Staph. aureus from the faeces of patients with diarrhoea was a result rather than a cause of the diarrhoea.

The Effect of the Duration of Hospital Stay on the Incidence of Cl. welchii and Staph. aureus

Despite promises of clinical and nursing co-operation - and much personal pleading and persuasion - it was not possible to collect a series of specimens from a sufficient number of patients throughout their stay in hospital. However, a review of the 45 patients from whom more than one specimen was received, shows that the incidence of Cl. welchii and of Staph. aureus

showed only a slight increase during the period which elapsed between examining the first and last specimens. These results are summarised in table XV.

In addition, it was determined in what week during the patients' stay in hospital each of 102 samples of normal faeces was examined (this is noted in Appendix II) and the results were divided as shown in table XVI. The numbers in each group are unfortunately small but nevertheless no consistent increase of Cl. welchii or Staph. aureus was found according to the week in which the specimen was taken. It is doubtful, therefore, if the increase in incidence of Staph. aureus in the 7th-13th week group or the apparent acquisition of heat-resistant Cl. welchii after the first week is significant. The results indicate that the hospital patients examined did not acquire either Cl. welchii or Staph. aureus to any great extent during their stay in hospital but I appreciate that a more detailed survey would be necessary to make this a valid conclusion.

Conclusions

This investigation did not afford a single bacteriological explanation of "hospital diarrhoea". The diarrhoeal stools examined were specimens from patients suffering from a wide range of complaints in both the surgical and medical wards of the hospital and in the

TABLE XV

The incidence of Cl. welchii and Staph. aureus in the first and last faecal specimens from hospital patients

	Cl. welchii			Staph. aureus
	haemolytic	nonhaemolytic	heat-resistant	
First specimen	30 (66.7%)	4 (8.8%)	7 (15.5%)	5 (14.7%)
Last specimen	33 (73.3%)	8 (17.7%)	8 (17.7%)	8 (23.5%)
Increase	3 (6.6%)	4 (8.8%)	1 (2.2%)	3 (8.8%)
No. examined	45	45	45	34

TABLE XVI

The incidence of Cl. welchii and Staph. aureus in faecal specimens collected on different weeks during patients' stay in hospital

Week of stay	No. examined	No. containing			
		Cl. welchii			Staph. aureus
		haem-olytic	nonhaem-olytic	heat-resistant	
1st	25	16 (64%)	3 (12%)	0	1 of 21 (4.7%)
2nd	23	15 (65.2%)	5 (21.7%)	5 (21.7%)	1 of 21 (4.7%)
3rd	15	8 (53.3%)	1 (6.6%)	2 (13.3%)	0 of 11
4th	14	7 (50%)	0	1 (7.1%)	1 (7.1%)
5th-6th	14	9 (64.2%)	0	2 (14.2%)	0
7th-13th	11	5 (45.4%)	1 (9%)	1 (9%)	3 (27.2%)

vast majority no specific intestinal pathogen was found and no individual organism was present in abnormally large numbers to account for the diarrhoea. Cl. welchii was not found to be more prevalent in the diarrhoeal patients than it was in the non-diarrhoeal patients and although the incidence of Staph. aureus was higher in the former group, the presence of this organism was not, in most cases, regarded as significant due to the small numbers isolated. Many cases of hospital diarrhoea do not, therefore, appear to be of bacterial origin.

P A R T VI

CHARACTERISTICS OF THE CL. WELCHII AND STAPH. AUREUS ISOLATED FROM FAECES

Some investigations were made into the characteristics of the strains of Cl. welchii and Staph. aureus which were isolated in the survey of the faeces of hospital patients reported above in Part V. Note was also made of the morphology and colonial appearance of all strains.

Clostridium welchii

The morphology of all strains of Cl. welchii which were smeared and stained by Gram's method was identical apart from slight variations in size. No spores were seen in smears from any of the cultures on blood agar or Robertson's meat medium and although the direct smears of faeces frequently showed spores their presence could not certainly be attributed to Cl. welchii as Cl. sporogenes was often present.

Both haemolytic and nonhaemolytic Cl. welchii were isolated from faeces; all heat-resistant strains were nonhaemolytic. The strains designated as haemolytic Cl. welchii gave a marked zone of β-haemolysis on horse-blood agar. The nonhaemolytic, including the nonhaemolytic heat-resistant strains, were generally

nonhaemolytic on primary isolation but after allowing the plates to stand at room temperature for a few days a faint zone of α-haemolysis sometimes developed. Similarly some initially nonhaemolytic strains after being subcultured in Robertson's meat broth produced slight α-haemolysis when replated on blood agar.

A variety of colonial forms, from smooth colonies with an entire edge to rather rough colonies with an irregular edge and radial striations, were noted in cultures of both haemolytic and nonhaemolytic strains and strains with different types of colonial form were, on occasions, isolated from the one specimen of stool.

All the strains isolated gave a positive test for the production of α-toxin specifically inhibited by type-A antitoxin. In culture some of the heat-resistant strains isolated retained their heat-resistance although spores were not seen in direct smears. Others, however, did not survive boiling for even 5 minutes.

54 nonhaemolytic strains (33 from normal stools and 21 from diarrhoeal stools) were sent to Dr. Betty Hobbs of the Food Hygiene Laboratory of the Public Health Laboratory Service at Colindale for serelogical typing. Both heat-resistant strains and strains isolated on direct and enrichment culture were

included. 46 of the strains were untypable, 2 were type 3, 1 was type 5, 1 type 6, 1 type 8, 1 type 9, 1 type 10 and one was autoagglutinable. No relation was found between the typability of the strain and the source (whether diarrhoeal or normal stool) or the method of primary isolation (whether heated or not). It was noted, however, that more than one serelogical type was frequently isolated from the one specimen and different types from a single patient in succesive specimens. It seems likely therefore, that the carriage of Cl. welchii in the faeces is of a transient nature.

As the majority of the strains were untypable by Dr. Hobbs's sera it was impossible to say whether one type was predominant in the patients within this hospital.

Staph. aureus

The morphology of all the strains of Staph. aureus isolated from faeces was typical. The colonial appearances of different strains showed marked variations in haemolysis on horse-blood agar and pigment production. Although haemolysin production on horse-blood agar is not significant as an indication of potential pathogenicity, it aids the identification in a mixed growth of different strains of Staph. aureus which have an otherwise similar colonial appearance.

The strains which were haemolytic on horse-blood agar showed different degrees of β-haemolysis, the remainder were nonhaemolytic. The production of pigment was marked in some strains which showed a deep golden colour, whereas others failed to produce any noticeable pigment even after standing at room temperature for some days. All cultures of Staph. aureus isolated from the faeces gave a positive tube coagulase test in 3-4 hours at 37°C.

All faecal strains of Staph. aureus were tested for antibiotic sensitivity and all save 3 were 'phage-typed. These results are shown in Appendix III and abstracted in tables XVII and XVIII.

As judged by haemolysis on horse-blood agar, antibiotic sensitivity pattern and 'phage-type some patients were found to be carrying two or three strains of Staph. aureus in their faeces but most carried only one. As a rule, individual patients were found to be carrying the same strain or strains on succesive examinations. 62 strains in all were isolated, 48 (75.9%) of which were resistant to penicillin; none was resistant to erythromycin. 13 strains (20.9%) were sensitive to all the antibiotics tested and 12 (19.3%) had a sensitivity pattern indicating a possible acquisition of a hospital strain of Staph. aureus, being sensitive to erythromycin or to chloramphenicol and erythromycin only. This evidence strengthens the

TABLE XVII

Antibiotic sensitivity of faecal strains of Staph. aureus

No. examined - 62		
Antibiotic	No. sensitive	%
P	15	24.1
C	60	96.7
T	46	74.1
St	43	69.3
E	62	100
P,C,T,St & E	13	20.9
E only	1	1.6
C & E only	11	17.7

P = penicillin
C = chloramphenicol
T = tetracycline
St = streptomycin
E = erythromycin

TABLE XVIII

Distribution of faecal strains of Staph. aureus in 'phage groups

No. examined - 59		
'phage group	No.	%
I	9	15.2
II	5	8.5
III	33	56.0
N.T.	12	20.3

N.T. = untypable

view that there was only a slight increase, if any, in the incidence of faecal carriage of Staph. aureus during the patients' stay in hospital.

59 of the above mentioned strains of staphylococci were 'phage-typed. As expected, 45 (76.3%) belonged to Group III or were untypable. The remainder were in Groups I & II, the majority of the multiple-antibiotic-resistant organisms being of Group III or untypable. No difference in the sensitivity patterns or 'phage type existed between the strains isolated from diarrhoeal and normal stools.

Conclusions

Both the Cl. welchii and Staph. aureus from the faeces of hospital patients proved to be of a wide range of strains and no predominating strain of either organism was found to exist throughout the patients. Although large numbers of multiple-antibiotic-resistant hospital strains of Staph. aureus are undoubtedly present in the hospital atmosphere, they did not appear, in the present investigation, to be acquired frequently by the hospital patients as part of their faecal flora. The evidence suggests that faecal carriage of Staph. aureus is more prolonged than that of Cl. welchii, which appears to be merely transient.

P A R T VII

A BACTERIOLOGICAL INVESTIGATION OF CASES OF NECROTISING ENTEROCOLITIS

By considering both the clinical and pathological features, the diagnosis of necrotising enterocolitis was confirmed in each of 7 fatal cases recorded below by Dr. A. Watson of the Pathology Department of the University and Western Infirmary of Glasgow. Kay, Richards, and Watson (Brit. J. Surg.; in press) included cases 1-4 in their report on this disease. For the purposes of this thesis only the bacteriological findings are considered relevant and few details of the individual clinical and pathological features are presented.

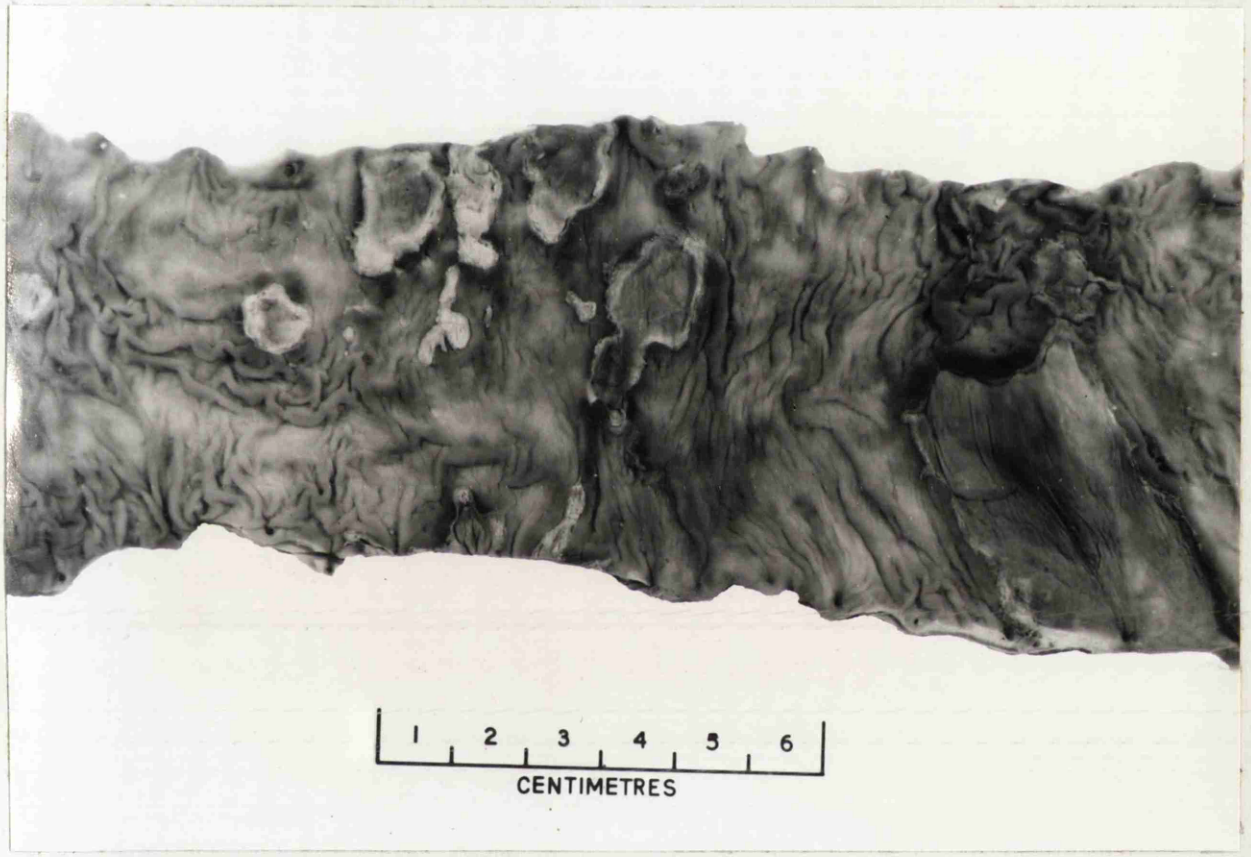
In 6 of the cases, acute necrotising enterocolitis followed a surgical operation usually on the 2nd to 5th post-operative day; in one (case 3) it was a sequel to myocardial infarction. The outstanding clinical feature was the development of acute peripheral circulatory failure which did not respond to treatment but persisted till death some 6 to 48 hours after the onset of the acute symptoms. Diarrhoea was not always present. 6 of the 7 patients (all except case 6) had received antibiotics, but in only one case (case 5) was the dosage excessive.

The gross morbid-anatomical and microscopic appearances

differed in detail from case to case according to the extent and distribution of the lesions. The pathological feature common to all cases was necrosis of the inner layer of the bowel wall, usually confined to the mucosa. The mucosal necrosis differed in extent from focal and patchy to diffuse (figs. XI and XII). Superficial inflammatory exudation was not a constant finding although in many cases it constituted a distinct pseudomembrane. In some cases the whole intestinal tract and even the stomach were extensively involved; in others the lesions were limited to the small or large bowel.

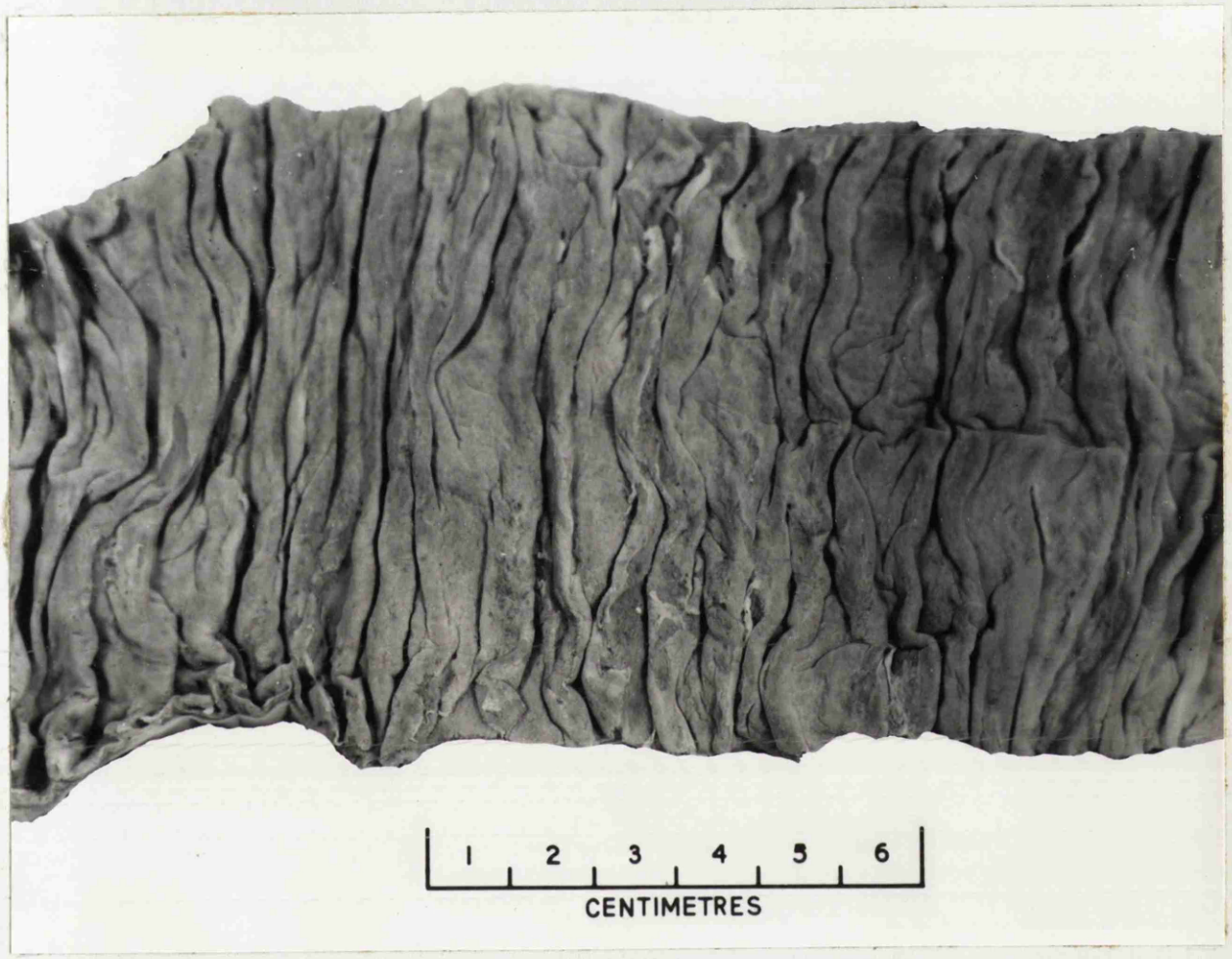
Specimens taken at autopsy for bacteriological investigation always included bowel contents from the affected section of the gastro-intestinal tract and in some cases specimens were also examined before death. The nature of the specimens examined is recorded for each case with the bacteriological results. When samples of liver and spleen were examined a section of the outer surface was seared and a sterile swab inserted through the seared surface; this swab was used for investigation. A smear of all specimens was made and stained by Gram's method and further examination was made according to the methods described for faecal specimens (p 41). Agar plus 6% NaCl was invariably included, however, because of the possible role of staphylococci as a causative

Figure XI



Segment of descending colon from case 1, showing the discontinuous patchy type of necrotising enterocolitis.

Figure XII



Segment of jejunum from case 4, showing the diffuse type of necrotising enterocolitis.

factor in necrotising enterocolitis.

The conclusion reached as a result of the examinations reported below was that there was no constant or significant association between this condition and any bacterium or group of bacteria. The findings are given, however, because the work formed a necessary part of the inquiry into hospital diarrhoea and involved the expenditure of a substantial amount of time and effort.

Case 1

Clinical history.- The patient, a male of 49 years, became shocked with cold cyanosed extremities on the 5th day after gastrectomy and died on the 6th day. Achromycin and aureomycin were administered only after collapse on the 5th post-operative day.

Pathological findings.- Patchy necrosis of mucosa of stomach, descending colon, and rectum, extending deeply in places. The mucosal lining of the whole intestinal tract was intensely congested.

Bacteriological findings.- At autopsy specimens of stomach, jejunum, rectum, spleen,and liver were taken. The stomach, jejunum and liver gave a heavy growth of coliforms, Pseudomonas pyocyanea, and a scanty growth of enterococci; coagulase-positive Staph. aureus of 'phage group III (sensitive to chloramphenicol and erythromycin only) were also isolated from these 3

sites. The rectal contents showed a heavy growth of coliforms and Ps. pyocyanea and a light growth of haemolytic Cl. welchii.

Case 2

Clinical history.- A male, aged 51, collapsed on the 2nd day after a partial gastrectomy. Laparotomy was performed on the 4th post-operative day and a portion of ileum removed; the patient died later on the same day. Oxytetracycline was administered.

Pathological findings.- Numerous irregular, almost confluent areas of mucosal necrosis were found in ileum, descending colon and rectum, often invading deeper layers of bowel wall.

Bacteriological findings.- Before death, gastro-intestinal aspirate, ileal contents, and a peritoneal swab, taken at the time of laparotomy, were examined. There was a heavy growth of coliforms and haemolytic Cl. welchii and a scanty growth of enterococci from the aspirate and ileal contents. The peritoneal swab showed a fair growth of micrococci.

Post-mortem specimens included caecal and rectal contents and liver. Large numbers of coliforms and a few enterococci were isolated from all three sites; in addition the rectal contents contained a large number of haemolytic Cl. welchii.

Case 3

Clinical history.- The patient, a male of 63 years, suffered shock after coronary thrombosis with massive infarction and died on the 7th day. Penicillin was the only antibiotic administered.

Pathological findings.- Extensive mucosal necrosis of descending colon and rectum with areas of deeper involvement.

Bacteriological findings.- Colonic contents were examined at autopsy. Numerous coliforms and haemolytic Cl. welchii and a few enterococci were isolated on direct culture, and a coagulase-positive Staph. aureus 'phage group III (sensitive to chloramphenicol and erythromycin only) was isolated after enrichment culture.

Case 4

Clinical history.- A male of 41 years collapsed and died on the 2nd day after gastrectomy. Intravenous erythromycin was given after the collapse.

Pathological findings.- Very extensive mucosal necrosis of jejunum and ileum with a slight fibrinous exudate.

Bacteriological findings.- Gastric aspirate and a rectal swab were received just before death. These specimens gave a heavy growth of coliforms, proteus, and haemolytic and nonhaemolytic Cl. welchii; in addition, staphylococci of 'phage group II (sensitive to all antibiotics tested) were isolated after enrichment

culture from the gastric aspirate.

At post mortem the contents of jejunum, ileum, caecum, and colon, and a specimen of liver were examined. The intestinal contents gave a heavy growth of proteus, coliforms, and haemolytic and nonhaemolytic Cl. welchii. Staph. aureus ('phage group III; sensitive to chloramphenicol and erythromycin) was isolated after enrichment culture from the jejunum only. The liver contained a fair number of coliforms and proteus.

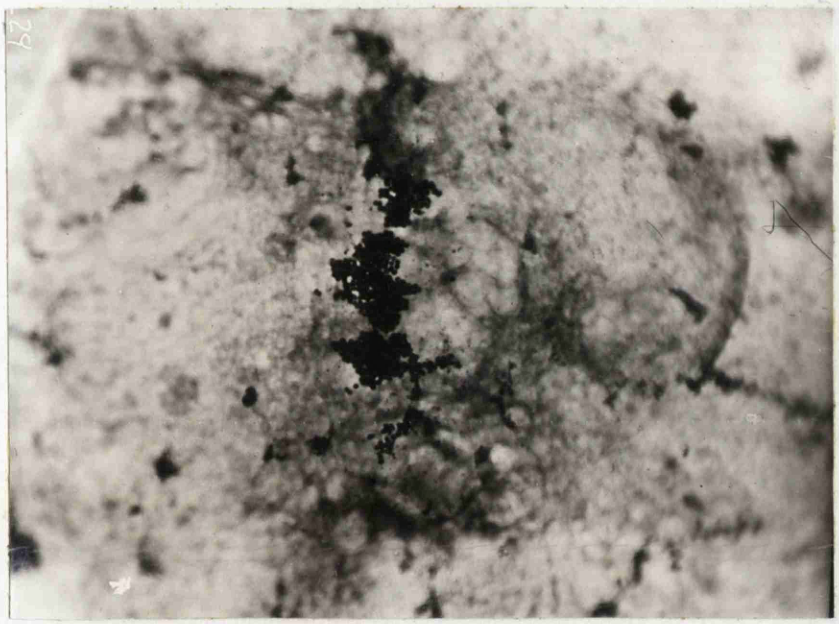
Case 5

Clinical history.- A man aged 28 had an operation for acute appendicitis and died 10 days after the operation with generalised peritonitis and bronchopneumonia. Penicillin, streptomycin, chloramphenicol, and chlortetracycline administered at intervals over the 10 days.

Pathological findings.- Mucosa of small bowel intensely congested. Extensive mucofibrinous exudate adhering to mucosa of jejunum. Contents of large bowel semi-fluid but no notable inflammation of wall.

Bacteriological findings.- Swabs were taken from the bronchus and ileum at post mortem. A direct film of both swabs showed a large number of pus cells and numerous Gram-positive cocci (fig. XIII).

Figure XIII



Smear of the ileal contents from case 5, showing numerous staphylococci. Gram's method x 750.

Staph. aureus was isolated in large numbers from both specimens in addition to smaller numbers of coliforms. The staphylococci belonged to 'phage group III and were sensitive to chloramphenicol and erythromycin only.

Case 6

Clinical history.- A man of 69 suffered irreversible shock after gastrectomy and died on the 2nd post-operative day. No antibiotics were administered.

Pathological findings.-Acute erosions of lower oesophagus; intestine dilated, very congested, and covered by a slight fibrinous exudate; patchy mucosal necrosis and membranous inflammation throughout small and large intestine.

Bacteriological findings.- At autopsy, specimens of bowel contents (from jejunum, ileum, ileocaecal valve, and transverse colon), liver, and spleen were examined. The bowel contents gave a heavy growth of coliforms, Ps. pyocyanea, and haemolytic and nonhaemolytic Cl. welchii, and a slight growth of bacteroides. Staphylococci (untypable by 'phage and sensitive to all antibiotics tested) were isolated after enrichment culture from all sites except the transverse colon. The liver contained a small number of coliforms and Ps. pyocyanea and the spleen a moderate number of coliforms and nonhaemolytic Cl. welchii.

Case 7

Clinical history.- Because of a bronchial infection, a female of 67 did not recover well after partial gastrectomy; she went into a state of severe collapse and died on the 8th post-operative day. Penicillin was administered for 6 days before death.

Pathological findings.- Bowel wall very congested with a loose muco-fibrinous membrane of patchy distribution in jejunum. Stomach mucosa congested and in places haemorrhagic, areas of mucosal necrosis.

Bacteriological findings.- A swab of the bronchus and contents of upper jejunum were taken at autopsy. The bronchial swab contained numerous coliforms, Ps. pyocyanea, and Staph. aureus ('phage group III and sensitive to chloramphenicol and erythromycin). Staphylococci were not present in the jejunal contents but large numbers of coliforms and Ps. pyocyanea and a few Cl. welchii were found.

Conclusions

In the 7 cases of necrotising enterocolitis which were investigated no one organism appeared with regularity. Cl. welchii, when present, was not of the heat-resistant variety and although staphylococci were isolated from the bowel contents in 5 of the 7 cases they were present in small numbers only except in case no. 5. No potentially pathogenic organisms were found to predominate in 6

of the 7 cases; one only (case no. 5) in which the signs of necrotising enterocolitis were minimal, harboured large numbers of Staph. aureus. From this investigation, therefore, it is concluded that the 7 cases did not have a common bacterial origin. This investigation, despite its negative conclusions, served the useful end of making it clear that necrotising enterocolitis, a dramatic clinical condition, should not be regarded simply as an unusually severe manifestation of the kind of 'hospital diarrhoea' which proved the main reason for undertaking the work described in this thesis.

D I S C U S S I O N

D I S C U S S I O N

The food purchased by the Western Infirmary of Glasgow was found to be relatively free from potentially pathogenic organisms and could not therefore be regarded as a source of food-poisoning or other food-borne infections. The purchased food frequently contained small numbers of Cl. welchii and Staph. aureus; although it would be desirable to keep food free of such organisms it is virtually impossible to do so because these organisms are so widespread. Only one of 127 samples of food examined (tables I and III), an uncooked sausage, contained a salmonella; the incidence of salmonellas in raw sausage was in fact 1 among 38 (2.6%). This figure compares favourably with those reported from America by Galton, Lowery and Hardy (1954) who found that the incidence of salmonellas in fresh and smoked pork sausages was 23% and 12.5% respectively.

After the food was cooked and prepared for serving in the hospital kitchens, the number of contaminating organisms was generally much reduced. Contamination with small numbers of Cl. welchii and Staph. aureus was more common in the foods which required further handling after they were cooked than in those not so handled (table II). This observation suggested that they were present mainly on the surface of the food due to air-borne contamination. Cold chicken, however,

was found to contain large numbers of Cl. welchii in 10 of 46 samples examined. Because of the large numbers of organisms isolated it was apparent that the Cl. welchii had actually grown in the fowls to some considerable extent after they were cooked.

Further observations and investigations on the methods of cooking and storing the fowls showed that the gross degree of contamination of the cooked fowls with Cl. welchii was explained by the fact that the methods employed in cooling the cooked fowls provided suitable conditions for the growth of such organisms. There was no evidence in experiments that chicken was more favourable to the growth of Cl. welchii than other meat dishes but in the main kitchens of the hospital the chickens were left to cool uncovered in the broth some 22 at a time in one container outside the cold room (fig. V) whereas other items of diet were usually refrigerated in small quantities after an initial cooling period. By comparing the incidence of Cl. welchii in the chicken from the main and special-diet kitchens in the hospital this was proved to be the cause of the high rate of contamination of cold chicken with Cl. welchii. All the samples of chicken which were heavily contaminated with Cl. welchii came from the main kitchen whereas the samples from the special-diet kitchen showed only minimal contamination. The cooking procedures were similar in both kitchens; but after cooking different

methods of cooling and storing were employed. As stated above the fowls in the main kitchen were left overnight unrefrigerated and uncovered all together with the broth in the one container. On the other hand, the chickens in the special-diet kitchen were removed from the broth after cooking and stored on shallow trays (fig. VII) in the refrigerator after an initial period of cooling. Experiments in the laboratory proved that this method of cooling prevented the growth of contaminating strains of Cl. welchii and even when large numbers of these organisms were present there was no gross invasion of the flesh if individual pieces of fowl were refrigerated immediately after cooking.

Contamination of the chickens with Cl. welchii took place either before cooking with heat-resistant strains or after cooking with strains which were not necessarily heat-resistant. 1 of 12 samples of uncooked fowl contained heat-resistant Cl. welchii. This is not an unduly high figure when compared with the incidence in other meats ranging from 0 to 20% as reported by Hobbs et al (1953). In addition, all samples of uncooked fowl examined contained non-heat-resistant Cl. welchii; but although the temperatures recorded during cooking would permit the survival of fairly heat-resistant strains the evidence presented in this thesis proved that non-heat-resistant strains were killed by the cooking. This is contrary to the

view held by McClung (1945) who concluded that Cl. welchii in the flesh before cooking might be protected by the fat and survive. Although McClung did not specify wether or not he was referring to heat-resistant strains it may be inferred that he was postulating the survival of non-heat-resistant strains; otherwise the heat-resistance of the organisms would have been sufficient in itself to explain their survival. The high rate of contamination of cold chicken was not due to the survival of heat-resistant strains alone because the strains of Cl. welchii isolated were usually non-heat-resistant. It is difficult to determine whether the nonhaemolytic strains were merely vegatative strains of originally heat-resistant organisms which survived the cooking. Notably, however, the most frequent isolates from cooked fowls were non-heat-resistant haemolytic strains, which must be presumed to have gained access after cooking. On these grounds, therefore, I consider that contamination with all kinds of Cl. welchii type A took place in most instances after cooking. The dust in the hospital kitchens, which was the most likely source of contamination after cooking, was proved to be heavily infected with Cl. welchii and due to the method of storing the chickens in the main kitchen unrefrigerated and uncovered there was ample opportunity for the contamination of the cooked fowls with Cl. welchii from this heavily infected dust.

The conditions in the cooked fowls were highly suitable for the growth of these anaerobic organisms and the temperature in the mass of 22 chickens in the broth in one container remained suitable for bacterial growth for some considerable time. In this way the flesh of the fowls was liable to be contaminated to a dangerous degree if Cl. welchii gained access.

The possibility of heavy contamination with Cl. welchii was not restricted to chickens. This was proved on one occasion when a batch of stewed sausages which was stored under suitable conditions was heavily contaminated with Cl. welchii the day after cooking. In order to reduce the risk of gross contamination with Cl. welchii it is necessary to store cooked food in conditions which are not suitable for bacterial growth. This is a well recognised principle but in practice the criteria necessary to ensure such conditions are sometimes not fully appreciated. In the main kitchen in this hospital for example the chickens were left to cool outside the cold room where the temperature was lower than that of the main kitchens but the danger of leaving all 22 fowls in one container with the broth was not realized. I consider that even if the pot containing the fowls had been refrigerated, the temperature in the centre of the mass may have remained suitable for long enough to allow considerable growth of Cl. welchii in the fowls deep in the pot. McClung

(1945) emphasised that the cause of the trouble in the outbreaks he reported was not lack of refrigeration but the failure of the pans of food to cool quickly enough to a safe temperature. Therefore, in order to eliminate the possibility of heat-resistant strains surviving cooking it is necessary to use a pressure cooker but after cooking it is important to keep the birds sterile in this container to prevent the subsequent contamination with either heat-resistant or non-heat-resistant strains. On the other hand, removal from the broth after cooking and immediate refrigeration of the fowls well separated on shallow trays as in fig. VII was proved to prevent any contaminating strains of Cl. welchii proliferating to a harmful extent.

The outbreaks of food-poisoning which were reported to me from within the hospital during the period of the present investigation supported the suspicions aroused by the examination of the hospital food. All of the outbreaks investigated showed an association between the eating of cold chicken and a clinical history of Cl. welchii food-poisoning. In addition to the outbreaks reported, the medical and nursing staff of the hospital freely admitted that cold chicken on the patients' menu was regarded with some apprehension and many cases of diarrhoea which they thought to be caused by it were not reported. In 5 of the outbreaks reported in the hospital, cold chicken was

the only item of diet common to the affected persons; it was not possible to examine the suspected chicken in any of these outbreaks, however, because it was either eaten by the ward staff or discarded before there was an indication that it was a possible cause of food-poisoning. On 3 of these 5 occasions heat-resistant Cl. welchii were isolated from the faeces of the affected persons; on the other 2 occasions the evidence was only circumstantial because no specimens were made available for examination. A further 2 incidents are reported in this thesis; one involved members of the laboratory who had eaten chicken cooked in the hospital kitchen and the second involved a member of staff's family who ate chicken cooked outwith the hospital. These 2 outbreaks indicated that haemolytic strains of Cl. welchii were capable of producing food-poisoning. On the former occasion the chicken eaten by the members of the laboratory staff was contaminated with large numbers of haemolytic Cl. welchii and the symptoms in the affected persons were typical of Cl. welchii food-poisoning. Unfortunately no samples of faeces were made available for examination. On the second occasion which involved a member of staff's family, however, large numbers of haemolytic Cl. welchii were isolated from the suspected chicken and the faeces of the affected person. These organisms were shown to be identical by serological investigation.

Since Hobbs et al (1953) published their evidence that nonhaemolytic heat-resistant strains of Cl. welchii caused food-poisoning, attention has been focused on such strains. This is shown by the fact that all the outbreaks reported in recent years were caused by heat-resistant strains. Before 1953, however, Knox and Macdonald (1943) and McClung (1945) reported outbreaks of Cl. welchii food-poisoning and made no mention of the characteristics of the organisms isolated. From this it can only be assumed that the Cl. welchii were of the normal haemolytic variety. Moreover, Hobbs et al (1953) mentioned 2 outbreaks in which haemolytic Cl. welchii were isolated from the suspected food and the faeces of the affected persons. The authors concluded that contamination in these instances took place after cooking but they made no further comment on the possibility of haemolytic strains producing food-poisoning throughout the rest of the paper. A diagnosis of Cl. welchii food-poisoning is more readily made when heat-resistant strains are involved because the incidence of heat-resistant strains in healthy people is fairly low (2.2% reported by Hobbs et al, 1953). On the other hand, haemolytic Cl. welchii are frequently present in human faeces and less attention is liable to be paid to the isolation of such strains from a number of people suspected of suffering from food-poisoning. This may account for the lack of

reports incriminating haemolytic strains. This difficulty could be overcome by serological tests on the organisms isolated.

It is evident to me that heat-resistance does not alone confer the power to produce food-poisoning. Although heat-resistant strains are more liable to survive cooking, food may be contaminated with either heat-resistant or non-heat-resistant after cooking. This is a hazard which is not often stressed with regard to Cl. welchii food-poisoning. Heat-resistance is a characteristic of non-haemolytic strains of Cl. welchii, that is strains which do not produce θ - toxin, but not all haemolytic strains are heat-resistant. On the other hand, haemolytic strains are rarely heat-resistant. Brooks, Sterne, and Warrack (1957) found that only two of 146 non-food-poisoning strains of Cl. welchii were heat-resistant but as several of the strains designated as belonging to this group were nonhaemolytic it is not possible from the details in the article to know whether the 2 heat-resistant strains were haemolytic or not. In addition, although the authors stated that the food poisoning strains formed a well-defined group, all showing heat-resistance and failing to produce θ - toxin, their subsequent figures did not agree with that statement. 5 of 56 strains tested produced θ - toxin and 34 of 52 survived 1 hour at 100°C. Neither heat-resistance nor the production of θ-toxin therefore

appear to be sufficiently constant criteria on which to define food-poisoning strains. Heat-resistance is moreover a variable characteristic when found in individual strains. It is generally considered to be due to the presence of spores. However, Halverson (1957) discussed the multiphase process of sporulation in which the development of heat-resistant cells appeared to be a final separate phase and conversely Knaysi (1957) stressed that heat-sensitivity was not an adequate criterion of germination because it took place before germination was complete. Vas and Proszt (1957) also found that descendants of spores which survived extreme heat treatment did not necessarily show extreme heat tolerance. These authors based their view mainly on experiments with B. cereus but observations were made in this laboratory which indicated that the arguments also applied to Cl. welchii. Under my supervision Mr. T. M. Joys attempted to induce sporulation of Cl. welchii in the medium recommended by Ellner (1956). This experiment was initially undertaken to find out if non-heat-resistant nonhaemolytic strains showed heat-resistance when in the sporing state. Although spores were seen on direct smears, however, the cultures showed no degree of heat-resistance (i.e. were killed by 100°C in even 5 minutes). Similar results were observed with strains which had lost their heat-resistance in culture but which were

heat-resistant on primary isolation. Finally, a strain isolated from an outbreak of food-poisoning was tested. This strain retained its heat-resistance (1 hour at 100°C) in Robertson's meat medium although no spores were visible on direct smear. In Ellner's medium, however, numerous spores were seen on the direct film but the culture was killed by 5 minutes at 100°C. The results were identical in a repeat experiment. I therefore consider that the criterion of heat-resistance for differentiating potential food-poisoning strains of Cl. welchii is not reliable and that gross contamination of food with haemolytic strains is just as liable to cause Cl. welchii food-poisoning as gross contamination with nonhaemolytic heat-resistant strains. There seems no doubt from the evidence reported by other workers based on experiments with human volunteers that individual strains differ in their ability to produce food-poisoning but what causes this variation is not yet determined.

These investigations indicated that Cl. welchii food-poisoning was responsible for a number of cases of 'hospital diarrhoea' in the Western Infirmary. Apart from 3 cases of sonne dysentery, however, the only other organism which was isolated from patients with diarrhoea in sufficiently large numbers or often enough to be suspected as a possible cause of unexplained diarrhoea was Staph. aureus. This organism was isolated

in large numbers from the faeces of 3 of the 170 diarrhoeal patients examined. All 3 patients were undergoing antibiotic therapy and this was thought to be the predisposing cause because the numbers of staphylococci fell rapidly after the treatment was discontinued. It is striking how seldom this condition arose in such a large hospital as the Western Infirmary (639 beds) where antibiotics must of necessity be used frequently, and this observation does not agree with that of Cook et al (1957) who found that Staph. aureus was the chief cause of post-operative diarrhoea in the Radcliffe Infirmary at Oxford. In the majority of cases of diarrhoea examined here no organism was found to predominate; the flora of most cases appeared to be normal, consisting of coliforms, haemolytic Cl. welchii, enterococci, and bacteroides. I consider, therefore, that the diarrhoea in these cases was not caused by bacteria.

The incidence of haemolytic and nonhaemolytic Cl. welchii in 272 hospital patients was 62.8% and 22% respectively. No figures are available, however, to determine if this is of the normal order. Heat-resistant Cl. welchii were isolated from 12.8%. This is somewhat higher than the incidence of 2.2% for healthy adults and children reported by Hobbs et al (1953) but is comparable to their figure of 15.1% for old people in hospital. Hobbs et al thought that the higher incidence

in the latter group might be due to some undetected cases of Cl. welchii food-poisoning because this group contained people with and without diarrhoea. This was not the explanation for the higher incidence which I observed, however, because a comparison of the incidence in the patients with and without diarrhoea showed that the incidence was higher in the patients without diarrhoea (15.6% as compared with 11.1% in the diarrhoeal group). Dische and Elek (1957), on the other hand, found some indication of an association between a higher carrier rate of heat-resistant Cl. welchii and the hospital environment and reported an overall incidence of 15.6% in hospital patients and members of hospital staff and their families. In my survey, however, I was unable to conclude that this was the reason for the high incidence of heat-resistant Cl. welchii which I observed in hospital patients. There is therefore as yet no conclusive evidence to support any one explanation for the differences in faecal incidence of heat-resistant Cl. welchii which have been reported between hospital inhabitants and the healthy people outside hospital.

The incidence of Staph. aureus in the faeces of 255 patients was 21.9%. This figure is not unduly high and falls within the range of figures quoted by other workers. The incidence in diarrhoeal specimens was markedly higher than that in nondiarrhoeal stools, being 27.6% and 10.5% respectively. This difference

agrees with the findings of Moustardier et al (1957) and Neuman et al (1957) who observed a higher faecal incidence of Staph. aureus in patients suffering from diarrhoea than in patients with no gastro-intestinal disturbance. In the present survey the staphylococci were usually present in the diarrhoeal and the non-diarrhoeal stools in small numbers only and could not therefore be incriminated as the cause of the diarrhoea. I consider that they were more probably present as a result of the diarrhoea because of the increased rate at which material passed through the alimentary tract. I found no conclusive evidence to support the view of Brodie et al (1956) and Matthias et al (1957) that the faecal carriage rate of Staph. aureus increased to any great extent with the duration of the patients' stay in hospital and the patients did not appear to acquire the multiple-antibiotic-resistant staphylococci present in the hospital atmosphere as part of their faecal flora.

A bacteriological investigation of 7 fatal cases of necrotising enterocolitis failed to support the view that this is a disease caused by one particular group of bacteria. In fact apart from the clinical features immediately before death and the pathological changes in the intestinal tract there was no known feature common to all 7 cases. Six of the 7 had antibiotic treatment before death and in 6 the condition followed an abdominal operation. No one organism appeared to

predominate in 6 of the 7 cases. Staphylococci were isolated from the bowel contents of 5 of the 7 cases but in 4 of the 5 cases from which it was isolated Staph. aureus was present in small numbers only. The one case which harboured large numbers of staphylococci showed minimal signs of necrotising enterocolitis; also, large quantities of antibiotics were administered before death in this case and most probably accounted for the increase in the numbers of staphylococci. In one of the cases where no staphylococci were found in the bowel there was a marked staphylococcal infection of the lungs. There was therefore ample opportunity for these organisms to invade the bowel and their absence from the bowel is a strong indication that Staph. aureus is not necessarily the cause of necrotising enterocolitis.

From the work reported here, it seems worth examining all outbreaks of unexplained diarrhoea in hospital very carefully to see whether they are, in fact, examples of Cl. welchii food-poisoning. In this connection the usefulness of the rectal swab to ensure sampling of all at risk should be kept in mind. In hospital the most difficult part of such an investigation may be to ensure that a stool sample is collected for examination from all at risk. It would be worth using the rectal swab in a thorough survey of patients during their stay in hospital to see if there is an actual increase in the faecal carriage of Cl. welchii during the patients'

stay in hospital. My results were largely negative in this respect but I was able to obtain more than one specimen from only 45 patients. The matter is clearly in need of further study especially in view of the methods of hospital-kitchen hygiene which may favour the inadvertent feeding of Cl. welchii to patients in some of their food.

S U M M A R Y

SUMMARY

1) Eighty-nine samples of uncooked food purchased by the Western Infirmary of Glasgow were examined. Salm. enteritidis was found in small numbers in one sample only: an uncooked sausage. No other specific intestinal pathogens were isolated. Haemolytic Cl. welchii were isolated from enrichment media in 73% of the samples, nonhaemolytic Cl. welchii from 17.9% and heat-resistant Cl. welchii from 2.2%. Staph. aureus was present in small numbers in 16.8%. Gross contamination with potentially pathogenic organisms was not observed in any sample.

2) One hundred and seventy-three samples of food which were cooked in the hospital kitchens were examined. The overall contamination was usually very much reduced by the cooking. No salmonellas or shigellas were isolated. Haemolytic Cl. welchii was isolated from enrichment media in 24.8% of the samples, nonhaemolytic Cl. welchii from 13.3%, and heat-resistant Cl. welchii from 0.6%. Staph. aureus was present in small numbers in 11.5%. Haemolytic and nonhaemolytic Cl. welchii were isolated on direct culture, sometimes in fairly large numbers; 5.2% of the samples yielded haemolytic Cl. welchii and 2.8% gave nonhaemolytic Cl. welchii. With the exception of one batch of sausages, gross

contamination with Cl. welchii was confined to cold boiled chicken; 10 of 46 samples of this food were heavily contaminated with Cl. welchii.

3) Thirty-eight samples of cooked food purchased by the Western Infirmary were examined. These items of food were very seldom contaminated with potentially pathogenic organisms. Haemolytic and nonhaemolytic Cl. welchii were each isolated from enrichment media in 2.6% of the samples and Staph. aureus from 7.8%.

4) Further observations were made on the contamination of chickens with Cl. welchii. Chicken-extract broth was not found to be a more favourable medium than meat-extract broth for the growth of Cl. welchii. However, a comparison of the chicken samples which came from the main kitchen of the hospital with the samples from the special-diet kitchen indicated that the high rate of contamination of chickens with Cl. welchii was caused by the methods employed in preparing the fowls for serving. All the samples of cold chicken which were heavily contaminated with Cl. welchii came from the main kitchen. The method of cooking the fowls was similar in the two kitchens but the cooked fowls in the main kitchens were left to cool overnight outside the cold room, some 22 together with the broth in an uncovered container, whereas the cooked birds

in the special-diet kitchen were separated from the broth after cooking; after a preliminary cooling period of some 3-4 hours on shallow trays, they were refrigerated until required. The method of cooling and storing the cooked chickens in the main kitchen thus provided suitable conditions for the growth of contaminating strains of Cl. welchii.

5) Cl. welchii was found in all 12 samples of uncooked chicken examined but a heat-resistant strain was isolated from only one of these. From the temperatures recorded during the cooking of the fowls and experiments in the laboratory with pieces of fowl, it was determined that the non-heat-resistant strains were killed by the cooking. Heat-resistant strains present in the fowls before cooking, however, constituted a potential hazard when the conditions after cooking were suitable for the growth of any surviving organisms. Thirty-four chicken viscera were examined for the presence of Cl. welchii in order to determine if the presence of such organisms in the flesh of fowls was due to spread from the visceral contents during the storage of the birds in the undrawn state. It was concluded that this was not necessarily the source of Cl. welchii in the fowl flesh but contamination also took place during and after evisceration.

6) Haemolytic non-heat-resistant Cl. welchii were frequently found in large numbers on the cooked chicken in the hospital. Contamination in these instances must be assumed to have taken place after cooking. A very probable source of such contamination was the kitchen dust. Fifty-two of 58 samples of dust from the main kitchen yielded haemolytic Cl. welchii and 47 of the 58 yielded nonhaemolytic Cl. welchii. Due to the method of storing and cooling the cooked fowls in the main kitchen there was ample opportunity for their contamination with this heavily infected dust and this was considered to be the main source of contamination of the fowls. Immediate refrigeration of the fowls well separated on shallow trays was proved to be an adequate method to prevent the growth of contaminating strains of Cl. welchii even when these were present in large numbers.

7) During the course of the present investigation 5 outbreaks of suspected food-poisoning were reported within the hospital. All 5 were associated with the distribution of cold chicken; the affected persons all showed typical symptoms of Cl. welchii food-poisoning. Heat-resistant Cl. welchii were isolated from the faeces of the affected persons in 3 outbreaks; the evidence was circumstantial in the remaining 2. The evidence incriminating cold chicken was fairly strong

in all 5 outbreaks.

8) A further 2 outbreaks are reported. One involved members of the laboratory staff who ate chicken prepared in the hospital kitchen and the other involved one of a member of staff's family who had eaten chicken cooked outwith the hospital. In both these instances the causal organism appeared to be a haemolytic Cl. welchii. In the latter, large numbers of haemolytic Cl. welchii were isolated from the suspected chicken and the faeces of the affected person. Serological tests proved these organisms to be identical. Haemolytic Cl. welchii as a cause of food-poisoning and the unreliability of heat-resistance as a criterion for differentiating potential food-poisoning strains are discussed.

9) The incidence of haemolytic, nonhaemolytic, and heat-resistant Cl. welchii in a survey of diarrhoeal and normal stools from 272 hospital patients was 62.8%, 22% and 12.8% respectively. The incidence of Staph. aureus in similar specimens from 255 patients was 21.9%. A comparison of the incidence of Cl. welchii and Staph. aureus in patients with and without diarrhoea showed that the incidence of Cl. welchii was of the same order (11.1% and 15.6% respectively yielded heat-resistant strains) whereas the incidence of Staph. aureus

was considerably higher in the diarrhoeal group (27.6% and 10.5% respectively). The reasons for this are discussed and it is concluded that in the majority of cases the presence of Staph. aureus in diarrhoeal stools was the result rather than the cause of the diarrhoea.

10) This investigation failed to reveal any particular organism as the single cause of all cases of unexplained hospital diarrhoea. From the investigation of the food-poisoning outbreaks some cases of diarrhoea were doubtless due to Cl. welchii food-poisoning. Three of 170 patients suffering from diarrhoea were found to have sonne dysentery. A further 3 had a staphylococcal enteritis caused presumably by antibiotic therapy. The majority of cases, however, appeared to have no obvious bacterial cause.

11) A bacteriological examination of the bowel contents of 7 fatal cases of necrotising enterocolitis showed that no pathogenic organism was common to all 7 cases and only in one case did any one organism predominate. From these results, therefore it is concluded that necrotising enterocolitis is not caused by or even associated with any one particular group of bacteria.

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A C K N O W L E D G E M E N T S

A C K N O W L E D G E M E N T S

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Key to Appendix I

Nature of food

(a), (b) and (c) refer to appropriate sections describing observations and experimental work

(a) uncooked food purchased by hospital

(b) food, cooked in hospital kitchens

(c) cooked food purchased by hospital

Lab. number

When an item of food was sampled before and after cooking the two samples are given the same number with the suffices A and B.

Degree of contamination

+	=	no growth on direct plates, growth in enrichment culture only
++	=	slight growth on direct plates
+++	=	moderate growth on direct plates
++++	=	heavy growth on direct plates

Predominating organisms

If there was no growth on direct culture the predominating organism after enrichment culture is stated.

Potentially Pathogenic organisms isolated

Cl. welchii	=	Cl. w.
Staph. aureus	=	St. a.
Salmonellas	=	Salm.

All staphylococci and salmonellas were isolated after enrichment culture only. Strains of Cl. welchii are noted as follows:-

DH = haemolytic strains isolated on direct culture

EH = haemolytic strains isolated after enrichment culture

Dnh = nonhaemolytic strains isolated on direct culture

Enh = nonhaemolytic strains isolated after enrichment culture

Htd = isolated after 15 minutes or more at 100°C.

Appendix I (Bacterial Contamination of Food)

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated	
Fish(a)	F1	+++	micrococci	-	
(uncooked)	F2	+++	micrococci	Cl.w.(EH)	
	F3	+++	micrococci	Cl.w.(EH)	
	F4	+++	micrococci	Cl.w.(EH)	St.a.
	F5A	++	micrococci	Cl.w.(EH)	St.a.
	F6A	++	micrococci	Cl.w.(EH)	St.a.
	F7	++	micrococci	-	
	F8	+++	micrococci		St.a.
	F9	+	micrococci	-	
	F10A	+++	micrococci	-	
	F11A	+++	micrococci	Cl.w.(EH)	
	F12	++	micrococci	Cl.w.(EH)	
	F13	++	micrococci	Cl.w.(EH)	
	F14	+++	micrococci	Cl.w.(EH,Enh)	
	F15	++++	micrococci	Cl.w.(EH,Enh)	
	F16	++	micrococci	-	
	F17	++	micrococci	-	
	F18	+++	micrococci	Cl.w.(EH)	
Fish(b)	F5B	+	micrococci	-	
(cooked)	F6B	+	anthracoids	-	
	F10B	+	micrococci	Cl.w.(EH)	
	F11B	+	anthracoids	-	
	F19	+	micrococci	-	
	F20	+	micrococci	-	
Liver(a)	F21	+++	micrococci	Cl.w.(Enh)	
(uncooked)	F22	++	micrococci	-	

See Key to Appendix I for explanation of symbols

Appendix I continued

Nature of food	Lab. No	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Liver(b) (cooked)	F23	+	micrococci	-
Steak & mince(a) (uncooked)	F24	++	micrococci	-
	F25	++	anthracoids	Cl.w.(EH)
	F26	++++	micrococci	Cl.w.(EH) St.a.
	F27	++++	micrococci	Cl.w.(EH)
	F28	++	micrococci	St.a.
	F29A	++	micrococci	Cl.w.(EH)
	F30A	++	micrococci	-
	F31A	++	micrococci	St.a.
	F32	+++	micrococci	Cl.w.(EH)
	F33A	++	micrococci	Cl.w.(EH)
Steak & mince (b) (cooked)	F29B	+	anthracoids	-
	F30B	+	anthracoids	-
	F31B	+	anthracoids	-
	F33B	++	coliforms	-
	F34	+	anthracoids	-
	F35	+	anthracoids	-
	F36	+	micrococci	-
	F37	++	anthracoids	-
	F38	++	anthracoids	Cl.w.(EH)
	F39	++	anthracoids	-
	F40	+	anthracoids	-
	F41	+	micrococci	-
	F42	+	anthracoids	Cl.w.(Enh)
	F43	+	anthracoids	-
	F44	+	micrococci	-

Appendix I continued

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Tripe(a) (uncooked)	F45	+++	micrococci	-
	F46	+++	anthracoids	-
	F47	+++	coliforms	St.a.
	F48A	++++	micrococci	Cl.w.(EH)
	F49A	+++	micrococci	-
	F50	++++	micrococci	Cl.w.(Enh)
Tripe(b) (cooked)	F48B	+	anthracoids	-
	F49B	++	anthracoids	-
	F51	+	anthracoids	-
	F52	++	anthracoids	Cl.w.(EH)
	F53	+	anthracoids	-
	F54	+	anthracoids	-
Sausage(a) (uncooked)	F55	++	micrococci	Cl.w.(EH)
	F56	+++	micrococci	Cl.w.(EH)
	F57	+++	micrococci	Cl.w.(EH)
	F58	++++	micrococci	Cl.w.(EH)
	F59	+++	micrococci	Cl.w.(Enh)
	F60	+++	anthracoids	Cl.w.(EH)
	F61	+++	micrococci	Cl.w.(Enh)
	F62	+++	micrococci	Cl.w.(EH) St.a.
	F63	+++	micrococci	Cl.w.(EH) St.a.
	F64	++	micrococci	Cl.w.(EH)
	F65A	+++	micrococci	Cl.w.(EH)
	F66A	+++	coliforms	Cl.w.(EH)
	F67A	+++	micrococci	Cl.w.(EH)

Appendix I continued

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Sausage(a) (uncooked) contd.	F68A	++++	micrococci	Cl.w.(EH)
	F69A	+++	micrococci	Cl.w.(EH)
	F70A	+++	micrococci	Cl.w.(EH,Enh)
	F71A	+++	anthracoids	Cl.w.(EH)
	F72A	+++	micrococci	Cl.w.(EH)
	F73	+++	micrococci	Cl.w.(EH)
	F74A	+++	micrococci	Cl.w.(EH,Enh)
	F75A	+++	micrococci	Cl.w.(EH)
	F76	+++	micrococci	Cl.w.(EH)
	F77A	+++	micrococci	Cl.w.(EH,Enh) Salm.
	F78	+++	micrococci	Cl.w.(EH,Htd)
	F79A	+++	coliforms	Cl.w.(EH)
	F80	+++	micrococci	Cl.w.(EH,Enh)
	F81	+++	micrococci	Cl.w.(EH,Enh)
	F82	+++	micrococci	Cl.w.(EH)
	F83	+++	micrococci	Cl.w.(EH)
	F84	+++	micrococci	Cl.w.(EH,Enh)
	F85	+++	micrococci	Cl.w.(EH) St.a.
	F86	+++	micrococci	Cl.w.(EH,Enh)
	F87A	++	micrococci	Cl.w.(EH) St.a.
	F88A	+++	micrococci	Cl.w.(EH)
	F89A	+++	micrococci	Cl.w.(EH)
	F90	++	micrococci	Cl.w.(EH) St.a.
	F91	++++	micrococci	Cl.w.(EH)
	F92	+++	micrococci	Cl.w.(EH)
Sausage(b) (cooked)	F65B	+	anthracoids	-
	F66B	+	anthracoids	-

Appendix I continued

Nature of food	Lab. No.	Degree of contam-ination	Predom-inating organisms	Potentially patho genic organisms isolated
Sausage(b) (cooked) contd.	F67B	+	anthracoids	-
	F68B	+	anthracoids	-
	F69B	+	anthracoids	-
	F70B	+	coliforms	-
	F71B	+	anthracoids	-
	F72B	+	anthracoids	-
	F74B	+	anthracoids	-
	F75B	+	micrococci	-
	F77B	+	anthracoids	-
	F79B	+	anthracoids	-
	F87B	+	anthracoids	-
	F88B	+	anthracoids	-
	F89B	+	anthracoids	-
	F93	+	anthracoids	-
	F94	+	anthracoids	Cl.w.(EH)
	*F95A	+	Cl.w.	Cl.w.(EH,Enh)
	F96	+	anthracoids	-
	F97	+	anthracoids	Cl.w.(EH)
	F98	+	anthracoids	-
	F99	+	anthracoids	-
	F100	+	anthracoids	-
	F101	+	anthracoids	-
	F102	+	anthracoids	Cl.w.(EH)
Sausage gravy(b)	*F95B	++++	Cl.w.	Cl.w.(DH,EH,Dnh,Enh)

* reported by kitchen staff as bad due to foul smell

Appendix I continued

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Black pudding(a) (uncooked)	F103	++	anthracoids	Cl.w.(EH)
	F104A	+++	anthracoids	Cl.w.(EH) St.a.
	F105	+++	anthracoids	-
	F106	+++	anthracoids	-
Black pudding(b) (cooked)	F104B	++	anthracoids	Cl.w.(EH)
	F107	++	anthracoids	Cl.w.(EH)
Ulster fry(a) (uncooked)	F108	++	anthracoids	Cl.w.(EH)
	F109	++++	anthracoids	-
	F110A	++	anthracoids	-
	F111	+	micrococci	Cl.w.(EH)
Ulster fry(b) (cooked)	F110B	+	anthracoids	-
	F112	+	anthracoids	-
Chicken(a) (uncooked)	F113	++++	coliforms	Cl.w.(EH)
	F114A	+++	coliforms	Cl.w.(DH,EH)
	F115A	+++	coliforms	Cl.w.(EH)
	F116	+++	micrococci	Cl.w.(EH,Enh) St.a.
	F117A	++++	coliforms	Cl.w.(EH,Enh)
	F118A	++++	coliforms	Cl.w.(EH, Htd)
	F119A	++++	coliforms	Cl.w.(EH,Enh)

Appendix I continued

Nature of food	Lab. No.	Degree of contam-	Predominating organisms	Potentially pathogenic organisms isolated
Chicken(b) (cooked)	xF114B	+	micrococci	-
	F115B	+	coliforms	-
	xF117B	+	micrococci	-
	xF118B	+	micrococci	Cl.w.(Htd)
	xF119B	+	micrococci	-
	F120	++	micrococci	Cl.w.(EH)
	F121	++	micrococci	St.a.
	F122	++	anthracoids	Cl.w.(EH) St.a.
	F123	+++	Cl.w.	Cl.w.(DH,EH) St.a.
	F124	++	micrococci	St.a.
	F125	+	micrococci	-
	F126	++	anthracoids	-
	F127	++	coliforms	-
	F128	+++	Cl.w.	Cl.w.(EH,Dnh,Enh) St.a.
	xF129A	+	coliforms	-
	F130	+	anthracoids	Cl.w.(Enh)
	F131	++	micrococci	Cl.w.(EH)
	F132A	++	micrococci	Cl.w.(EH) St.a.
	F133	+	micrococci	-
	xF134	++	micrococci	Cl.w.(EH)
	F135	++	Cl.w.	Cl.w.(DH,EH,Enh)
	*xF136	+	anthracoids	-
	xF137	+	coliforms	Cl.w.(EH,Enh)
	F138	++	coliforms	Cl.w.(EH) St.a.

x = chicken samples from special-diets kitchen

* = reported by kitchen staff as bad due to foul smell

Appendix I continued

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Chicken(b)	xF139	++	micrococci	St.a.
(cooked)	F140	++	micrococci	-
contd.	xF141	+	anthracoids	-
	xF142	+	micrococci	Cl.w.(Enh)
	xF143	++	micrococci	-
	xF144	++	micrococci	-
	xF145	+	micrococci	-
	F146	++	Cl.w.	Cl.w.(DH,EH,Enh)
	F147	++	micrococci	Cl.w.(EH)
	F148	+	micrococci	-
	xF149	+	micrococci	-
	*F150	++++	Cl.w.	Cl.w.(DH,EH)
	F151	++	micrococci	Cl.w.(EH)
	F152	++	micrococci	Cl.w.(EH)
	F153	+	micrococci	Cl.w.(EH,Enh)
	F154	+++	Cl.w.	Cl.w.(DH,EH;Dnh,Enh)
	F155	++	Cl.w.	Cl.w.(DH,EH,Dnh,Enh)
	F156	++	micrococci	Cl.w.(EH,Enh)
	F157	+++	micrococci	Cl.w.(DH,EH)
	F158	++++	Cl.w.	Cl.w.(Dnh,Enh)
	F159	+	coliforms	-
	F160A	++	micrococci	Cl.w.(DH,EH) St.a.
Chicken(b) broth	F129B	+	anthracoids	-
	F132B	+	anthracoids	-
	F160B	+	coliforms	-

x = chicken samples from special-diets kitchen

* = reported by kitchen staff as bad due to foul smell

Appendix I continued

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Cold	F161	++	coliforms	Cl.w.(EH)
brisket(b)	F162	++	micrococci	-
	F163	+	micrococci	Cl.w.(EH,Enh)
	F164	+++	micrococci	Cl.w.(EH)
	F165	+++	micrococci	Cl.w.(Enh)
Cold pork(b)	F166	++	micrococci	-
Cold mutton(b)	F167	+	anthracoids	-
Cold	F168	++	micrococci	-
roast beef	F169	++	micrococci	Cl.w.(EH)
(b)	F170	++	micrococci	St.a.
	F171	++	micrococci	-
	F172	++	anthracoids	-
	F173	+	coliforms	-
	F174	++	micrococci	-
	F175	++	micrococci	-
Sliced	F176	+++	micrococci	Cl.w.(EH)
tongue(b)	F177	++	micrococci	-
	F178	+	micrococci	St.a.
	F179	++	micrococci	St.a.
	F180	++	micrococci	-
	F181	++	micrococci	Cl.w.(EH)
	F182	++	micrococci	-
	F183	+	micrococci	-
	F184	+++	micrococci	-
	F185	+++	coliforms	-
	F186	+	micrococci	St.a.

Appendix I continued

Nature of food	Lab. No.	Degree of contam-ination	Predom-inating organisms	Potentially patho-genic organisms isolated
Sliced	F187	++	micrococci	-
tongue	F188	+	micrococci	St.a.
(b)contd.	F189	++	micrococci	-
Potted	F190	++	micrococci	-
hough(b)	F191	++	micrococci	-
	F192	++	micrococci	-
	F193	++	micrococci	-
	F194	+	micrococci	Cl.w.(EH,Enh)
	F195	+	anthracoids	-
	F196	+++	anthracoids	-
Galantine	F197	++	micrococci	St.a.
(b)	F198	+	micrococci	-
	F199	+	micrococci	-
	F200	++	micrococci	-
	F201	+	micrococci	-
	F202	+	micrococci	-
Boiled	F203	+++	micrococci	-
ham(b)	F204	+++	micrococci	-
	F205	++	micrococci	St.a.
	F206	++	micrococci	-
	F207	++	micrococci	Cl.w.(Enh)
	F208	++	micrococci	-
	F209	+	micrococci	Cl.w.(Enh)
	F210	+	micrococci	St.a.
	F211	+	micrococci	-

Appendix I continued

Nature of food	Lab. No.	Degree of contam-ination	Predom-inating organisms	Potentially patho-genic organisms isolated
Boiled ham(b) contd.	F212	++	micrococci	-
Fish cake(b) (uncooked)	F213A	++	micrococci	Cl.w.(EH) St.a.
Fish cake(b) (cooked)	F213B	+	anthracoids	-
Savoury croquette (b) (uncooked)	F214	++	anthracoids	Cl.w.(Enh)
	F215A	++	anthracoids	Cl.w.(EH)
	F216A	+++	anthracoids	Cl.w.(EH)
	F217	++	anthracoids	Cl.w.(EH)
	F218A	+++	anthracoids	Cl.w.(EH,Enh)
	F219	++	anthracoids	Cl.w.(Enh)
	F220	+++	anthracoids	Cl.w(Enh)
	F221	++	anthracoids	St.a.
Savoury croquette (b) (cooked)	F215B	+	anthracoids	-
	F216B	+	anthracoids	-
	F218B	+	anthracoids	-
	F222	+	anthracoids	-
Mutton pie(c)	F223	+	anthracoids	-
	F224	+	anthracoids	-
	F225	+	anthracoids	-

Appendix I continued

Nature of food	Lab. No.	Degree of contamination	Predominating organisms	Potentially pathogenic organisms isolated
Mutton pie(c) contd.	F226	+	anthracoids	-
	F227	+	anthracoids	-
	F228	+	anthracoids	-
	F229	+	anthracoids	-
Sausage roll(c)	F230	+	anthracoids	-
	F231	+	anthracoids	-
	F232	+	anthracoids	-
	F233	+	anthracoids	-
	F234	+	anthracoids	-
Corned beef(c)	F235	+	anthracoids	-
	F236	++	micrococci	-
	F237	+	anthracoids	-
	F238	+	micrococci	-
	F239	++	anthracoids	St.a.
Jellied veal(c)	F240	+++	anthracoids	-
	F241	++	anthracoids	-
	F242	++	micrococci	St.a.
	F243	+	micrococci	Cl.w.(EH)
	F244	++	micrococci	-
	F245	++	micrococci	-
	F246	++	micrococci	-
	F247	+	micrococci	-
	F248	+	micrococci	-
	F249	++	micrococci	-
	F250	+++	micrococci	-

Appendix I continued

Nature of food	Lab. No.	Degree of contam-ination	Predom-inating organisms	Potentially patho genic organisms isolated
Jellied	F251	+	micrococci	-
veal(c)	F252	+	micrococci	-
contd.	F253	+	micrococci	St.a.
	F254	++	micrococci	-
	F255	++	micrococci	-
	F256	+	micrococci	-
Pressed	F257	++	micrococci	Cl.w.(EH,Enh)
beef(c)	F258	++	micrococci	-
	F259	+	micrococci	-
Spam(c)	F260	+	micrococci	-

APPENDIX II

Incidence of Cl. welchii and Staph. aureus in the faeces of hospital patients

Key to Appendix II

Lab. number

Each patient is given a number and suffices A, B etc. added for subsequent specimens. Patients with diarrhoea have prefix D and patients without diarrhoea prefix N.

Isolation of Cl. welchii and Staph. aureus is denoted as follows:-

- \- = not isolated
- \+ = isolated in enrichment culture only
- ++ = isolated in direct and enrichment culture
- //// = not tested

An indication of the aetiology of the diarrhoea is given when known and the week during the patient's stay in hospital in which the specimen was collected is given for some of the normal stools.

Appendix II (Incidence of *Cl. welchii* & *Staph. aureus* in the faeces of hospital patients)

Lab. No.	*Cl. welchii*			*Staph. aureus*	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D1	-	-	-	+	ulcerative colitis
D2	-	-	-	-	ulcerative colitis
D2A	+	-	-	-	ulcerative colitis
D3	++	-	-	-	ulcerative colitis
D3A	-	-	-	+	ulcerative colitis
D3B	+	-	-	-	ulcerative colitis
D4	++	+	-	+	ulcerative colitis
D5	-	-	-	-	ulcerative colitis
D5A	+	-	-	-	ulcerative colitis
D6	-	-	-	-	ulcerative colitis
D7	-	+	-	-	ulcerative colitis
D8	+	+	-	+	ulcerative colitis
D9	-	-	+	-	ulcerative colitis
D10	++	+	-	+	ulcerative colitis
D11	-	-	-	-	ulcerative colitis
D12	-	-	-	-	ulcerative colitis
D13	++	-	-	-	ulcerative colitis
D13A	++	++	-	-	ulcerative colitis
D13B	++	-	-	-	ulcerative colitis
D13C	++	-	-	-	ulcerative colitis
D14	+	-	-	-	ulcerative colitis
D14A	-	-	-	+	ulcerative colitis
D14B	-	-	-	++	ulcerative colitis
D14C	-	-	-	++	ulcerative colitis
D14D	+	++	-	++	ulcerative colitis
D14E	++	++	-	+	ulcerative colitis

See key to Appendix II for explanation of symbols

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D14F	++	-	-	+	ulcerative colitis
D15	-	+	-	+	ulcerative colitis
D15A	++	-	+	-	ulcerative colitis
D15B	++	-	+	-	ulcerative colitis
D15C	++	-	-	-	ulcerative colitis
D16	-	-	-	-	ulcerative colitis
D17	+	+	-	+	post-operative
D18	-	-	-	-	post-operative
D19	-	-	-	+	post-operative
D20	-	+	-	-	post-operative
D21	++	-	-	-	post-operative
D22	-	-	-	-	post-operative
D23	++	-	-	-	post-operative
D24	-	-	-	-	post-operative
D25	+	++	-	-	post-operative
D26	++	+	-	-	post-operative
D27	-	-	-	-	post-operative
D28	+	-	-	+	post-operative
D29	-	-	-	-	post-operative
D30	++	-	-	-	post-operative
D31	++	-	-	+	post-operative
D32	-	-	-	-	post-operative
D33	++	-	-	+	post-operative
D34	-	++	-	-	post-operative
D35	-	-	-	+	post-operative
D36	-	-	-	-	post-operative
D37	++	-	-	-	post-operative
D38	-	+	-	+	post-operative

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D39	-	-	-	-	post-operative
D40	+	-	-	-	post-operative
D41	++	-	+	-	post-operative
D42	-	++	-	-	post-operative
D43	-	-	-	-	post-operative
D43A	++	++	-	-	post-operative
D44	-	-	-	-	post-operative
D45	-	-	-	-	post-operative
D46	-	-	-	-	post-operative
D47	+	-	-	-	post-operative
D48	++	-	-	+	post-operative
D49	+	-	+	-	post-operative
D50	+	+	-	-	post-operative
D51	+	-	-	+	post-operative
D52	+	-	-	-	post-operative
D53	-	-	-	++	staphylococcal enteritis
D54	+	-	-	-	sonne dysentery
D55	+	-	-	-	sonne dysentery
D56	+	-	-	-	sonne dysentery
D57	+	-	-	-	unknown
D58	-	-	-	-	unknown
D59	-	-	-	-	unknown
D60	++	-	-	-	unknown
D61	++	-	+	+	unknown
D61A	-	-	-	-	unknown
D62	-	-	-	+	unknown
D63	-	-	-	-	unknown

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D63A	-	+	-	-	unknown
D64	-	-	-	-	unknown
D65	++	-	-	++	unknown
D66	-	-	-	-	unknown
D67	+	-	-	+	unknown
D67A	+	-	-	+	unknown
D67B	-	-	-	+	unknown
D68	+	-	-	-	unknown
D69	-	-	-	-	unknown
D70	-	-	-	+	unknown
D71	+	+	-	+	unknown
D72	++	+	+	-	unknown
D73	++	-	-	+	unknown
D74	+	++	-	+	unknown
D75	++	-	-	-	unknown
D76	++	++	-	+	unknown
D77	-	++	-	+	unknown
D77A	+	++	-	+	unknown
D77B	-	++	-	+	unknown
D78	-	-	-	-	unknown
D79	+	-	-	-	unknown
D80	++	++	+	-	unknown
D81	+	++	-	-	unknown
D82	++	-	-	-	unknown
D83	++	-	-	+	unknown
D84	-	-	-	-	unknown
D85	+	-	-	-	unknown
D86	-	-	-	+	unknown

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D87	+	+	-	+	unknown
D88	+	+	-	-	unknown
D89	++	++	+	-	unknown
D90	-	-	+	++	unknown
D91	-	-	-	-	unknown
D92	-	-	-	+	unknown
D93	-	-	-	-	unknown
D94	+	-	+	-	unknown
D95	-	-	-	-	unknown
D96	++	-	-	-	unknown
D97	++	-	-	-	unknown
D98	-	-	-	+	unknown
D99	-	-	-	+	unknown
D100	+	-	-	+	unknown
D101	++	-	-	-	unknown
D102	-	-	-	-	unknown
D103	++	-	+	-	unknown
D103A	++	-	+	-	unknown
D104	-	-	-	-	unknown
D105	++	++	+	-	unknown
D106	+	-	-	-	unknown
D107	+	-	-	-	unknown
D108	-	-	-	+	unknown
D109	+	+	+	-	unknown
D110	+	-	-	-	unknown
D110A	++	+	+	-	unknown
D110B	++	++	-	-	unknown
D111	++	++	-	-	unknown

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D112	-	-	-	-	unknown
D113	+	-	-	-	unknown
D113A	+	-	-	-	unknown
D114	++	++	-	-	unknown
D115	-	-	-	-	unknown
D116	++	-	-	-	unknown
D117	-	-	-	-	unknown
D118	-	-	-	-	unknown
D119	-	-	-	-	unknown
D120	+	-	-	-	unknown
D121	-	-	-	-	unknown
D122	-	-	-	-	unknown
D123	++	++	-	-	unknown
D124	+	-	-	-	unknown
D125	+	-	+	+	unknown
D126	+	-	-	-	unknown
D127	-	-	-	-	unknown
D128	++	++	-	-	unknown
D129	++	-	+	-	unknown
D130	+	-	-	-	unknown
D131	-	-	-	-	unknown
D132	++	-	+	-	unknown
D133	+	+	-	+	unknown
D134	+	-	-	-	unknown
D135	+	-	-	-	unknown
D136	+	-	-	-	unknown
D137	+	-	-	-	unknown
D138	++	-	-	+	unknown

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D139	+	-	-	-	unknown
D140	++	-	-	-	unknown
D141	-	-	-	-	unknown
D142	-	-	-	-	unknown
D143	++	-	-	-	unknown
D144	-	-	-	-	unknown
D145	-	-	-	+	unknown
D146	-	-	-	-	unknown
D147	++	++	-	-	unknown
D148	+	-	-	+	unknown
D149	-	-	-	-	unknown
D150	-	-	-	+	unknown
D151	-	-	-	-	unknown
D152	+	-	-	+	unknown
D153	-	+	-	-	unknown
D154	+	-	-	-	unknown
D155	+	-	-	-	unknown
D156	+	-	-	-	unknown
D157	++	++	-	-	unknown
D158	-	-	-	-	unknown
D159	+	-	-	-	unknown
D160	-	-	-	+	unknown
D161	+	-	-	+	unknown
D161A	+	-	-	+	unknown
D162	-	-	-	-	unknown
D163	++	+	-	-	unknown
D164	++	-	-	-	unknown
D165	+	-	-	-	unknown

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Aetiology of diarrhoea
	haem-olytic	non-haem-olytic	heat-resistant		
D166	+	-	+	-	unknown
D167	++	+	+	-	unknown
D167A	+	-	-	-	unknown
D168	++	-	-	-	unknown
D168A	+	++	-	+	unknown
D169	-	+	-	-	unknown
D170	++	-	-	-	unknown

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Week of stay (if known)
	haem-olytic	non-haem-olytic	heat-resistant		
N1	++	-	-	////	
N1A	++	-	+	////	
N1B	+	-	+	////	
N2	-	-	+	////	
N2A	++	-	+	////	
N2B	-	-	+	////	
N2C	-	-	+	////	
N3	+	-	-	////	
N3A	+	-	-	////	
N3B	+	-	-	////	
N3C	++	-	+	////	
N4	++	-	+	////	
N4A	+	-	+	////	
N4B	++	-	+	////	
N4C	++	-	+	////	
N5	+	-	-	////	
N5A	++	++	+	////	
N6	+	-	-	////	
N6A	++	-	-	////	
N7	-	-	-	////	
N7A	-	-	-	////	
N9	-	-	-	////	3rd
N9A	-	-	-	////	4th
N10	++	-	-	////	1st
N10A	+	-	-	////	3rd
N11	++	-	-	////	3rd
N12	-	-	-	////	2nd
N13	+	-	-	////	8th

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Week of stay (if known)
	haem-olytic	non-haem-olytic	heat-resistant		
N14	+	-	-	////	1st
N14A	+	-	-	////	3rd
N15	+	-	-	////	2nd
N16	+	-	-	////	1st
N17	+	-	-	////	1st
N17A	-	-	-	////	3rd
N18	-	-	+	-	
N19	++	-	-	-	3rd
N20	-	-	-	-	3rd
N20A	++	-	-	+	4th
N21	-	-	-	-	4th
N22	-	-	-	-	8th
N23	+	-	-	-	2nd
N24	++	-	-	-	2nd
N25	++	-	-	-	3rd
N26	-	-	-	-	1st
N27	-	-	-	-	1st
N28	-	-	-	-	4th
N29	++	-	-	-	4th
N30	+	-	-	-	1st
N31	+	-	-	-	1st
N32	+	-	-	-	4th
N33	++	++	-	-	1st
N34	-	-	-	-	4th
N35	++	-	-	-	1st
N35A	++	-	-	-	1st
N36	-	-	-	-	4th
N36A	-	-	-	-	5th

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Week of stay (if known)
	haem-olytic	non-haem-olytic	heat-resistant		
N36B	+	-	-	-	6th
N37	++	-	-	-	
N37A	++	++	-	-	
N38	-	-	-	-	2nd
N38A	-	-	-	-	3rd
N39	-	-	-	-	4th
N39A	-	-	-	-	5th
N39B	++	-	-	-	6th
N40	-	-	-	-	2nd
N41	++	-	-	-	3rd
N42	++	-	-	-	2nd
N43	+	-	-	-	1st
N44	++	-	-	-	6th
N44A	++	-	-	-	6th
N45	++	-	-	-	5th
N45A	--	-	-	-	6th
N46	++	-	-	-	2nd
N47	++	-	-	-	4th
N47A	-	-	-	-	6th
N47B	-	-	-	-	6th
N47C	-	-	-	-	8th
N47D	-	-	-	-	9th
N47E	-	-	-	-	11th
N48	+	-	-	-	
N48A	+	-	-	+	
N48B	++	-	-	+	
N49	++	-	-	-	6th

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Week of stay (if known)
	haem-olytic	non-haem-olytic	heat-resistant		
N50	++	-	-	-	12th
N50A	+	-	-	-	13th
N51	++	-	-	-	4th
N52	-	-	-	-	1st
N53	-	-	-	-	1st
N53A	-	-	-	-	2nd
N54	-	-	-	-	1st
N55	++	-	-	-	1st
N56	++	-	-	-	2nd
N56A	++	++	-	-	2nd
N57	-	-	-	-	1st
N58	-	-	-	-	1st
N59	++	-	-	-	1st
N60	-	-	-	-	1st
N61	++	-	-	-	1st
N61A	++	++	+	-	2nd
N61B	+	-	-	-	5th
N62	-	-	-	-	4th
N62A	+	-	+	-	6th
N62B	-	-	-	+	8th
N62C	++	-	+	+	9th
N63	+	-	-	-	4th
N64	+	+	+	-	2nd
N64A	+	-	+	-	2nd
N65	++	-	+	-	4th
N65A	++	-	+	-	5th
N66	+	+	-	-	2nd
N67	-	-	-	-	1st

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Week of stay (if known)
	haem-olytic	non-haem-olytic	heat-resistant		
N68	++	-	-	-	1st
N69	+	+	-	+	10th
N70	+	-	-	-	3rd
N71	++	++	-	-	1st
N72	++	++	-	-	1st
N73	-	-	+	-	3rd
N74	-	-	-	-	2nd
N75	++	-	+	+	2nd
N76	++	++	+	-	3rd
N77	-	-	-	-	2nd
N78	++	-	+	-	2nd
N79	-	-	-	-	3rd
N80	-	-	-	-	3rd
N81	-	-	-	+	7th
N82	-	-	-	-	2nd
N83	++	++	-	-	2nd
N84	-	-	-	-	2nd
N85	-	-	-	-	
N86	++	-	-	-	2nd
N87	+	-	-	-	
N88	+	+	+	-	
N89	+	-	-	-	
N90	-	-	-	-	
N91	+	-	-	-	
N92	+	-	-	-	
N93	+	+	-	-	
N94	+	+	-	-	
N95	-	-	-	-	

Appendix II continued

Lab. No.	Cl. welchii			Staph. aureus	Week of stay (if known)
	haem-olytic	non-haem-olytic	heat-resistant		
N96	+	+	-	+	
N97	+	+	-	-	
N98	+	+	-	-	
N99	-	-	-	-	
N100	+	-	-	-	
N101	+	-	-	-	
N102	-	+	-	+	

APPENDIX III

Antibiotic sensitivity and 'phage groups of faecal strains of Staph. aureus

Key to Appendix III

Lab. number

The Staph. aureus are given the numbers of the patients from whom they were isolated.

Antibiotic sensitivity

P	=	penicillin	S	=	sensitive
C	=	chloramphenicol	R	=	resistant
T	=	tetracycline			
St	=	streptomycin			
E	=	erythromycin			

'Phage groups

I, II and III denote the main 'phage groups.

NT = untypable

///// = not tested

Appendix III (Antibiotic sensitivity & 'phage groups of faecal strains of *Staph. aureus*)

Lab. no.	Antibiotic sensitivity					'Phage group
	P	C	T	St	E	
D1(1)	R	S	S	R	S	III
D1(2)	S	S	S	S	S	I
D3	R	S	R	R	S	III
D4	R	S	S	S	S	III
D8	R	S	S	R	S	III
D10	R	S	R	R	S	N.T.
D14	R	S	R	R	S	III
D15	S	S	S	S	S	III
D17	R	S	S	S	S	III
D19	R	S	R	R	S	III
D28	R	S	S	R	S	III
D31	R	S	S	S	S	III
D33	R	S	S	S	S	III
D35	R	S	S	S	S	III
D38	R	S	S	S	S	I
D48	R	S	S	S	S	I
D51	R	S	S	S	S	III
D53	R	S	S	R	S	/////
D61	S	S	S	S	S	III
D62	R	S	S	S	S	II
D65	R	S	S	S	S	III
D67	R	S	S	S	S	III
D70	R	S	S	S	S	III
D71	R	S	S	S	S	III
D73(1)	R	S	S	S	S	III
D73(2)	S	S	S	S	S	I
D74	R	S	R	R	S	III
D76	R	S	S	S	S	N.T.
D77	R	S	S	S	S	II

See key to Appendix III for explanation of symbols

Appendix III continued

Lab. no.	Antibiotic sensitivity					'Phage group
	P	C	T	St	E	
D83	R	S	S	R	S	III
D86	R	S	S	S	S	I
D87	R	S	S	S	S	I
D90(1)	R	S	S	S	S	I
D90(2)	S	S	R	S	S	III
D90(3)	S	R	R	S	S	III
D92	S	S	S	S	S	N.T.
D98	S	S	S	S	S	N.T.
D99	R	S	R	R	S	III
D100	R	S	S	S	S	III
D108	R	S	R	R	S	III
D125	R	S	S	S	S	III
D133	R	S	S	S	S	I
D138	R	S	R	R	S	N.T.
D145	S	S	S	S	S	/////
D148	R	S	S	S	S	N.T.
D150	S	S	S	S	S	N.T.
D152	S	S	S	S	S	II
D160	R	R	R	R	S	III
D161	R	S	R	R	S	N.T.
D168	R	S	R	R	S	III
D170	R	S	R	R	S	/////
N20	S	S	S	S	S	II
N31	S	S	S	S	S	I
N48	R	S	S	S	S	III
N62(1)	S	S	S	S	S	N.T.
N62(2)	R	S	S	R	S	III
N69	R	S	S	S	S	N.T.
N75(1)	R	S	S	S	S	III
N75(2)	R	S	S	S	S	N.T.

Appendix III continued

Lab. no.	Antibiotic sensitivity					'Phage group
	P	C	T	St	E	
N81	R	S	R	S	S	III
N96	S	S	S	S	S	II
N102	R	S	R	R	S	N.T.