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MICROBIAL CONTAMINATION OF ENTERAL FEEDS

BY

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DECLARATION

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ABBREVIATIONS

FAO	Food and Agricultural Organisation of the United Nations.	
ICMSF	International Commission on Microbiological Specifications for Foods	
ICU	Intensive Care Unit	These terms are broadly equivalent but usage differs from hospital to hospital.
ITU	Intensive Therapy Unit	
PCA	plate count agar	
PEMBA	polymyxin pyruvate egg yolk mannitol bromothymol blue agar	
PVC	polyvinylchloride	
SBA	sheep's blood agar	
UHT	ultra-high temperature	

TERMINOLOGY

The terminology for enteral feeding equipment follows the definitions given in the British Standard (British Standards Institution 1983) where possible. For details of this and other terminology used in the thesis see page 166.

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SUMMARY

A series of studies were made to examine various aspects of the microbial contamination of enteral feeds. These studies included surveys of hospital practice and laboratory investigations of the quality of feed ingredients, the levels of contamination in feeds in a local hospital and the growth of contaminants in enteral feeds under laboratory and simulated ward conditions.

Surveys carried out during 1982-1984 revealed a variety of practices in the preparation and administration of enteral feeds in hospitals and also several potential sources of microbial contamination. These included the feed ingredients, inadequately cleaned kitchen equipment and poor handling procedures such as re-use of nutrient containers and storage of non-sterile feeds at ambient temperature for up to 12h prior to administration.

The microbiological quality of 19 commonly used enteral feeds and feed ingredients was examined. Thirteen of the products yielded no viable micro-organisms, while six, all of which were powders containing milk or whey proteins, gave aerobic viable counts from 50-3000 cfu g^{-1}. The main organisms isolated were Staph. albus and aerobic sporeformers such as B. cereus.

Studies of the growth of bacteria in experimentally contaminated enteral feeds showed no increase in numbers of any of the organisms in any of the feeds over 24h at 4°C. However, all the organisms multiplied rapidly in both Clinifeed ISO and Triosorbon at 25 and 37°C. There was less rapid growth in half-strength Vivonex Standard

at 25°C, although at 37°C all strains grew rapidly except for those of Staph. aureus. In full strength Vivonex Standard at 25°C only Ps aeruginosa showed any increase in numbers over 24h, whereas Ps aeruginosa, K. aerogenes and Ent. cloacae all multiplied at 37°C. Thus bacteria survive and may multiply even in feeds with low pH and high osmolarity.

The growth of E. coli and Staph. aureus during 8h under simulated ward conditions was compared in feeding systems containing either Clinifeed ISO or Nutrient Broth. In both, counts increased from 10^2 to 10^7 cfu ml^{-1}. Other systems containing Clinifeed ISO were inoculated with E. coli or Staph. aureus and sampled over 24h. At 8 and 16h the contaminated nutrient container was replaced, refilled or replaced together with the giving set. When the container was replaced or refilled it was found always to have been recontaminated by residual organisms. Even when the nutrient container and giving set were replaced, although the contents of the container were sterile, E. coli or Staph. aureus were still detected in feed collected from the end of the fine-bore tube. Experiments with varying numbers of E. coli or Staph. aureus in the inoculum demonstrated that even a single viable bacterial cell in the nutrient container could, within 16h, multiply to a level that might be harmful, especially to compromised patients.

A six month survey of the microbial quality of enteral feeds administered in the Intensive Therapy Unit of a local hospital revealed levels of contamination which ranged from 4.5×10^2 - 2.2 x

10^3 cfu ml^{-1} of residual feed in the nutrient container. These low levels of contamination may have been because only sterile, commercially prepared feeds and sterile feeding sets were used in the ITU. Nonetheless they demonstrated that contamination was occurring during the handling of the feeding sets in the ward.

The feasibility of adding food colouring to enteral feeds as indicators of microbial contamination was examined. Experiments using Triosorbon, Clinifeed ISO or Vivonex Standard plus amaranth, carmoisine, ponceau 4R, sunset yellow FCF, tartrazine or erythrosine demonstrated that although the change in appearance of coloured feeds could be linked with the presence of high numbers of bacteria in the feeds, the converse was not always true.

Adhesion studies on the equipment used for the administration of enteral feeds showed that residual feed and/or micro-organisms adhering to the inner surfaces of used nutrient containers could contaminate fresh sterile feed used to re-fill the container. Scanning electron microscope studies of the inner and outer surface topography of enteral feeding tubes revealed that a wide range of commercially available tubes have surface irregularities large enough to trap micro-organisms and food particles. Both the inner and outer surfaces of PVC, radio-opaque PVC and radio-opaque polyurethane tubes had many surface irregularities including longitudinal ridges, reticulations, fissures, pits and roughness due to particles of the radio-opaque material. By contrast, the surfaces of the silicone tubes appeared fairly smooth. Tubes which had been perfused with or immersed in

Triosorbon or Clinifeed ISO experimentally contaminated with E. coli or Staph. aureus for 24h showed patches of residual feed and bacterial cells on their surfaces.

Finally, in the light of the experimental studies and the surveys of hospital practice, recommendations for the handling and administration of enteral feeds are presented which should reduce levels of microbial contamination and minimise any clinical effects if contamination occurs.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Good nutrition is essential for recovery from illness or injury but many hospital patients are unable to eat sufficient food to meet their nutritional requirements. Enteral feeding by the naso-gastric or naso-enteric route provides an effective form of nutritional support for patients whose gastro-intestinal tract is capable of normal absorption (Allison and Walford 1979). The enteral route is considered to be safer and is also cheaper than total parenteral nutrition (Blackett et al. 1978, Silk 1980).

The administration of nutrients via a catheter into the pharynx or oesophagus dates back at least as far as a report by Capivacceus in 1598 of feeding through a hollow tube attached to an animal bladder (His 1925). Nearly two centuries later, in 1776, John Hunter described the use of a hollow 'bougie' and syringe for conveying spirits of hartshorn, peppermint water, juice of horse-radish, balsams and turpentines into the stomachs of patients recovering from drowning. Hunter later achieved total naso-gastric nutrition in a man with paralytic dysphagia (Hunter 1790). Hunter proposed that

> *'a hollow flexible tube should be passed into the stomach, through which he (the patient) might receive nourishment and medicines'* and *'recommended when the tube was made, to inject jellies, eggs beat up with a little water, sugar, and milk, or wine, by way of food, and that the medicines might be mixed with it'.*

In a description of the procedure employed, Hunter (1790) reported that

'The instrument made use of was a fresh eel-skin, of rather a small size, drawn over a probang, and tied up at the end where it covered the sponge, and tied again close to the sponge where it is fastened to the whale-bone, and a small longitudinal slit was made into it just above this upper ligature. To the other end of the eel-skin was fixed a bladder and a wooden pipe, similar to what is used in giving a clyster, only the pipe large enough to let the end of the probang pass into the bladder without filling up the passage. The probang, thus covered, was introduced into the stomach, and the food and medicines were put into the bladder, and squeezed down through the eel skin'.

Hunter also stated that

'The instrument did not produce irritation in the fauces or oesophagus; in such cases the parts losing their natural sensibility allow greater liberties to be taken with them'.

This statement would receive little support today! For instance, Bateman (1977) reports that before the introduction of modern plastic naso-gastric tubes, less flexible tubes of rubber were used and gastrointestinal intubation was often complicated by unpleasant side

effects due to inflammation alongside the tube which could result in severe ulceration and stricture formation.

Since Hunter's time, isolated reports have advocated similar feeding procedures (reviewed by Peaston 1967) but it was not until 1916 that naso-gastric feeding was introduced as a regular procedure in the management of specific diseases. In that year Jones advocated intraduodenal feeding by continuous drip for the treatment of a peptic ulcer. Subsequently, Winkelstein (1933) described the use of a standard naso-gastric milk drip.

In recent years, the availability of fine-bore and flexible plastic tubes, and an increasing awareness of the deficiencies of prolonged intravenous feeding as the sole source of nourishment for patients has led to a steady increase in the use of naso-gastric and naso-enteric feeds (Anon 1982, Bastow, Greaves and Allison 1982). However, the composition of these feeds is such that once contaminated with micro-organisms luxuriant growth is liable to occur (White et al. 1979, Furtado, Parrish and Beyer 1980, Simmons 1981, Bastow et al. 1982, Stanek et al. 1983). There is anxiety, therefore, that tube feeds may provide sources of infection which are a particular danger to the debilitated or compromised patient (Casewell and Phillips 1978a, 1978b, Casewell 1979, Pottecher et al. 1979, Casewell, Cooper and Webster 1981, Bastow et al. 1982). Potential hazards to the patient include colonisation and infection by opportunistic pathogens (Casewell et al. 1981) and/or food poisoning due to bacterial

enterotoxins. Recent reports indicate that gross contamination of both commercial and home-made feeds may occur, with bacterial counts up to 10^9 organisms ml^{-1} (Fason 1967, Casewell and Phillips 1978a, 1978b, Casewell 1979, Pottecher et al. 1979, Schreiner et al. 1979, Furtado et al. 1980, Casewell et al. 1981, Bastow et al. 1982, Hoestetler et al. 1982). Some of the micro-organisms isolated from enteral feeds are listed in Table 1.

1.2 CONTAMINATION OF ENTERAL FEEDS : RECOGNITION OF THE PROBLEM

Contamination of naso-gastric feeds was first reported by Fason (1967) who, in a study of disposable feeding bags, found that the residue after 24h had a count of 1.7×10^6 organisms ml^{-1}. The organisms were predominantly coliforms with a few Gram-positive cells. Although these results might be queried because prior to bacteriological examination the test sample was left at room temperature for an extended period of time, a potential hazard had been highlighted. Subsequently, milk-based feeds were found to be contaminated during investigations of feeds prepared in hospital diet kitchens (Cooke et al. 1970, Shooter et al. 1971). In 1970, Montgomerie et al. stated that large numbers of klebsiellae had been found in milk shakes prepared in a contaminated mixer in a hospital. Surprisingly, therefore, little detailed work on types and sources of contamination seems to have been carried out until Casewell (1977) demonstrated that naso-gastric feeds were a source of Klebsiella infection for intensive care patients.

TABLE 1 : Examples of micro-organisms isolated from enteral feeds

Organism	References
Enterobacter spp.	3, 4, 5, 6, 11, 12, 13
Escherichia coli	3, 4, 8, 12, 13
Klebsiella spp.	1, 2, 3, 4, 11, 12, 13
Proteus spp.	3, 8, 12
Salmonella enteritidis	7
Pseudomonas spp.	3, 4, 11, 12, 13, 14
Moraxella spp.	3
Bacillus spp.	4, 8, 11, 12, 13
Staphylococcus aureus	4, 8, 10, 11, 12
Staphylococcus epidermidis	4, 5, 8, 9, 11, 12, 13
Streptococcus spp.	4, 8, 12, 13
Yeasts	10, 12, 13, 14

Key to references :
1. Casewell 1977.
2. Casewell and Phillips 1978a, b.
3. Pottecher *et al.* 1979.
4. Schreiner *et al.* 1979
5. White *et al.* 1979
6. Casewell *et al.* 1981.
7. Gill and Gill 1981.
8. Bastow *et al.* 1982.
9. Hoestetler *et al.* 1982.
10. Keighley *et al.* 1982.
11. Gibbs 1983.
12. Schroeder *et al.* 1983.
13. Anderson *et al.* 1984.
14. Mandal *et al.* 1985.

In the early 1970's there appears to have been little concern expressed in the literature about contamination of naso-gastric feeds. Notwithstanding the statement by Cheek and Staub (1973) that bacterial complications were not common in neonates fed by the nasojejeunal route, Challacombe (1974) noted that within 24h of insertion of the tube there was an increase in the E. coli in the duodenal microflora of infants fed by the nasoduodenal route. Also Chen and Wong (1974) reported intestinal complications in four low-birth-weight infants who died while being fed by the nasojejeunal route, enteropathogenic E. coli being isolated from a blood culture of one of these infants. Conversely, Wells and Zachman (1975) stated that bacterial complications were not seen in a controlled study of nasojejeunal and naso-gastric tube feeding in 21 low-birth-weight infants although they did not examine gastrointestinal cultures to detect possible changes in flora as suggested by Challacombe (1974).

In the first detailed study of contamination of naso-gastric feeds (Casewell 1977, Casewell and Phillips 1978a, 1978b, Casewell 1979) it was found that of 47 samples of feeds prepared for patients in an intensive care unit, 32 (68%) were contaminated with up to 10^4 klebsiellae ml^{-1}. Contamination was traced to the liquidiser, dish cloths and work surfaces in the diet kitchen, and it was also demonstrated that the hands of the staff of the intensive care unit were contaminated with Klebsiella spp. (Casewell and Phillips 1977). Naso-gastric feeds contaminated with Enterobacter cloacae were

reported by Casewell *et al*. (1981) as the cause of septicaemia in a patient in an intensive care unit, 10^5 - 10^6 mixed coliforms ml^{-1} being detected in a sample of the feed. The source of contamination was a detergent dispenser in the diet kitchen which had contaminated the blender head, sieve and jug used in the preparation of the feeds.

Contamination of naso-gastric feeds was also demonstrated by Pottecher *et al*. (1979) during an investigation of infectious enterocolitis in an intensive care unit where it was found that feeds prepared in the diet kitchen were contaminated with 10^5 - 10^6 organisms ml^{-1}, including *Klebsiella* spp., *E. coli*, *Enterobacter* spp., *Proteus* spp., *Ps aeruginosa* and *Moraxella* spp. The gravity of the problem was emphasised by the counts of 10^6 - 10^9 organisms ml^{-1} in the feeds given to patients in the intensive care unit, the increase in numbers apparently occurring during the non-refrigerated transport to the unit and further handling of the feed in the unit prior to administration.

Many of the recent reports of contamination such as those discussed above, refer to feeds given to patients in intensive care units. These patients are very susceptible to colonisation and infection, infants being particularly at risk. For example, examination of the naso-gastric drip feed administered to an infant that had become colonised with both β-haemolytic streptococci and *Staph. aureus* yielded counts of more than 10^4 organisms ml^{-1} of feed

for both organisms (Schreiner et al. 1979). A further survey in the same neonatal intensive care unit revealed that 176 (33%) of 576 feed samples obtained from the drip chambers were contaminated. Among the organisms isolated were Bacillus spp., Staph. epidermidis, Ps aeruginosa, K. pneumoniae, Enterobacter spp. and E. coli, the numbers ranging from 20 to more than 5×10^3 organisms ml^{-1}. The hands of nurses working in this unit were colonised with a similar range of organisms, all but one of the 93 nurses examined having Staph. epidermidis on one or both of their hands.

Staph. epidermidis was isolated from naso-gastric feeds in a preliminary study into sources of bacterial contamination carried out by White et al. (1979). Commercially available sterile formulae were repackaged into 5 x 1 litre plastic containers in the pharmacy departments of two separate hospitals following which they were sampled and analysed microbiologically at regular intervals over a period of 48h. All 5 of the plastic bags from one hospital ultimately showed growth of Staph. epidermidis while only one plastic bottle from the other hospital showed growth, Ent. agglomerans being detected in the 12h sample. White et al. (1979) suggested that Staph. epidermidis may have originated from the pharmacist's hands or from the outside of the tubing used to transfer the feed from the can to the plastic bag since, although the can and can opener were swabbed and flamed and the procedure was carried out under a laminar flow hood, no particular attempt was made to ensure that either the pharmacist's hands or the

from contamination
tubing were free . In the second hospital, the Ent. agglomerans could have reached the feed from the hands of the pharmacist, from air contamination since there was no laminar flow hood, or from the can opener which was not swabbed. Thus even commercially prepared sterile formulae are likely to become contaminated during the procedures of mixing or diluting the feed and filling the nutrient container.

Bastow et al. (1982) made a detailed comparison of bacterial contamination of feeds prepared in the diet kitchen with commercial feeds poured straight from the can on the ward. All feeds prepared in the diet kitchen were contaminated with 10^2 - 10^3 organisms ml^{-1} immediately after preparation, but no viable organisms were detected in the sterile commercial feed. Organisms isolated from the home-made feeds included K. aerogenes, Staph. epidermidis, Staph. aureus, Str. faecalis and Proteus spp. Similarly, in a comparison of a commercial preparation and a home-made feed Keighley et al. (1982) reported that there was no contamination of the commercial feed but that the contamination of the home-made feeds with yeasts and Staph. aureus led to severe intestinal disturbance in the patients to which they were administered.

Gill and Gill (1981) reported a case of Salmonella enteritidis infection associated with an enteral feed prepared in a hospital diet kitchen. They suggested that contamination of the feed may have occurred from an undetected human source, from the surface of the raw

eggs used or by cross-contamination in the diet kitchen. Bacteriological examination of enteral feeds yielded counts of 10^4 - 10^6 organisms ml^{-1}, the predominant organisms being coliforms and Ps aeruginosa. Conversely, Broom and Jones (1981) reported that all of the home-made feeds (all of which contained raw eggs) that they sampled were completely free from Gram-negative bacteria; however, they do not mention whether any other micro-organisms were isolated.

1.3 SOURCES OF CONTAMINATION

In examining the routes by which naso-gastric feeds may become contaminated, it should be noted that there are many similarities between administration sets used for naso-gastric feeds and those used for intravenous infusions and many of the opportunities for contamination are also similar. The problem of microbial contamination of intravenous infusions has been the subject of intensive study (Michaels and Ruebner 1953, Phillips, Meers and D'Arcy 1976, Johnston 1978, Holmes and Allwood 1979) and many of the findings in this area can equally well be applied to naso-gastric feeds. It is also worth noting that although the route of administration of naso-gastric feeds is the digestive tract rather than the bloodstream, these enteral feeds provide a much richer source of nutrients for contaminating micro-organisms than that provided by intravenous infusions and, once contaminated, rapid growth may occur. For example White et al. (1979) inoculated a commercial feed with a pure strain of

Staph. aureus, adding 10^3 organisms to 1000 ml of solution. After 24h at 37°C a viable count of 8.2×10^5 ml^{-1}, or nearly 10^9 organisms in the whole container, was obtained.

Potential sources of contamination of these feeds are summarised in Figure 1 and will now be discussed in more detail.

Hospital Kitchens

Casewell (1977) reported that the main source of klebsiellae in food was the hospital kitchens where liquidisers, ice-cream utensils, dishcloths and work surfaces provided a reservoir of these organisms which were of the same capsular type as isolated from the feeds. Naso-gastric feeds were prepared in a separate diet kitchen and klebsiellae could not be demonstrated in any of the naso-gastric feed ingredients. However, type 10 (commonly found in ward naso-gastric feeds) was isolated from the cleaned homogeniser, the working surface and a dishcloth in a diet kitchen (Casewell 1977). Organisms were also isolated from a cutting knife, a scraping machine and a chopping board. In a survey of a hospital kitchen by Cooke et al. (1980) Klebsiella spp. were isolated from surfaces, utensils, sinks and washing-up water.

In a study of eight hospitals, eleven canteens and two schools, Shooter et al. (1971) isolated E. coli from 37 of 214 samples, Ps aeruginosa from 43 of 214 samples and Klebsiella spp. from 24 of 125

CONTAMINATION OF ENTERAL FEEDS : SOURCES, ROUTES AND PRINCIPAL MICRO-ORGANISMS

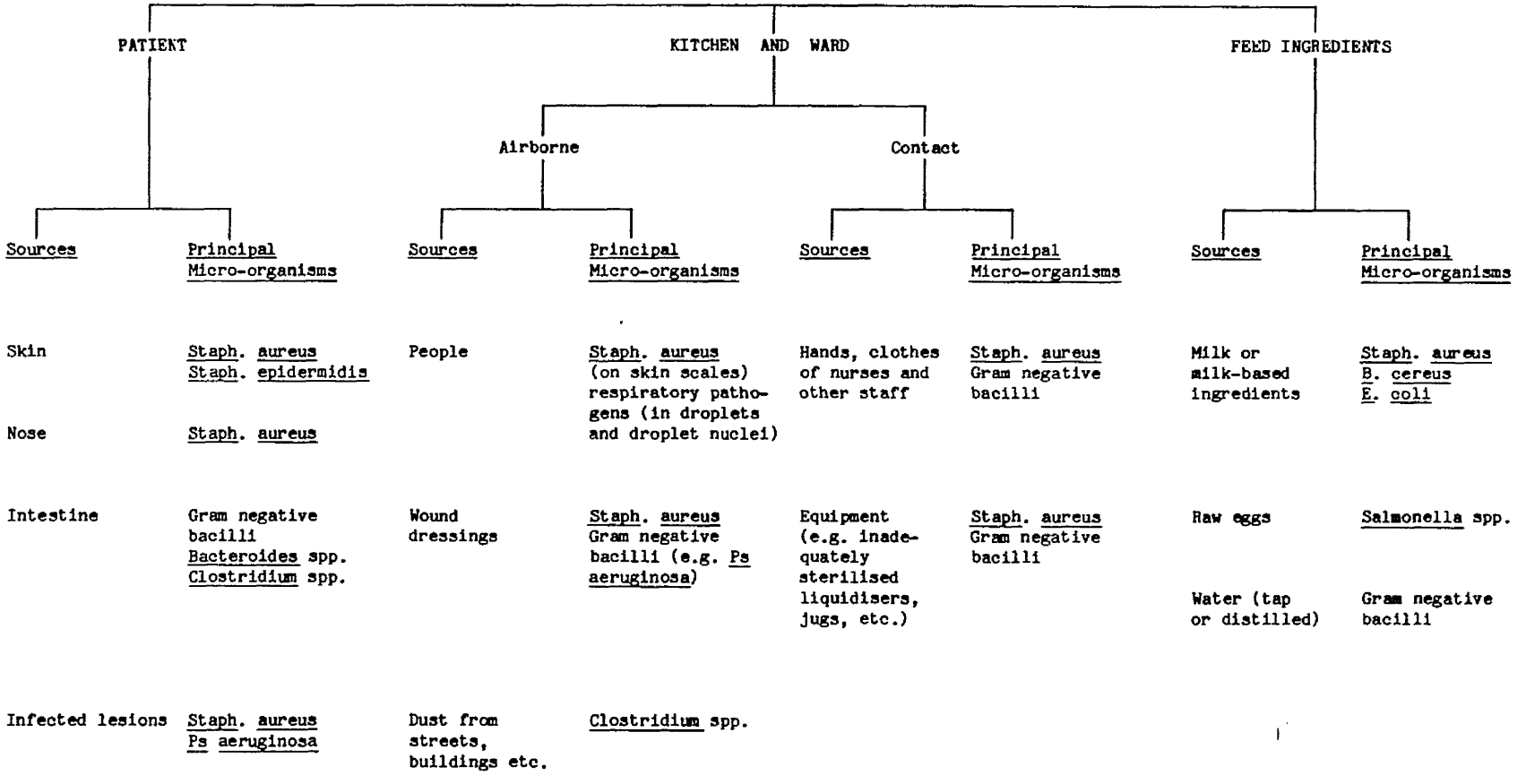


FIGURE 1 - Summary diagram of sources, routes and principal micro-organisms involved in the contamination of enteral feeds

samples taken in a limited environmental survey in which swabs from taps, sinks, washing-up water, puddles of water in bowls, chopping blocks and slicing machines were examined.

Contamination of feeds often occurs during mixing and/or diluting (Casewell et al. 1981, Bastow et al. 1982) and contamination of the mixers and liquidisers used in the preparation of hospital food has been reported by a number of authors (Montgomerie et al. 1970, Casewell 1977, Casewell and Phillips 1977, Sharpe, Collier and Gilbert 1979, Casewell et al. 1981, Bastow et al. 1982). In 1979 a hospital outbreak of salmonella food poisoning was traced to a contaminated liquidiser (Sharpe et al. 1979).

Naso-gastric feeds may sometimes be left at room temperature for several hours before refrigeration, and feeds are often ingested by patients the day following preparation. Delay in transport to the ward provides a further opportunity for bacterial multiplication (Pottecher et al. 1979, Anon 1982).

Hospital Wards

Environmental samples that have yielded Klebsiella spp., Ps aeruginosa and other Gram-negative bacilli include 'sterile water', hexachlorophene soap dispensers and sink traps (Kresky 1964). Klebsiella spp. have been isolated from ward sinks, soap, dishcloths, sink handles, a pH meter, a flower vase, horizontal surfaces, floors and a bathtub (Adler et al. 1970, Selden et al. 1971, Casewell 1977).

The water in hospital flower vases has been shown to contain Gram-negative bacilli, with counts ranging from 10^4 to 10^{10} organisms ml^{-1} and species identified including Ps aeruginosa, Ps cepacia, Ps alcaligenes, Aeromonas hydrophila, Acinetobacter spp., Flavobacterium spp., E. coli, K. ozoena and Proteus mirabilis (Taplin and Mertz 1973). Hughes (1974) demonstrated that although bacteria did not disperse from the flowers themselves, the vase water became heavily contaminated with up to 10^{10} organisms ml^{-1}. As a result the ward sinks where the water was changed became contaminated. He suggested that flower vase water should be treated with 1% hypochlorite, should not be disposed of in hand basins or ward sinks, and spilled flower vase water should be mopped up and disinfected as carefully as blood or excreta.

Feed ingredients

There are two main types of enteral feed available, those that are commercially made and those produced locally in the hospital itself (often called home-made feeds). Many hospitals are now using the commercially made feeds. In a survey of 72 hospitals in the United Kingdom, Tredger, Bazin and Dickerson (1981) found that 17 (24%) of hospitals used mainly commercial preparations and another 17 used commercial preparations as often as hospital-prepared feeds. The commercially-made feeds are nutritionally adequate, ready-made and sterile (White et al. 1979, Bastow et al. 1982, Mandal et al. 1985) but are relatively expensive. Tredger et al. (1981) found that the

remaining 38 (53%) hospitals in their survey used hospital-prepared feeds most frequently. They also report that of the 55 hospitals preparing their own tube feeds, 43 used Complan (Farley Health Products Ltd.) as the main protein source, Prosparol (Duncan Flockart Ltd.) was the fat source used by 44 and Caloreen (Roussel Laboratories Ltd.) contributed to the carbohydrate content in 42. Thirty-one of those making their own feed added a vitamin supplement, 13 added a mineral supplement and 22 added electrolytes. Bastow et al. (1982) identified B. cereus and other spore formers in Complan although these organisms failed to grow significantly during administration.

Other ingredients used in hospital-prepared feeds include pasteurised milk, dried whole milk, dried skim milk, raw eggs, sugar and water.

In a milk-based feed made with ordinary pasteurised milk there is the likelihood that the milk itself might be contaminated. B. cereus may remain after pasteurisation (Hobbs and Christian 1973) and both it and E. coli have been found in spray-dried skim milk (Thomson, Harmon and Stine 1978).

The eggs used in tube feeds may be contaminated with Salmonella spp. Normally contamination of eggs occurs during and after laying, e.g. from the faecal matter of the hen or the nest lining (Hobbs and Gilbert 1978). However, Gordon and Tucker (1965) found that eggs may become

contaminated before laying. This is the result of the chicken being a carrier of Salmonella spp. which pass from the alimentary tract via the bloodstream to the ovaries thus contaminating the ova.

Both commercially-prepared and hospital-prepared feeds may become contaminated if unsterilised distilled water is added to them. Some species of Pseudomonas, Serratia and also E. coli have been shown to multiply in distilled water (Bigger and Nelson 1943, Favero et al. 1971, Carson et al. 1973, Crichton 1973) and Holmes and Allwood (1979) observed that freshly distilled water permitted the multiplication of E. coli, Ps aeruginosa and Ent. aerogenes. Water may also be contaminated as a result of growth of micro-organisms on plumbing materials. Burman and Colbourne (1977) found that Citrobacter freundi, Aeromonas hydrophila, Ps aeruginosa and a number of fungi could grow on a wide range of plumbing materials. They also reported that in 1974 the neurosurgical unit in a London hospital was unusable until all the joints in the system were remade, the bacterial count at 21°C being 2.5×10^5 ml^{-1} in the water supplying the surgeon's scrub-up area.

Air and Dust

Air in hospital wards may contain 1-5 micro-organisms l^{-1} (Davies and Noble 1962, Greene et al. 1962a, 1962b) and so the possibility of micro-organisms gaining access to the contents of the naso-gastric feed containers from the air would appear to be quite low. However,

the number of bacteria and the distribution of various bacterial species depends on where the air samples are taken in the hospital (Greene et al. 1962a, 1962b). The predominant types of airborne micro-organisms are Gram-positive cocci and diphtheroids (Greene et al. 1962b) which originate from hospital staff and patients (Speers et al. 1965), airborne bacteria such as Staph. epidermidis being liberated from the human body surface during the shedding of skin scales of the stratum corneum (Davies and Noble 1962). A close relationship has been demonstrated between ward activity, especially bedmaking, and the number of airborne contaminants (Noble 1962); dust from clothing is known to disseminate airborne micro-organisms (Duguid and Wallace 1948).

Holmes and Allwood (1979) reported that Staph. epidermidis is present extensively in the hospital atmosphere and in a preliminary study of bacterial growth in enteral nutrient solutions, White et al. (1979) isolated Staph. epidermidis from all 5 bags of feed examined from one hospital. Staph. epidermidis has also been reported as a contaminant in enteral feeds examined by Schreiner et al. (1979) and Bastow et al. (1982). Staph. epidermidis has recently been implicated as a significant cause of surgical infection and of bacteraemia in immunocompromised hosts (Christensen et al. 1982a, Winston et al. 1983).

Holmes and Allwood (1977) demonstrated that skin scales are attracted on to the connector needles of intravenous infusion administration sets. They suggested that this attraction is electrostatic in nature (Lees and Brighton 1972). Holmes and Allwood (1977) also observed that skin scales were equally attracted to the surfaces of other materials such as borosilicate glass, natural rubber and aluminium. Thus, it seems that an attraction exists between skin scales and solid surfaces and so the surfaces of naso-gastric feed giving sets are equally at risk. It should also be noted that Noble and Davies (1965) reported that skin scales shed from the body of a person assembling or manipulating a system carry a greater number of viable micro-organisms than are present in the environmental air. Holmes and Allwood (1979) suggested that the longer the connector needle of an intravenous infusion set is left exposed to the environment, the greater the chance of it becoming contaminated, and this comment could equally be applied to the connecting spikes and airlines of enteral feeding sets (Fig. 2).

Micro-organisms associated with skin scales trapped in the connection made by needle and container and not directly introduced to the solution may gain access to this solution on subsequent movement of the tubing of the giving set, or when the joint is broken and re-made when, for example, a second container is attached to the same giving set. This problem has been described by Meers (1976) for intravenous infusion sets and similar problems can be envisaged in the

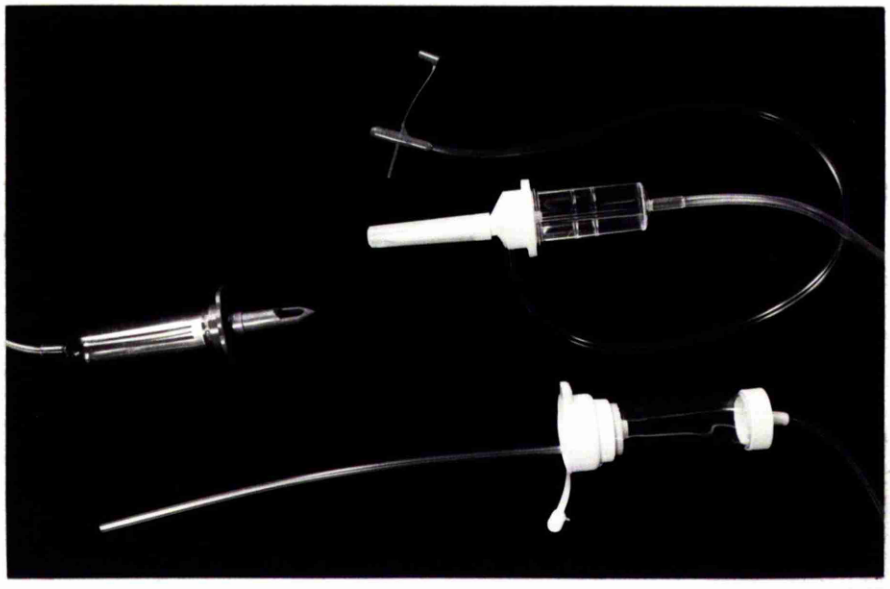


FIGURE 2 Examples of connectors used to join giving sets and nutrient containers.

Top : Clinifeeding System 3 spike connector, note cover to protect spike prior to use.

Middle : Viomedex spike connector.

Bottom : Nutrauxil cap connector and airline.

assembly of the nutrient containers and giving sets of naso-gastric feeding systems.

Once the nutrient container of feed is hanging at the bedside it would seem that the possibility of micro-organisms gaining access to its contents are quite low. In a study of naso-gastric feeding bags by Miller and Logan (1979), the feed from bags left open to the atmosphere for up to 8h was contaminated with a variety of Gram-negative rods and yeasts whereas no contamination was detected in the closed bags. Also, in a survey of closed (a filter is included in the air inlet of the administration set) and open (unfiltered air allowed to bubble through the solution) intravenous systems, Hansen and Hepler (1973) concluded that the closed system gave significantly better protection against microbial contamination.

Touch

Closures of glass bottles may be contaminated with micro-organisms if they are touched accidentally during the assembly of a feeding system. Studies with intravenous infusions (Letcher et al. 1972, Holmes 1978) suggest that the higher level of contamination with glass bottles could be a reflection of the increased amount of handling needed to set them up prior to administration. Possible routes of contamination of feeds in a range of feeding systems are illustrated in Fig. 3. The sterile contents of the bottle of feed in Fig. 3a may become contaminated if the surface of the airline

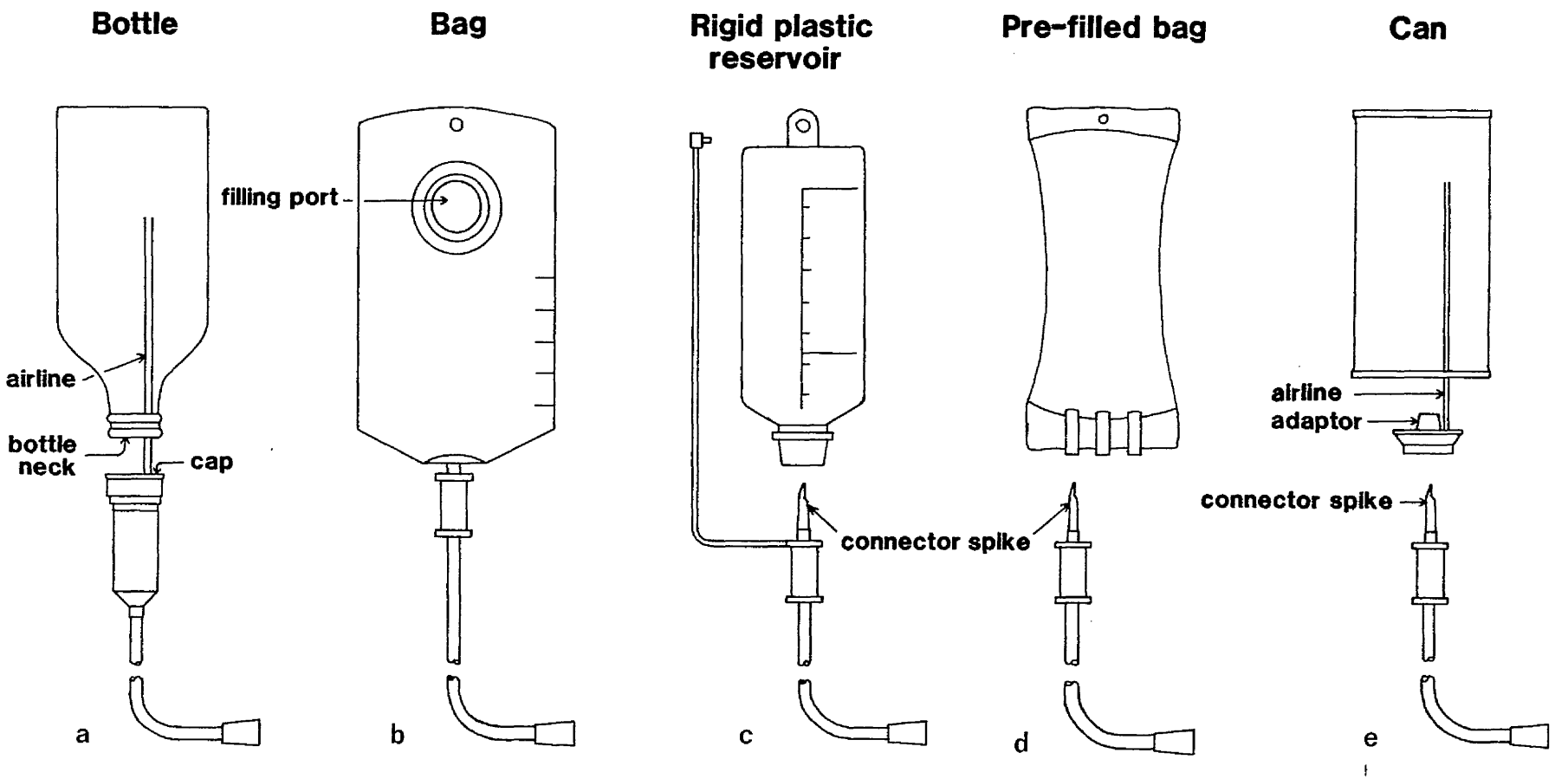


FIGURE 3 Diagrams of different types of feeding systems illustrating possible routes (arrowed) by which the feeds may be contaminated during assembly of the systems.

and/or bottle neck are handled when the giving set is attached to the bottle. Contamination of the inside of the bag illustrated in Fig. 3b may occur if the person assembling the system touches the inside surface of the bag with their fingers or even 'blows' into the bag to open it up prior to pouring the feed into it. Also, in bag systems where the giving set is attached by means of a connector spike, micro-organisms may be transferred by touch to the surface of the spike and then 'inoculated' into the contents of the bag when the spike is inserted into it; a similar problem may occur with the plastic reservoirs in Fig. 3c and the pre-filled bag system illustrated in Fig. 3d. In the system shown in Fig. 3e the giving set is attached to a can of sterile feed by means of a naso-gastric adaptor. Contamination of the sterile contents of the can could occur if the can opener or adaptor were incorrectly handled, if the surface of the can was not swabbed prior to insertion of the adaptor and if the spike on the giving set was handled when the system was being assembled.

Casewell and Phillips (1977) found little evidence that air or dust-borne particles were a route of transmission for klebsiellae but their results show that the capsular types contaminating the hands of staff and patients were the same as those colonising or infecting patients in the ward. Only slight contact with the patients' skin was required for the transfer of 10^2 - 10^3 viable klebsiellae onto the nurses hands (Casewell and Phillips 1977). Schreiner et al. (1979)

isolated Bacillus spp., Staph. epidermidis, Staph. aureus, Ps aeruginosa, K. pneumoniae, Enterobacter spp. and E. coli from continuous naso-gastric drip feeds administered in a neonate special care nursery, one infant being colonised with both β-haemolytic streptococci and Staph. aureus for over a month. Results of cultures of organisms taken from the hands of nurses working in this unit showed a high incidence of organisms that were very similar to those isolated from the feeding chambers. Knittle et al. (1975) demonstrated that hands, through acting as carriers of organisms may become reservoirs of nosocomial infections.

The insertion of the tube into the patient may also result in contamination of the feed since the nasal mucus membranes are reservoirs of Staphylococcus spp. (Hobbs and Gilbert 1978) and organisms may become trapped in the tube during its passage. In an account of two outbreaks of staphylococcal enterocolitis (Cook et al. 1957) the majority of adult patients with the disease also had an indwelling stomach tube. Also, a study by Gutman et al. (1976) of four newborn infants fed with nasoduodenal or naso-gastric feeding catheters that had been passed through a nasal or gastronomy site colonised with Staph. aureus revealed that all four infants developed staphylococcal enterocolitis or excreted Staph. aureus in their stool, whereas infants not fed by nasoduodenal or gastronomy catheters or not colonised by Staph. aureus at the site of the feeding catheter did not develop the disease.

Routes by which contaminants may be introduced when systems are being assembled are illustrated in Fig. 4.

1.4 FACTORS CONTROLLING THE GROWTH OF MICRO-ORGANISMS IN ENTERAL FEEDING SYSTEMS

Duration of Feeding

There are two methods of administration of naso-gastric feeds, i) the bolus method (small amounts of feed given at regular intervals) and ii) continuous drip feeding. The second method is becoming more popular as it appears to allow better fluid absorption in the gut (Gibbs 1983).

In the continuous drip method the feed is usually held in a gravity drip container above the patient and the flow rate can be controlled, thus altering the time the feed is left standing in the nutrient container. Flow rate may vary between 25 - 300 ml h^{-1}, a rate of 125 ml h^{-1} being commonly used (Sagar, Harland and Shields, 1979). At this flow rate a 1000 ml container will empty in 8h and a 1500 ml bag in 12h, ample time for the multiplication of micro-organisms to result in gross contamination (Simmons 1981, Bastow et al. 1982).

Temperature

The temperature at which the feed is left to stand is an important factor, since temperatures of between 20 - 25°C which are

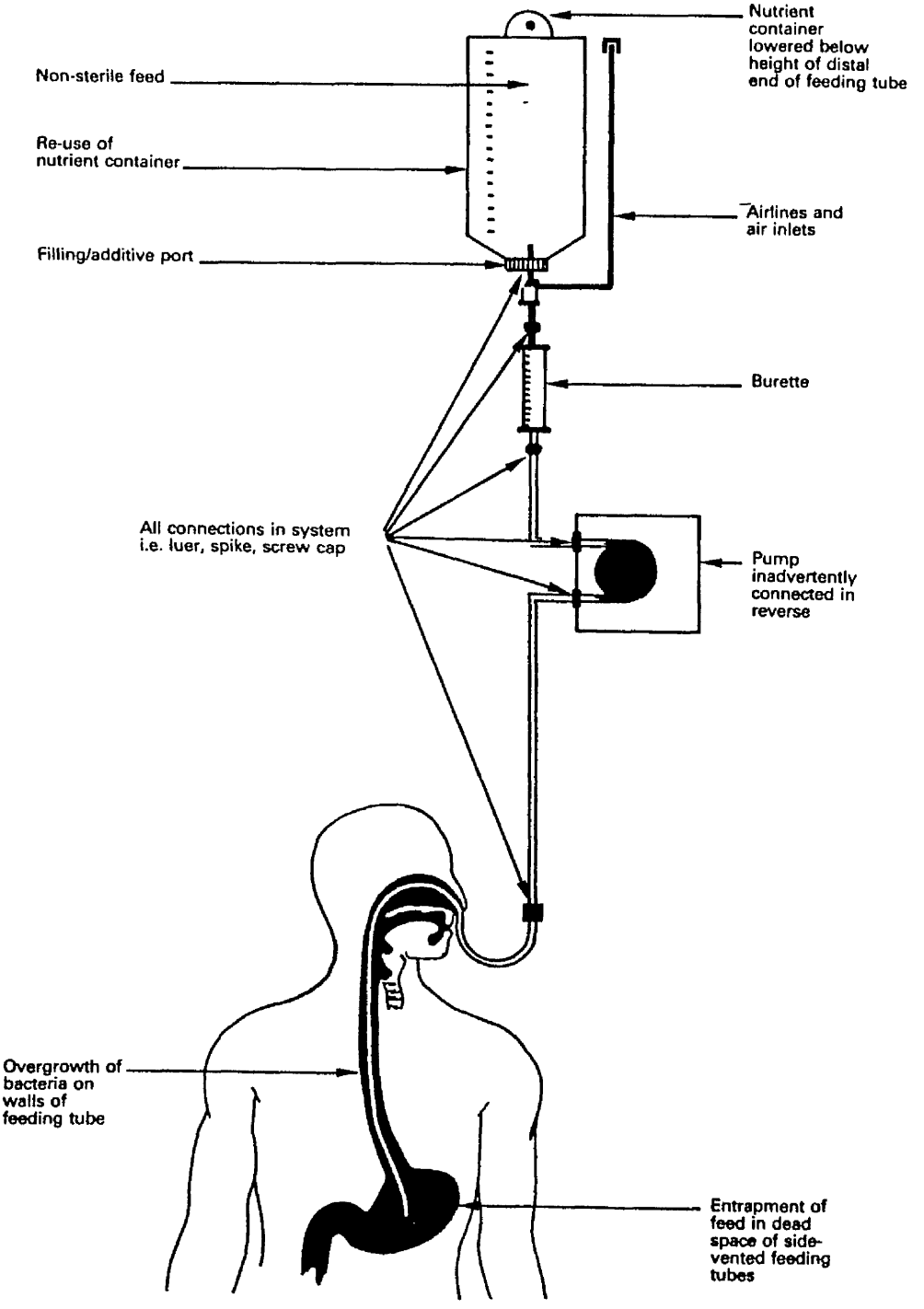


FIGURE 4 Illustration showing routes for microbial contamination of enteral feeds.

commonly found in hospitals (Maki 1976, Bastow et al. 1982) provide a suitable growth temperature for contaminating micro-organisms. Also, the feeding tube inside the patient is at the patient's body temperature and therefore there is a period of time when the feed is flowing through this tube when the temperature is even better suited to the growth of pathogenic micro-organisms. This problem will be aggravated if flow of feed through the system is stopped for any reason (e.g. accidentally due to blockage of the tube, or on purpose, to give the patient's digestive system a rest) since this will result in a static column of feed being maintained at approximately 37°C for extended periods.

Adhesion of micro-organisms to surfaces

Enteral feeds are generally administered through a soft plastic tube which is passed via the nostril and oesophagus into the stomach, facilitated by the patient's own swallowing reflexes, except when the patient is delirious or unconscious when the tube is manually directed into the oesophagus (Davidson et al. 1975). Metz, Dilawari and Kellock (1978) reported that fine-bore radio-opaque polyvinyl chloride tubing might remain in situ in the patient for periods ranging from 1 week to up to 16 months. However, they found that after 2-3 months the tube became less flexible and consequently recommended that it be changed every 2 months. Allison and Walford (1979) stated that the tubing tends to harden and should, therefore, be replaced after 2-3 weeks and, more recently, Roussel Laboratories Ltd (1980) recommended

that fine-bore tubes be replaced every 10 days since there is no deterioration (e.g. hardening) of the tubes in that time (J.C. Harkness 1982 : pers. comm.).

The practice of leaving the tube in place for 10 days or more presents a further possible hazard resulting from microbial adhesion to the plastic. The phenomenon of the adhesion of micro-organisms to solid surfaces is well documented (Zobell and Allen 1935, Zobell 1943, Fletcher and Floodgate 1976, Berkeley et al. 1980, Fletcher 1980). In a study of the growth of micro-organisms on plumbing materials in contact with potable water supplies, Burman and Colbourne (1977) found that of the plastics they examined, those most frequently supporting growth were the plasticised polyvinyl chlorides and resins used in the manufacture of glass-reinforced plastics.

Leech and Hefford (1980) examined bacterial deposition from a flowing suspension in a narrow rectangular glass capillary tube (0.2 x 2.0 x 30 mm) and found that although a close packed layer of bacteria (Streptococcus sanguis NCTC 7868) was never achieved by deposition alone, as soon as they passed a solution of nutrient broth over the deposited bacteria, growth occurred and multilayer coverage of the tube surfaces was observed.

Detailed studies have been made of the microbial colonisation of intravenous catheters (Locci, Peters and Pulverer 1981a, 1981b,

Peters, Locci and Pulverer 1981) which suggest that small numbers of organisms initially lodge in irregularities along the surface of the catheter and subsequently colonies build up. Christensen et al. (1982b) have also demonstrated that slime-producing strains of Staph. epidermidis were encased in an adhesive layer on the surface of catheters examined during an investigation of an outbreak of catheter-associated infections (Christensen et al. 1982a).

Similar adhesion of micro-organisms to the fine-bore tubing used in enteral feeding could lead to the patient being continually dosed with micro-organisms and/or their metabolites. Gemeinhardt and Kirchberger (1971) reported thrush fungi (Candida albicans and Cand. tropicalis) on feeding tubes following removal from the upper intestinal tract of children but no further work on the adhesion of micro-organisms to enteral feeding tubes has been reported.

1.5 SIGNIFICANCE OF MICROBIAL CONTAMINATION

Septicaemia caused by Ent. cloacae derived from enteral feeds has been reported by Casewell et al. (1981) and high levels of microbial contamination of tube feeds have been observed by a number of authors, counts recorded being 10^6 organisms ml^{-1} (Casewell 1979), 10^6 - 10^9 ml^{-1} (Pottecher et al. 1979), 10^5 - 10^6 ml^{-1} (Casewell et al. 1981) and 10^2 -10^{10} ml^{-1} (Bastow et al. 1982). The disease potential of a number of reported and possible bacterial contaminants of enteral feeds is presented in Table 2.

TABLE 2 : Disease potential of possible bacterial contaminants of enteral feeds, compiled from the data of Burton (1979), Ketchum (1984) and Sleigh and Timbury (1981)

Principal Division	Genus	Disease potential
Gram negative bacteria		
Enterobacteriaceae	Shigella e.g. Sh. sonnei	- Shigella dysentery
	Escherichia e.g. E. coli	- opportunistic pathogen - gastroenteritis
	Salmonella e.g. Salm. typhimurium	- gastroenteritis - septicaemia
	Klebsiella e.g. K. pneumoniae	- respiratory tract infections - septicaemia - U.T.I.
	Enterobacter Serratia Proteus Yersinia	- opportunistic pathogens - gastroenteritis
Bacteroidaceae	Bacteroides	- infections of soft tissues and wounds
Pseudomonadaceae	Pseudomonas e.g. Ps aeruginosa	- respiratory and wound infections
Campylobacter	Campylobacter	- gastroenteritis
Gram positive bacteria		
Micrococcaeceae	Staphylococcus e.g. Staph. aureus	- toxic food poisoning - wound infections - septicaemia
Bacillaceae	Bacillus e.g. B. cereus	- toxic food poisoning
	Clostridium e.g. Cl. difficile	- antibiotic associated colitis
	Cl. perfringens	- food poisoning - wound infections
Lactobacillaceae	Streptococcus e.g. Str. faecalis	- gastroenteritis - septicaemia

Patients receiving naso-gastric or naso-enteric tube feeds are very vulnerable to colonisation and subsequent infection (Fig. 5) and Pottecher et al. (1979) suggested that 10^4 organisms ml^{-1} of feed are sufficient to result in the colonisation of the digestive tract of hospital patients. However, the number of organisms required for colonisation may be much lower than this since a total dose of 10^3 - 10^4 Ps aeruginosa organisms (Shooter et al. 1969) or less than 10^4 E. coli organisms (Cooke et al. 1970) in hospital food consumed by patients led to them acquiring the same serotypes in their faeces.

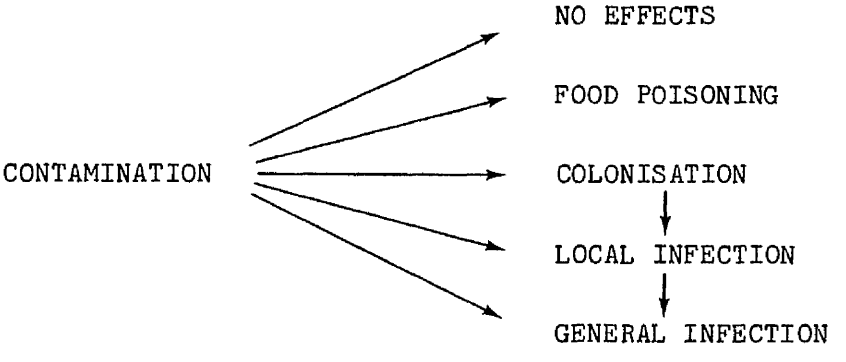


FIGURE 5 : Consequences of Contamination for Patient

Preliminary experiments by Cooke et al. (1970) with volunteers showed that the ingestion of a total of 10^4 E. coli in milk may result in colonisation of the bowel for a period of two to eight weeks. Other

workers have also shown that the ingestion of 10^4 - 10^6 organisms (E. coli, Klebsiella or Pseudomonas) by healthy volunteers produced detectable faecal counts (Buck and Cooke 1969, Montgomerie et al. 1970, Shooter et al. 1971).

The serotypes of E. coli that Cooke et al. (1970) isolated from hospital food included some that are commonly found to cause urinary tract infection and they suggested that the report by Spencer et al. (1968), that groups of female patients developed urinary infection with similar serotypes of E. coli, could be explained by these groups of patients originally acquiring their strains from the hospital food. Shooter et al. (1966, 1969) found that patients, during their stay in hospital, acquired new strains of Ps aeruginosa from hospital food and suggested that the patients might develop clinical infections from their own strains, contaminate the ward environment and infect other patients. Subsequently, Atherton and White (1978) demonstrated that overgrowth of bacteria in the stomach provided a reservoir for colonisation of the oesophagus, mouth and naso-pharynx, and suggested that this was probably facilitated by the inability of some patients to swallow and by the presence of a naso-gastric tube. Du Moulin et al. (1982) then examined the gastric and upper-airway flora of 60 patients treated with antacids or cimetidine in a respiratory/surgical intensive therapy unit and reported that in 52 (87.0%) patients one or more of the same species of organisms were cultured simultaneously from both the upper airway and stomach. It can therefore be

hypothesised that the administration of a contaminated enteral feed could lead to colonisation of the stomach followed by transmission of bacteria from the stomach to the upper airway with subsequent colonisation and infection of the respiratory tract.

The work of du Moulin et al. (1982) also highlights the fact that many patients on naso-gastric feeds are treated with antacids or cimetidine which raise the pH of the stomach from 2.0 to approximately 5.5, thus favouring the growth of bacteria and inhibiting the action of pepsin on staphylococcal enterotoxins (Bergdoll 1970). Treatment with antibiotics, steroids and immunosuppressants may also alter the natural flora of the patients, predisposing them to infection with Gram negative bacilli (Rose and Schreier 1968, Pollack et al. 1972), Staph. aureus (Dearing and Needham 1960) or Cand. albicans (Huppert, MacPherson and Cazin 1953), particularly when the patients are receiving naso-enteric feeds which pass directly into the duodenum or intestine.

Another potential hazard, to which the patient may be subjected, is that of food poisoning due to enterotoxins produced by bacteria present in the feed. Hobbs (1962) reported that the minimum number of organisms that must be present to achieve this may be as low as 5×10^5 g^{-1} of food. Although the minimum dose of staphylococcal enterotoxin that can produce food poisoning symptoms in man is not known accurately, Bergdoll (1973) and Reiser, Conway and Bergdoll

(1974), consider that less than 1.0 μg may be sufficient and as this is likely to be consumed in a 100g portion of food by a healthy individual, toxin concentrations of 0.01 μg g^{-1} of food would be hazardous (Crowther and Holbrook 1976). However, patients being fed by continuous drip feeding do not receive 100g portions of food but up to ten times this, viz. 500 - 1000 ml of feed, and therefore even lower concentrations of toxin might be harmful. Gilbert's (1974) estimates of the minimum amount of staphylococcal enterotoxin necessary to cause illness ranged from 0.015 to 0.357 μg enterotoxin kg^{-1} body weight. This relationship between toxin concentration and body weight highlights the potential dangers to neonates, children and seriously ill hospital patients who are frequently underweight. The susceptibility of young children to enterotoxins is further illustrated in a report of two outbreaks of B. cereus gastroenteritis (Bodnar 1962) where adults and children both ate portions of the same B. cereus-contaminated food. The adults suffered only mild food poisoning symptoms whereas the children required hospital treatment.

In conclusion it must be stressed that patients receiving enteral feeds are usually in a poor state of health, their natural resistance to infection often being altered by broad spectrum antibiotics, immunosuppressants or antacids. Administration of contaminated feeds to these patients presents potential hazards of colonisation, infection and/or food poisoning. Therefore, it can be seen that it is important that the persons preparing and administering these feeds

understand the potential sources of microbial contamination and take precautions to minimise it.

1.6 OBJECT OF RESEARCH

The composition of enteral feeds is such that if they are contaminated with micro-organisms, luxuriant growth may occur (White et al. 1979) and there may be an infection risk if they are fed to patients. By 1979 the published literature was indicating that gross contamination of both commercial and hospital-prepared feeds was occurring, with reported numbers ranging up to 10^9 organisms ml^{-1} (Fason 1967, Casewell 1979, Pottecher et al. 1979, Schreiner et al. 1979). These reports indicated the need for a rigorous and systematic examination of the growth of micro-organisms in naso-gastric and naso-enteric feeding systems.

The aims of this investigation as formulated in 1980 were therefore :

1. To survey current methods of preparation and handling of naso-gastric and naso-enteric feeds in hospitals in the United Kingdom; to survey the microbial quality of a range of commonly used enteral feeds and feed ingredients at time of purchase; and to determine the level and type of contamination of feeds administered in a local hospital.

2. To quantify and compare the multiplication of selected micro-organisms (opportunistic and food-borne pathogens) in a range of enteral feeds under laboratory and simulated ward conditions in order to determine the conditions that give levels of contamination that are potentially unacceptable due to (a) the presence of sufficient numbers to result in colonisation and infection of the patient and/or (b) release sufficient enterotoxin to cause food poisoning; and to determine whether micro-organisms adhere and grow on the surfaces of the giving sets and enteral feeding tubes.

3. Finally, to suggest guidelines for the safe handling and administration of enteral feeds.

CHAPTER 2

THE USE OF ENTERAL FEEDS IN HOSPITALS : A SURVEY OF HANDLING PRACTICE

2.1 INTRODUCTION

In recent years there have been many changes in methods of enteral tube feeding and in the range of commercially-prepared, ready-to-use, sterile feeds available (Bateman 1977, Tredger et al. 1981). The purpose of this part of the study was to gather information on current practices in U.K. hospitals.

2.2 MATERIALS AND METHODS

A questionnaire was devised to elicit information on current practices in enteral tube feeding and a pilot study was conducted in 1981 to check that the questions were clear. These trial questionnaires were completed by 16 students who had just returned from their hospital training period. The format of the questionnaire and the wording of individual questions were then discussed with the students and appropriate modifications made.

The modified questionnaire (Appendix A) was used in surveys carried out in 1982, 1983 and 1984. A letter of explanation was sent to Hospital Dietitians in charge of the practical training of students requesting their co-operation in allowing the students to complete the questionnaires. Fifteen questionnaires were given out each year with a request for all students involved with enteral tube feeding during their hospital placement to complete and return them. In order to maintain confidentiality, the name of the hospital was not identified on the questionnaire. However, it is unlikely that more than two or

three hospitals have been surveyed more than once during the three years because there was only a slight overlap in the distribution of hospitals used for student placements from year to year.

2.3 RESULTS

The results of the surveys carried out in 1982, 1983 and 1984 are summarised in Tables 3-7, the numbers of correctly completed questionnaires being 12, 7 and 8 respectively. Tables 4-6 detail the handling procedures employed for commercial feeds. Procedures used for hospital-prepared (home-made) and commercial feeds were the same in all the hospitals surveyed and so the results for hospital-prepared feeds are not presented separately.

Tables 3a-3c show that the average percentage of patients on enteral feeds over the three years was 1.7 -1.8% and the period of time during which the patients were tube fed varied considerably, ranging from a few days to several years (median 14-21 days).

The number of hospitals that used only ready-prepared commercial feeds ranged from 2 out of 7 (29%) in the 1983 group to 6 out of 8 (75%) in 1984. One of the hospitals surveyed used only hospital-prepared feeds (Table 3b). All of the others either used commercial feeds only or a combination of commercial and hospital-prepared feeds. The brands of commercial feeds used are listed in Tables 4a-4c and included Clinifeed ISO, Clinifeed 400,

TABLE 3a : Reported use and type of enteral feeding in U.K. hospitals (1982 survey)

Type of hospital	Number of beds	Number of patients on enteral feeds on any one day	Period of time for tube feeding	Type of feed used	% ready prepared commercial feeds used
Large teaching	600	15-25	24-48h → 1-2 months	Commercial	100
Large teaching	1400	15	varies greatly	Commercial	100
Large teaching	800	10	14-21 days	Commercial/Home-made	90
Large teaching	920	10-15	1 week → several months	Commercial/Home-made	30
Large teaching	900	5	3- 7 days	Commercial	100
Large teaching	650-700	20-25	few days → several months	Commercial/Home-made	20
Large teaching	1100	10	2 days → 2 years	Commercial/Home-made	60
Childrens'	200	10	2-3 weeks → 4-5 years	Commercial	100
Large teaching	630	13	22 days (average)	Commercial/Home-made	98
Large teaching	600	50	14 days (average)	Commercial	100
Large teaching	800	10-15	1 week → 6 months	Commercial/Home-made	60
Large teaching	1200	8	7 days (average)	Commercial	100

Average % of patierts on enteral feeds = $\frac{\text{Total number of patients on enteral feeds}}{\text{Total number of hospital beds}} \times 100\% = \frac{181}{9825} \times 100\% = 1.8\%$

Range of time patients are on enteral feeds = few days → several years

Average % of ready prepared commercial feeds used = 80%

TABLE 3b : Reported use and type of enteral feeding in U.K. hospitals (1983 survey)

Type of hospital	Number of beds	Number of patients on enteral feeds on any one day	Period of time for tube feeding	Type of feed used	% ready prepared commercial feeds used
General	850	10	14 days (average)	Commercial	100
General	700	3	7 - 21 days	Commercial	100
General	600	8	2 - 14 days	Home-made	0
Large teaching	600	15	7 - 14 days	Commercial/Home-made	90
Large teaching	900	20	2 - 3 days → 6 months	Commercial/Home-made	80
Large teaching	600	2	7 days (average)	Commercial/Home-made	50
Large teaching	1000	30	7 - 14 days → several years	Commercial/Home-made	75

Average % of patients on enteral feeds = $\frac{\text{Total number of patients on enteral feeds}}{\text{Total number of hospital beds}} \times 100\% = \frac{88}{5250} = 1.7\%$

Range of time patients are on enteral feeds = few days → several years

Average % of ready prepared commercial feeds used = 71%

TABLE 3c : Reported use and type of enteral feeding in U.K. hospitals (1984 survey)

Type of hospital	Number of beds	Number of patients on enteral feeds on any one day	Period of time for tube feeding	Type of feed used	% ready prepared commercial feeds used
Large teaching	600	8	data not available	Commercial/Home-made*	5
Large teaching	1000	30	few days → 4-5 years	Commercial	100
General	750	10	14 days (average)	Commercial	100
Large teaching	800	10-12	5 days → several years	Commercial	100
Large teaching	660	10-20	few days → year	Commercial	100
General	1000	10-15	few days → several years	Commercial	100
Large teaching	850	10-15	few days → several years	Commercial	100
General	360	6- 8	4 weeks (average)	Commercial/Home-made	90

* includes liquidised ward food

Average % of patients on enteral feeds = $\frac{\text{Total number of patients on enteral feeds}}{\text{Total number of hospital beds}} \times 100\% = \frac{106}{6020} = 1.8\%$

Range of time patients are on enteral feeds = few days → several years

Average % of ready prepared commercial feeds used = 87%

Clinifeed 500, Clinifeed Favour, Clinifeed Protein Rich, Ensure, Ensure Plus, Isocal and Nutrauxil. The hospital-prepared feeds were based on pasteurised milk, pasteurised milk plus skimmed milk or Complan (Table 5). Whole raw egg was added to feeds prepared in two of the hospitals in the 1982 group and one in 1983. Neither of the two hospitals in the 1984 group added raw eggs to their recipes.

Of the hospitals surveyed over the three years 80% added substances to commercial feeds, such as water, sodium chloride, potassium chloride, Calogen, Liquigen, Hycal and trace elements (Tables 4a-4c). Ordinary tap water was the type of water most commonly used in both hospital-prepared and commercial feeds. However, distilled water was used in one hospital in the 1982 group and one in 1984. One hospital used boiled water and another used sterile water for patients in the Intensive Care Unit (Table 4a).

Equipment used for mixing the feeds included liquidisers (glass, metal or plastic goblets), jugs and spoons or bowls and whisks. Staff in two hospitals actually mixed the feeds in the nutrient container (Tables 4a and 4c). Equipment was usually washed with hot or very hot water (Table 4a-4c) but one hospital in each yearly survey group used warm soapy water. In the 1982 group one hospital included a Milton soak and another rinsed the equipment with Savlon (Table 4a). Of those hospitals that used liquidisers to mix the feeds, approximately 50% (Tables 4a-4c) used the liquidiser for all types of food.

TABLE 4a : Ingredients and methods of preparation for commercial feeds (1982 survey)
(For manufacturers of feeds see Appendix B).

Brands of feed used	Substances added to feed	Type of water	Method of mixing	Other uses of liquidiser	Method for cleaning the liquidiser
Ensure Isocal Nutrauxil Vivonex	Water	Tap water	Jug Liquidiser	Milk foods	Hot water
Clinifeed 400 Clinifeed 500 Ensure Isocal	Water NaCl, KCl Calogen, Liquigen Hycal Trace Elements	Tap water (general) Sterile water (ICU)	Metal jug/stirred with spoon	N/A	N/A
Isocal	Water	Tap water	Liquidiser (plastic goblet)	None	Very hot water/Milton
Ensure Ensure plus Nutrauxil	Water NaCl, KCl	Tap water	Liquidiser (metal goblet	Milk-based drinks	Boiling water
Clinifeed ISO Ensure Ensure plus	Water NaCl, KCl	Tap water	Added and mixed in nutrient container	N/A	N/A
Clinifeed ISO Ensure Ensure plus Isocal Triosorbon	Water	Tap water	Liquidiser (metal goblet)	All types of food	Very hot water
Clinifeed ISO Clinifeed 500 Ensure Isocal Nutrauxil	Water Caloreen	Tap water	Liquidiser (glass goblet)	None	Savlon

TABLE 4a (Cont'd.)

Brands of feed used	Substances added to feed	Type of water	Method of mixing	Other uses of liquidiser	Method for cleaning the liquidiser
Clinifeed 400 Clinifeed 500	Water Pasteurised cow's milk Maxijul Prosparol NaCl Vitamins Minerals	Boiled water	Whisked in sterile stainless steel bowl with sterile stainless steel whisk	N/A	N/A
Clinifeed 400 Clinifeed Favour Clinifeed Protein Rich Clinifeed ISO Isocal Nutrauxil	Water Caloreen Drugs (paediatric ward)	Tap water Distilled water Boiled water	Liquidiser (plastic goblet) Jug and spoon Mix in nutrient container	All types of food	Hot soapy water
Clinifeed ISO Express	None	N/A	N/A	N/A	N/A
Clinifeed 400 Clinifeed 500 Ensure Plus Triosorbon	Water Calogen Maxijul NaCl	Tap water	Liquidiser (metal goblet)	None	Warm water
Ensure Plus Isocal Nutrauxil	Water	Tap water	Liquidiser (glass/metal goblets)	All types of food	Hot water plus detergent

ICU = Intensive Care Unit
N/A = Not applicable

TABLE 4b : Ingredients and methods of preparation for commercial feeds (1983 survey)

Brands of feed used*	Substances added to feed	Type of water	Method of mixing	Other uses of liquidiser	Method for cleaning the liquidiser
Express Isocal	None	N/A	N/A	N/A	N/A
Isocal Vivonex	Water	Tap water	Liquidiser (metal goblet)	All types of food	Warm water
Clinifeed ISO Ensure Express Standard Express High Energy	None	N/A	N/A	N/A	N/A
Clinifeed ISO Isocal	None	N/A	N/A	N/A	N/A
Express Standard Triosorbon Vivonex	Water	Tap water	Mixed in stainless steel jug	N/A	N/A

N/A = not applicable

* a) This section not completed in one questionnaire
b) One hospital did not use commercial feeds

TABLE 4c : Ingredients and methods of preparation for commercial feeds (1984 survey)

Brands of feed used	Substances added to feed	Type of water	Method of mixing	Other uses of liquidiser	Method for cleaning the liquidiser
Isocal	None	N/A	N/A	N/A	N/A
Ensure Ensure Plus Isocal Peptisorbon Triosorbon	Water	Tap water	Liquidiser (glass goblet)	None	Warm water
Fortison Nutrauxil Vivonex	Water	Tap water	Mixed in jug Liquidiser (glass goblet)	All types of food	Very hot water
Clinifeed ISO Ensure Fortison Nutrauxil Nutranel Triosorbon	Water	Tap water	Liquidiser (metal goblet)	All types of food	Hot water plus detergent
Ensure Nutrauxil Triosorbon	Water Methyl cellulose NaCl, KCl	Tap water (softened)	Liquidiser (metal/ glass goblet)	All types of food	Hot water plus detergent followed by dishwasher
Clinifeed Favour Nutranel Peptisorbon Triosorbon	Water	Tap water	Liquidiser (metal goblet)	All types of food	Hot water
Clinifeed ISO Express Isocal Triosorbon	Water NaCl Maxijul	Distilled water stored in container	Liquidiser (metal goblet)	None	Very hot water
Clinifeed ISO Clinifeed Favour Clinifeed Protein Rich Clinifeed 400 Isocal	Water Maxijul	Tap water	Mixed in nutrient container	N/A	N/A

N/A = not applicable

TABLE 5a : Some of the ingredients used in hospital-prepared feeds (1982 survey)

Type of water	Type of milk	Type of egg
Tap water from mains	Pasteurised	None
Tap water from mains	Pasteurised	Whole raw egg
Tap water from tank	Pasteurised Dried skim	None
Tap water from mains	Pasteurised Dried skim	Whole raw egg
Tap water from mains Distilled water from container	Pasteurised Dried skim	None
Tap water from mains	Complan	None

TABLE 5b : Some of the ingredients used in hospital-prepared feeds (1983 survey)

Type of water	Type of milk	Type of egg
Tap water from mains	None	None
Tap water from mains	Dried skim	None
Tap water from mains	Pasteurised	Whole raw egg
Tap water from mains	Pasteurised	None

TABLE 5c : Some of the ingredients used in hospital-prepared feeds (1984 survey)

Type of water	Type of milk	Type of egg
Tap water from mains	Pasteurised Dried skim	None
Tap water from mains	Dried skim	None

In most hospitals the feeds were refrigerated for most of the time between preparation and administration (Tables 6a-6c). However, in one hospital the feed was left for up to 4h in the general kitchen area (Table 6b) and in two hospitals the feed was sometimes left on the top of the patient's locker in the ward for up to 12h (Table 6a). Also in one hospital the feed was transported from the kitchen to the ward on a warm breakfast trolley (Table 6b).

Patients received an average of 2000 ml of feed per day over a period of 12-24h (Tables 6a-6c). One exception to this was a paediatric hospital where the feed was administered over 3h periods (Table 6a). In seven (58%) of the hospitals in the 1982 group the feeds were administered solely by the continuous drip method, in 4 (33%) a combination of continuous drip and bolus feeding was used and in 1 (8%) only bolus feeding was used (Table 7a). In all of the hospitals in the 1983 group the continuous drip method was used (Table 7b). The continuous drip method was the method of administration in all of the 1984 group of 8 hospitals but in one hospital bolus feeding was carried out as well (Table 7c).

The volume of feed put into the nutrient container when feed was being administered by the continuous drip method varied from 400-1500 ml. Nutrient containers took from 2-16h to empty, the average flow rate being approximately 120-125 ml h^{-1} (Tables 7a-7c). In the one hospital where only bolus feeding was used the amount of feed put into the nutrient container was 50 ml and this was administered over a period of 0.5 h (Table 7a).

TABLE 6a : Storage and administration of commercial feeds (1982 survey)

Time between preparation and administration of feed (h)	Storage of feed prior to reaching ward	Storage of feed on ward	Time at ambient temperature prior to administration (h)	Quantity of feed per patient per day (ml)	Time per day for administration (h)
<1	cool area in kitchen	refrigerator	0.5	2400	24
0	N/A	N/A	N/A	1800 - 3500	18
≤4	refrigerator	refrigerator	0.75	2000	24
≤20	refrigerator	refrigerator	0	2000 - 2500	20
0	N/A	N/A	N/A	2000	18
<4	N/A	refrigerator	1	1000 - 3000	24
≤18	refrigerator	refrigerator	0.5	2000	16-24
≤24	refrigerator	refrigerator	1	depends on child	3
12-24	N/A	top of locker/ refrigerator	0.25 - 12	1500	16
2	N/A	refrigerator	2	2000	24
4-12	refrigerator	top of locker/ refrigerator	≤8	2000	24
≤6	cool room	refrigerator	0.25	2000	24

N/A = not applicable

Range of time between preparation and administration of feed = 0-24h

Range of time at ambient temperature prior to administration of feed = 0-12h

Average quantity of feed per patient per day = 2100 ml

Range of time per day for administration of feed = 3-24h

TABLE 6b : Storage and administration of commercial feeds (1983 survey)

Time between preparation and administration of feed (h)*	Storage of feed prior to reaching ward	Storage of feed on ward	Time at ambient temperature prior to administration (h)	Quantity of feed per patient per day (ml)	Time per day for administration (h)
0	N/A	N/A	N/A	2000	24
4	cool area in kitchen	refrigerator	0	1500	24
4	refrigerator	refrigerator	0	2000	24
24	refrigerator/ breakfast trolley	refrigerator	0	1500	24
4	general kitchen area	refrigerator	1	2000	24

N/A = not applicable

* a) This section not completed in one questionnaire
  b) One hospital did not use commercial feeds

Range of time between preparation and administration of feed = 0-24h

Range of time at ambient temperature prior to administration of feed = 0-1h

Average quantity of feed per patient per day = 1800ml

Time per day for administration of feed = 24h

TABLE 6c : Storage and administration of commercial feeds (1984 survey)

Time between preparation and administration of feed (h)	Storage of feed prior to reaching ward	Storage of feed on ward	Time at ambient temperature prior to administration (h)	Quantity of feed per patient per day (ml)	Time per day for administration (h)
4	refrigerator	refrigerator	1	N/C	12
6	N/A	refrigerator	N/C	3000	24
0	N/A	N/A	N/A	2000	16
1-16	refrigerator	refrigerator	N/C	2000	24
6	cool area in kitchen	refrigerator	N/C	2000	12
4	refrigerator	refrigerator	1-2	1500-2000	24
4	refrigerator	refrigerator	N/C	N/C	N/C
0	N/A	N/A	N/A	2500	24

N/A = not applicable

N/C = this part of questionnaire not completed

Range of time between preparation and administration of feed = 0-16h

Range of time at ambient temperature prior to administration of feed = 1-2h

Average quantity of feed per patient per day = 2200 ml

Range of time per day for administration of feed = 12-24h

TABLE 7a : Administration procedures used for commercial feeds (1982 survey)

Method of administration	Re-use/cleaning of nutrient containers	Volume of feed in nutrient container (ml)	Time taken for nutrient container to empty (h)	Type of feeding tube	Time feeding tube left in situ
Clinifeed System 3	washed, refilled over 24h	1000	4	Clinifeed System 1	10 days
Clinifeed System 2/ Clinifeed System 3	sterilised/washed, refilled over 24h	1000	8	Clinifeed System 1/ Ryles tube	6 weeks
Vygon Bag	refilled over 24h	400	5-6	Vygon gauge 6	1-7 weeks
Bag	washed, refilled over 24h	500	4-5	Clinifeed System 1	7 days
Bag/Clinifeed System 3	refilled over 24h	⩽1000	6	Clinifeed System 1	2 weeks
Bag/Clinifeed System 3	refilled over 24h, then sterilised using Milton	500	varies	Portex/Ryles	Only changed if tube blocked
Clinifeed System 3/Bolus	used once, sterilised, re-used	500	4-6	Clinifeed System 1/ Ryles/Dobhoff	2 weeks
Bolus	washed, refilled over 24h	50	0.5	Clinifeed System 1/ Ryles/Portex	7 days
Clinifeed System 3/Bolus	refilled over 24h	1000	7	Clinifeed System 1/ Ryles/Levins	1-3 weeks
Clinifeed System 3	used once, discarded	1000	12	Clinifeed System 1	N/C
Bag/Clinifeed System 3/ Bolus	washed, refilled over 24h, sterilised, re-used	500-750	3-5	Ryles/Vygon FG8	Few days → several months
Nutrauxil System/Bag/Bolus	Used once, discarded/ washed, refilled over 24h	500-1500	2/4	Clinifeed System/Vygon/ Ryles	2-3 days

N/C = this part of questionnaire not completed

Average volume of feed in nutrient container = 750 ml

Average time taken for nutrient container to empty = 6h

Range of time feeding tube left in situ = few days → several months

TABLE 7b : Administration procedures used for commercial feeds (1983 survey)

Method of administration*	Re-use/cleaning of nutrient containers	Volume of feed in nutrient container (ml)	Time taken for nutrient container to empty (h)	Type of feeding tube	Time feeding tube left in situ
Bag	Used once, discarded	1000	N/C	Clinifeed System 1/Ryles	N/C
Bag	Used once, discarded	500	varies	Clinifeed System 1	7 days
Bag	Used once, sterilised, re-used	500	4	Clinifeed System 1	N/C
Bag/Clinifeed System 3	N/C	500	N/C	N/C	N/C
Clinifeed System 3	Used once, discarded	500	6	Clinifeed System 1	7 days

N/C = this part of questionnaire not completed

* a) This section not completed in one questionnaire
b) One hospital did not use commercial feeds

Average volume of feed in nutrient container = 600ml

Average time taken for nutrient container to empty = 5h

Time feeding tube left in situ = 7 days

TABLE 7c : Administration procedures used for commercial feeds (1984 survey)

Method of administration	Re-use/cleaning of nutrient containers	Volume of feed in nutrient container (ml)	Time taken for nutrient container to empty (h)	Type of feeding tube	Time feeding tube left in situ
Bag	Used once, discarded	N/C	N/C	Clinifeed System 1	N/C
Clinifeed System 3	Used once, sterilised, re-used	1000	8	Clinifeed System 1	2 weeks
Nutrauxil or Fortison System	Used once, discarded	500	4	N/C	N/C
Nutrauxil or Fortison System/Bag	Used once, discarded/ refilled over 24h	500	4	Ryles/Viomedex	N/C
Nutrauxil System/Bag	Used once, discarded/ refilled over 24h	500	4-6	Clinifeed System 1	1 week
Clinifeed System 3/Bolus	Refilled over 24h	1000	8-12	Clinifeed System 1/Ryles	2 weeks
Clinifeed System 3	Used once, discarded	1000	8	Clinifeed System 1	Few days → several months
Bag/Clinifeed System 3	Refilled over 24h/refilled over 24h	1500/1000	8-16	Clinifeed System 1	1 week

N/C = this part of questionnaire not completed

Average volume of feed in nutrient container = 875ml

Average time for nutrient container to empty = 7h

Range of time feeding tube left in situ = few days → several months

In the 1982 group the nutrient containers were re-used in 11 (92%) of the hospitals (Table 7a). In the 1983 and 1984 groups the figures were 1 (20%) and 5 (63%) respectively (Tables 7b and 7c). Of those hospitals where the nutrient containers were re-used they were sterilised after use in one hospital in each of the 1983 and 1984 groups. In the remainder of the hospitals the nutrient containers were either washed prior to refilling or simply refilled over 24h without washing. In two hospitals the nutrient containers were used for more than 24h (Table 7a).

Fine-bore enteral feeding tubes (e.g. Clinifeed, Vygon, Viomedex) were used in all of the hospitals (Tables 7a-7c). Ryles tubes were also used in a number of hospitals (Tables 7a-7c). The times for which the tubes were left in situ in the patient varied considerably, ranging from a few days to several months.

General comments inserted at the ends of the questionnaires included two references to feeds with a curdled appearance being administered to patients and a report that in one hospital a feed solution turned green during the course of administration. Also, in one hospital contamination of a feed was detected and traced to an improperly cleaned liquidiser. The rubber rings at the base of the blades had not been taken apart for cleaning and as a result a build-up of feed had occurred at the base of the liquidiser.

CHAPTER 3

MICROBIOLOGICAL QUALITY OF ENTERAL FEEDS AND FEED INGREDIENTS

3.1 INTRODUCTION

The isolation of Staph. epidermidis, Ps stutseri and Bacillus spp. from feed ingredients by Gibbs (1983) and of Bacillus cereus from Complan by Bastow et al. (1982) indicates that the feeds or feed ingredients themselves may provide a source of potentially hazardous organisms. Therefore the microbiological quality, at the time of purchase, of a range of commonly used naso-gastric feeds and feed ingredients was investigated.

3.2 MATERIALS AND METHODS

Enteral feeds and ingredients - collection and preparation of samples

The feeds and dietary supplements used in this study were selected to be representative of the large number of commercially prepared liquid and powdered formulas now available. Products examined included 10 complete feeds (6 UHT processed, milk-based liquid feeds; one dried milk protein based feed; one dried, low residue feed containing whey protein; and two dried, elemental, minimal residue feeds), 4 protein sources (all dried, milk protein based products), 3 fat sources and 2 carbohydrate sources (Table 8). Products were ordered from the manufacturers/suppliers at intervals over a 6 month period in order to obtain different lot numbers for each unit sampled. Five units (i.e. bottles, cans or packets) of each product were sampled; 3 samples were taken from each unit. The procedures outlined by the International Commission on Microbiological

TABLE 8 Proprietary feeds and feed constituents examined for microbiological quality

Complete foods	Protein sources	Fat sources	Carbohydrate sources
Clinifeed ISO (Roussel Laboratories Ltd.)*	Build-Up (Carnation Ltd.)	Calogen (Scientific Hospital Supples Ltd.)	Caloreen (Roussel Laboratories Ltd.)
Ensure (Abbott Laboratories Ltd.)	Casilan (Farley Health Products Ltd.)	Liquigen (Scientific Hospital Supplies Ltd.)	Maxijul (Scientific Hospital Supplies Ltd.)
Enteral 400 (Scientific Hospital Ltd.)	Complan (Farley Health Products Ltd.)	Prosparol (Duncan Flockhart Ltd.)	
Express enteral feed (Express Nutrition)	Maxipro HBV (Scientific Hospital Supplies Ltd.)		
Fortison (Cow & Gate Ltd.)			
Isocal (Bristol-Myers Co. Ltd.)			
Nutrauxil Liquid (KabiVitrum Ltd.)			
Triosorbon (E. Merck Ltd.)			
Vivonex Standard (Eaton Laboratories Ltd.)			
Vivonex HN (Eaton Laboratories Ltd.)			

* Addresses given in Appendix B.

Specifications for Foods (ICMSF 1974) were followed when withdrawing samples from the bottles, cans or packets in order to ensure that the samples were as representative as possible of the unit from which they came. Thus, surface contamination on the outside of packets of dried powder was removed by wiping with 70% ($^{v}/v$) alcohol. Each packet was then opened with a dry sterile scalpel. Special care was taken to avoid contaminating the working area or the samples by avoiding sudden movements and keeping the laboratory doors and windows closed (ICMSF 1974). A modification of the method described by Harrigan and McCance (1976) for the sampling of bulk milk powders was then used for sampling the dried powders. The contents of each packet were transferred to a dry sterile jar of sufficient size to allow mixing by shaking.

Using a dry sterile metal spatula a 10g sample of powder was weighed out aseptically onto sterile aluminium foil and transferred to a wide-mouthed bottle containing 90ml sterile water and glass beads. The bottle was shaken 25 times in 12s with an excursion of 30cm. The reconstituted product was left to stand at room temperature for 15min. The bottle was inverted several times and the contents examined immediately. Sterile distilled water was used as the initial diluent because the specimens being prepared were highly soluble dry powders and there was therefore likely to be a low a_w at the lowest dilution (Silverstolpe et al. 1961).

Bottles and cans of feed were shaken vigorously, 25 times in 12s, with an excursion of 30cm. The surface of the can or the neck and lid of the bottle were then wiped with 70% (V/v) alcohol and flamed. The containers were inverted several times and then opened with a dry, sterile can/bottle opener where necessary.

Microbiological examination of samples

a) Viable counts

The number of viable cells present in the enteral feeds was determined by surface plating, as subsequently recommended by Greenwood et al. (1984) after they had carried out an inter-laboratory evaluation of molten agar and surface methods for the enumeration of micro-organisms in foods. Surface spread plates were prepared following the procedure described by the ICMSF (1978).

Serial decimal dilutions of the samples were prepared in 0.1% (w/v) peptone water (pH 7.0, Oxoid). This was chosen as the diluent throughout this study because it has been demonstrated to be less toxic to micro-organisms than the other commonly used diluents which include tap water, distilled water, saline solution, phosphate buffers and quarter strength Ringer solution (Straka and Stokes 1957, King and Hurst 1963, Farwell and Brown 1971, ICMSF 1978). Samples of 0.1 and 0.5 ml from at least four dilutions were spread over the surface of pre-dried (50°C for 2h) plates of PCA, using a separate sterile glass spreader for each plate. Three plates were prepared at each dilution. Inoculation of the plates was always carried out within 15-30min of

the preparation of dilutions (Harrigan and McCance 1976). The surfaces of the inoculated plates were allowed to dry for 15min and the plates were then inverted and incubated at 37°C for 24h or 30°C for 48h.

After incubation the dilutions giving colony counts between 30 and 300 colonies per plate were counted (ICMSF 1978) and the mean count expressed as cfu ml^{-1} or cfu g^{-1} of the original sample.

For dried samples with low levels of contamination (viable counts $<10^1$ cfu g^{-1}) the residual powder was sampled again and 3 further packets bearing the same lot number were also sampled. For liquid samples with viable counts $<10^1$ cfu ml^{-1} three further containers with the same lot number were sampled.

b) Coliforms

Portions (0.5 ml) of each feed were spread over the surface of two pre-dried plates of Violet Red Bile agar (Oxoid) and two of MacConkey agar (Oxoid). The plates were incubated at 37°C for 24h and lactose-fermenting colonies counted.

c) Staphylococcus aureus

Portions (0.5 ml) of each feed were spread over the surface of two plates of Baird-Parker agar (Oxoid Baird-Parker Medium CM 275 plus Oxoid Egg Yolk Tellurite Emulsion SR 54). The plates were incubated at 37°C for 24-48h. Typical black colonies surrounded by a zone of

opalescence or clearing were counted, subcultured, and confirmed as Staph. aureus by testing for coagulase and/or deoxyribonuclease production (Cowan 1974).

d) Bacillus cereus

Portions (0.5 ml) of each feed were spread over the surface of two plates of polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA, Holbrook and Anderson 1980). Plates were incubated at 37°C and examined after 24h of incubation and then left at room temperature for 24h and re-examined. Typical turquoise or peacock blue colonies surrounded by an egg yolk precipitate of similar colour were counted. The staining method developed by Holbrook and Anderson (1980) was used to confirm the identification of B. cereus. This method demonstrates microscopically both the presence of lipid globules in vegetative cells and spore morphology of isolates and was shown by Holbrook and Anderson (1980) to give complete agreement with a range of biochemical tests used for this purpose.

Incubation of samples of feed

Normal procedures for the administration of these feeds involve the prepared solutions being hung at the patient's bedside at hospital ward temperature for 8h and, in some instances, up to 24h (Bastow et al. 1982). Feeds prepared in advance may also be stored in a refrigerator overnight (Anon. 1982).

To determine the rate of growth of indigenous micro-organisms, 100ml samples of all the feeds and feed constituents were incubated at 4, 25 or 37°C for 24h. These temperatures were selected to represent the temperatures to which the feeds would be subjected in normal practice, namely that of the refrigerator (4°C), ambient (ward) temperature (25°C) and the patient's body temperature (37°C). It should also be noted that the temperature of the incubators used for neonatal care is approximately 37°C. Also, ambient temperatures in tropical climates may be substantially higher than 25°C. For example, Singh et al. (1980) quote a temperature as high as 37.5°C for ambient/room temperature in India.

Dried powders were first rehydrated according to the manufacturers' instructions. Sterile distilled water was used in order to avoid introducing possible contaminants from the water supply. Portions (100ml) of each feed were then transferred to sterile 250ml Erlenmeyer flasks. Three samples of each feed were incubated at each of the temperatures. Viable counts were made at 0, 8 and 24h.

3.3 RESULTS

Microbial content of enteral feeds and feed ingredients

No coliforms or Staph. aureus were isolated from any of the 19 products and no contamination at all was detected in 13 of the 19 products examined.

Viable counts for the remaining six products are summarised in Table 9 and ranged from 50-5444 cfu g^{-1}. The contaminated products were all dried powders containing milk and whey proteins. Four of these were protein sources (Complan, Build-Up, Casilan and Maxipro) and the remaining two were complete feeds (Enteral 400 and Triosorbon).

The organisms isolated were mainly aerobic spore-forming bacilli. B. cereus was detected (at levels of $\leqslant 10^1$ cfu g^{-1}) in at least one sample of all six products and in all of the samples of Build-Up and Complan. Staph. albus was isolated from three of the products (Build-Up, Casilan and Complan) but counts did not exceed 10^1 g^{-1}. The colonial and microscopic appearance of the predominant contaminants in all of the samples of Triosorbon examined were very similar. The organisms were therefore identified using the API system described by Logan and Berkeley (1984), and were found to belong to the B. sphaericus - B. brevis group.

It is important to note that wiping the outside of the packets and cartons of feed with 70% alcohol prior to opening does not necessarily achieve sterilisation (Harrigan and McCance 1976). However, it seems unlikely that the organisms isolated were introduced during handling in the laboratory since no micro-organisms were detected in any of the UHT processed, milk-based complete liquid feeds nor in the two dried complete feeds that did not contain milk or whey

TABLE 9 : Viable counts of enteral feeds and feed ingredients

Product	Viable Counts (cfu g^{-1})[1]		No. of units in which specific bacteria detected	
	range	median	*B. cereus*[2]	*Staph. albus*[3]
Build-Up	994 - 1334	1034	5	5
Casilan	147 - 1454	538	4	5
Complan	1422 - 5444	2456	5	5
Enteral 400	41 - 67	60	3	0
Maxipro	50 - 168	69	3	0
Triosorbon	32 - 68	43	1	0

[1] For each product, number of units = 5; 3 samples taken from each unit and plate counts made in triplicate, i.e. 9 plates per unit.

[2] *B. cereus* = $\leqslant 10^1$ cfu g^{-1} in all instances where detected

[3] *Staph. albus* = $\leqslant 10^1$ cfu g^{-1} in all instances where detected

proteins (Vivonex and Vivonex HN). Also no contamination was detected in any of the fat or carbohydrate sources examined.

Incubation of samples of feeds

The 13 feeds and feed ingredients originally found to be free of contamination showed no growth after 24h at 4, 25 or 37°C. In the remaining six products, initial contamination of the reconstituted feeds, ranged from 10^0 to 10^2 cfu ml^{-1} (Fig. 6). No increase or decrease in microbial load was observed after 24h at 4°C. Storage at 25°C resulted in counts ranging from 10^1 to 10^4 cfu ml^{-1} after 8h and this increased to 10^7 and 10^8 cfu ml^{-1} after 24h. After 8h and 24h at 37°C, the counts ranged from 10^2 to 10^4 and 10^9 to 10^{10} cfu ml^{-1} respectively (Fig. 6).

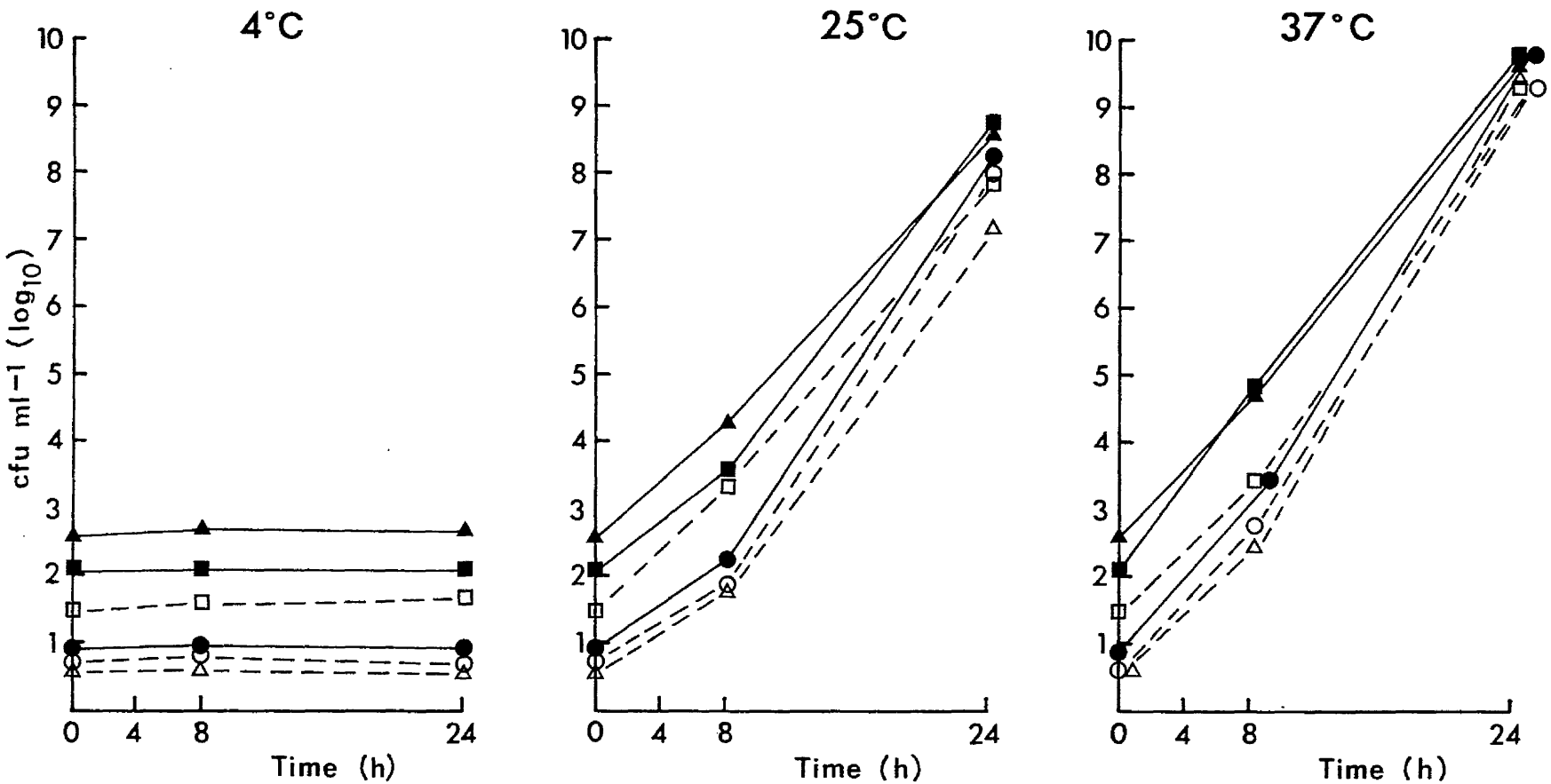


FIGURE 6 Growth of contaminants in reconstituted enteral feeds.
Build-Up (■——■), Casilan (□——□), Complan (▲——▲), Enteral 400 (O- - -O),
Maxipro (●——●), Triosorbon (△- - -△).

CHAPTER 4

CONTAMINATION OF ENTERAL FEEDS DURING ROUTINE HOSPITAL USE

4.1 INTRODUCTION

Septicaemia caused by Ent. cloacae derived from an enteral feed contaminated in the diet kitchen has been reported by Casewell et al. (1981) and hospital-prepared feeds have been suspected to be the source of Salm. enteritidis which has caused hospital-acquired gastro-enteritis (Gill and Gill 1981).

Many of the recent reports of contamination refer to feeds given to patients in intensive care units (Pottecher et al. 1979, Schreiner et al. 1979, Casewell et al. 1981). These patients are very susceptible to food poisoning and/or colonisation and infection by micro-organisms present in the feeds. This part of the study was undertaken to examine the microbial quality of the feeds administered in the Intensive Therapy Unit (ITU) of the Glasgow Western Infirmary and to ascertain if there was any correlation between organisms isolated from feeds and those isolated from patients.

4.2 MATERIALS AND METHODS

Collection of enteral feeding sets

Discarded Clinifeed System 3 enteral feeding sets (i.e. the nutrient container plus giving set) were collected from the ITU of the Glasgow Western Infirmary over the period from 9th June to 19th December, 1983.

The feeds used in the Western Infirmary ITU during this period were Isocal (Mead Johnson Ltd.), Nutrauxil (KabiVitrum) and Ensure (Abbott Laboratories Ltd.). These are all UHT processed, low residue, milk-based nutritionally complete liquid feeds supplied in cans (Isocal, Ensure) and bottles (Nutrauxil, Ensure). The feeds were administered either undiluted, or diluted with sterile distilled water in the nutrient container. The final quantity of prepared feed in the nutrient container ranged from 500-1000ml.

The nursing staff of the ITU collected the discarded feeding sets, closed the air vents and flow regulators and sealed the ends of the giving sets with sterile caps.

The feeding sets were transferred to a cold room in the Bacteriology Department of the Western Infirmary prior to collection and transfer to The Queen's College, Glasgow.

Feeding sets collected on the day they were discarded were assayed for viable bacteria quantitatively and qualitatively, those that were more than one day post-discard were examined qualitatively only.

Isolates from enteral feeding sets

For those feeding sets to be examined quantitatively, viable counts were made on samples of residual feed from the nutrient container and the giving set of the discarded feeding set. Duplicate

0.1ml samples were spread over the surface of pre-dried plates (50°C for 2h) of Sheeps' Blood Agar (SBA; Oxoid Blood Agar Base CM55 plus Oxoid Defibrinated Sheep Blood SR52) and the plates were incubated aerobically at 37°C for 24h. Representative colonies were subcultured and purified by streaking on Sheeps' Blood Agar plates and incubated at 37°C for 24h.

For feeding sets which were only to be examined qualitatively, duplicate samples of residual feed from the nutrient container and giving set were streaked onto plates of Sheeps' Blood Agar, incubated at 37°C for 24h and representative colony types purified as described above.

Gram-stained films were prepared on young cultures (18-24h) of all isolates. Further identification of isolates was done by standard bacteriological techniques (Cowan 1974, Harrigan and McCance 1976). Gram negative organisms were tested using the API 20E system (API Laboratory Products Ltd.) following the procedures recommended by the manufacturer. The organisms were identified using the coder and profiler index (Anon. 1979).

Where appropriate, an uninoculated tube or plate was incubated with each test in order to detect any false positive results due to impurities in, or a deterioration of, media or reagents. Strains of bacteria (NCTC cultures; Appendix C) known to give positive results

were also used to check that each batch of medium was satisfactory. Antibiotic sensitivity was tested with Multodisks (Oxoid).

Isolates from patients

Over the period of the study a record was kept of isolates from gastric aspirates, stools etc., collected from patients receiving enteral feeds. These isolates were identified by standard techniques (Cowan 1974). Antibiotic sensitivity was tested with Multodisks (Oxoid).

4.3 RESULTS

A total of 68 discarded feeding sets was collected from 14 patients. The results are summarised in Table 10. Twelve of the sets were not correctly sealed (air vent left open or giving set not sealed) and were therefore not examined further, since contamination of these sets could possibly have occurred after removal from the patient. Of the remaining 56 sets a large proportion (45) were not received for examination until one or two days after removal from the patients' bedside and, since they had been stored at ward temperature for indeterminate periods of time during which any contaminants could have multiplied, no viable counts were made.

Fifty of the feeding sets were found to be contaminated and the most frequently isolated organisms were Bacillus spp., Micrococcus

TABLE 10 : Micro-organisms isolated from enteral feeds and from patients receiving enteral feeds in the ITU of the Glasgow Western Infirmary between 9.6.83 and 19.12.83

Patient No.	No. of feeding sets (total)	No. of feeding sets not sealed	No. of feeding sets sampled	No. of feeding sets found to be contaminated	No. of feeding sets with no contamination	MICRO-ORGANISMS ISOLATED FROM: Residual feed	Patient (gastric aspirate and/or stool)
1	11	5	6	6	0	B, Ca, D, S.al, Str.	Ca
2	2	0	2	0	2	-	-
3	2	0	2	2	0	-	-
4	2	0	2	2	0	Co, K.a, S.al	-
5	2	0	2	2	0	Ac, S.al, S.au	Y
6	3	0	3	2	1	Al, B, S.al, S.au	-
7	2	0	2	2	0	B, M, S.al	-
8	5	0	5	5	0	Ac, B, K.a, S.al, Str.	Co, T
9	5	1	4	4	0	M, S.al	-
10	2	0	2	2	0	B, S.al, Str.	Ca, E.cl, P, Str.
11	18	3	15	15	0	B, E.sa, M	Ac, E.c
12	2	1	1	1	0	B, M	-
13	5	2	3	3	0	B	-
14	7	0	7	4	3	B	-

KEY

Ac	Acinetobacter calcoaceticus var. anitratus	E.cl	Enterobacter cloacae	P	Pseudomonas aeruginosa
Al	Alcaligenes spp.	E.sa	Enterobacter sakazakii	S.al	Staphylococcus albus
B	Bacillus spp.	E.c	Escherichia coli	S.au	Staphylococcus aureus
Ca	Candida albicans	K.a	Klebsiella aerogenes	Str.	Streptococcus spp.
Co	Coliform	K.o	Klebsiella oxytoca	T	Torulopsis glabrata
D	Diptheroid	M	Micrococcus spp.	Y	Yeasts

spp. and Staph. albus. Other organisms isolated included Cand. albicans, Staph. aureus, Acinetobacter calcoaceticus var. anitratus, K. aerogenes, K. oxytoca, Ent. sakazakii, Alcaligenes spp. and Streptococcus spp (Lancefield Group D). In all the feeding sets examined the same organisms were isolated from the residual feed in both the nutrient container and the giving set of each system and therefore contaminants will be referred to as occurring in residual feed in the feeding set rather than in either the nutrient container or giving set.

In Table 11 the feeds given to three of the patients are listed together with a list of the micro-organisms isolated from the residual feed in the feeding sets. It can be seen that there are examples of contamination both in undiluted feeds and feeds diluted with water (patients 6 and 11). Also, there are cases in which there was no contamination in either undiluted or diluted feeds (patients 2 and 6).

Eleven feeding sets were sampled on the same day that they were removed from the patient. Viable counts for the residual feed in these sets ranged from 4.5×10^2 to 2.2×10^3 cfu ml^{-1} of feed (Table 12). The counts for samples of residual feed from the nutrient container and giving set were very similar and therefore their average is given in Table 12. The antibiotic sensitivity of the micro-organisms isolated is also detailed in Table 12 and it can be seen that Staph. albus, which was resistant to all the antibiotics

TABLE 11 : Examples of the feeds given to patients in the ITU of the Glasgow Western Infirmary between 9.6.83 and and 19.12.83, together with the micro-organisms isolated from residual feeds

Patient No.	Date	Feed	Micro-organisms isolated from residual feed
2	7.07.83	2 cans Isocal + 600 ml H_2O	None
	7.07.83	3 cans Isocal made up to 1000 ml with H_2O	None
6	25.07.83	1 bottle Nutrauxil	Staph. aureus, Alcaligenes spp. Bacillus spp.
	27.07.83	2 bottles Nutrauxil	None
	27.07.83	2 bottles Nutrauxil	Staph. albus, Bacillus spp.
11	19.11.83	2 cans Isocal made up to 750 ml with H_2O	Staph. albus
	25.11.83	2 cans Isocal + 2 cans Ensure	Ent. sakazakii
	26.11.83	" " " " " "	" "
	26.11.83	" " " " " "	" "
	28.11.83	4 cans Ensure	Micrococcus spp.
	30.11.83	2 cans Isocal + 2 cans Ensure	Bacillus spp.
	2.12.83	4 cans Isocal	Ent. sakazakii
	3.12.83	" " "	Micrococcus spp.
	4.12.83	2 cans Isocal + 2 cans Ensure	Ent. sakazakii
	5.12.83	" " " " " "	Micrococcus spp. Bacillus spp.
	5.12.83	4 cans Isocal	Bacillus spp.
	6.12.83	" " "	" "
	6.12.83	" " "	Micrococcus spp.
	8.12.83	" " "	Ent. sakazakii
	8.12.83	" " "	" "

TABLE 12 : Viable counts and details of micro-organisms isolated from feeds collected on the day of removal from the patient. All patients were receiving enteral feeds in the ITU of the Glasgow Western Infirmary

Patient No.	Date	Viable count cfu ml^{-1}	Organisms isolated	Antibiotic Sensitivity*
5	7.07.83	2.1×10^3	Acinetobacter calcoaceticus var anitratus	SXT / D1 | D4
			Staph. aureus	D1 / PPN | CN CTX
7	29.07.83	2.2×10^3	Micrococcoccus spp.	D1 | D4
			Staph. albus	E / D1 | D4
8	15.08.83	4.5×10^2	Acinetobacter calc. var anitratus	SXT / D1 | D4
			Streptococcus spp.	PN E SXT / D1 | D4 / CN
8	16.08.83	1.4×10^3	Bacillus spp.	D4 / E | D1
8	16.08.83	1.8×10^3	K. aerogenes	D4 / TIC | SXT CE / D1
			Streptococcus spp	PN E SXT / D1 | D4 / CN
8	17.08.83	2.0×10^3	Staph. albus	E / D1 | D4
9	4.09.83	4.8×10^2	Staph. albus	D1 | D4
11	8.12.83	2.3×10^3	Ent. sakazakii	SXT / D1 | D4
11	8.12.83	1.5×10^3	Ent. sakazakii	SXT / D1 | D4
14	13.12.83	7.3×10^2	Bacillus spp.	
14	16.12.83	6.8×10^2	Bacillus spp.	

*KEY

D1	Antibiotics	D4	Antibiotics
P	Penicillin G	CN	Gentamicin
PN	Ampicillin	PRL	Piperacillin
E	Erythromycin	TIC	Ticarcillin
SXT	Cotrimoxazole	MEZ	Mezlocillin
CB	Methicillin	CTX	Cefotaxime
CE	Cephradine	AZL	Azlocillin

Bacteria are sensitive to antibiotics above the line, resistant to those below.

tested except for erythromycin, was isolated from residual feed from patient 7 on 29.7.83 and three weeks later on 17.8.83 similar organisms were isolated from the residue of the feed given to patient 8. Acinetobacter calcoaceticus var. anitratus organisms with similar antibiotic sensitivity were isolated from the feed given to patient 5 on 7.7.83 and from patient 8, five weeks later on 15.8.83.

Faecal streptococci with similar antibiotic sensitivity were isolated from the residual feed of patient 8 on consecutive days (15.8.83 and 16.8.83) and Ent. sakazakii organisms with similar antibiotic sensitivity were isolated from two consecutive feeds given to patient 11 (8.12.83). In fact, the latter organism was isolated from residual feed in seven of the discarded sets from patient 11 over the period from 25.11.83 to 8.12.83 (Table 11). Ent. sakazakii was not isolated from any of the samples from the patient during this time.

Details of the organisms isolated from gastric aspirates and faeces are given in Table 13. In one instance the organism Cand. albicans was isolated from both the feed and the gastric aspirate of a patient (patient 1). Both the organisms isolated from the feed and those isolated from the patient were resistant to all the antibiotics tested except for nystatin, amphotericin B and flucytosine. Five months later Cand. albicans organisms with similar antibiotic sensitivity were isolated from stool samples from patient 10, but were not detected in any of the feed samples from this patient.

TABLE 13 : Micro-organisms isolated from patients receiving enteral feeds in the ITU of the Glasgow Western Infirmary between 9.6.83 and 19.12.83

Patient No.	Date	Sample	Micro-organisms isolated
1	16.05.83	gastric aspirate	Cand. albicans
	19.05.83	" "	" "
	23.05.83	" "	" "
	26.05.83	" "	" "
	30.05.83	" "	" "
	6.06.83	" "	" "
5	4.07.83	" "	yeasts
8	28.07.83	" "	lactose fermenting coliforms
	1.08.83	" "	Torulopsis glabrata
10	25.10.83	rectal swab	Streptococcus spp., Ps aeruginosa
	27.10.83	stool	Cand. albicans, Ps aeruginosa, Ent. cloacae
11	28.11.83	gastric aspirate	E. coli, Acinetobacter calcoaceticus var. anitratus.

CHAPTER 5

EXPERIMENTAL CONTAMINATION OF ENTERAL FEEDS

5.1 GROWTH OF MICRO-ORGANISMS IN ENTERAL FEEDS UNDER LABORATORY CONDITIONS

5.1.1 INTRODUCTION

The rapid multiplication of micro-organisms in enteral feeds has been reported by a number of authors (White *et al*. 1979, Furtado *et al*. 1980, Simmons 1981, Bastow *et al*. 1982, Gibbs 1983). However, both Furtado *et al*. (1980) and Stanek *et al*. (1983) demonstrated that not all enteral feeds support microbial growth. Stanek et al. (1983) reported that when samples of an elemental diet were artificially contaminated with 10^2 organisms ml^{-1} of *Staph. aureus*, *Salm. enteritidis*, *Y. enterocolitica*, *Ps aeruginosa*, *Camp. jejuni*, *Cand. albicans* or *Cand. tropicalis* and incubated at 22°C for 24h, only *Ps aeruginosa* and the *Candida* spp. showed any growth, the numbers of the remaining organisms either remaining constant or, in the case of *Camp. jejuni*, being reduced. Furtado *et al*. (1980) also noted that in full strength Vivonex HN, *Staph. aureus* and *Staph. epidermidis* remained viable but did not proliferate and that neither *Ps aeruginosa* nor group B streptococci survived when incubated at 37°C for 24h.

It would appear that more information is needed on the growth of micro-organisms in enteral feeds and, therefore, a study was carried out to compare the rates of growth in enteral feeds of a variety of commonly encountered bacterial contaminants.

5.1.2 MATERIALS AND METHODS

Strains and Inocula

Two strains of each of the following bacterial species were used: Staph. aureus (NCTC 10652, 10657), Ps aeruginosa (NCTC 6750, 10332), Ent. cloacae (NCTC 10005, 8155), E. coli (NCTC 8007, 8603) and K. aerogenes (NCTC 8172, 9997).

Bacteria were grown and maintained from the original freeze-dried cultures according to the procedure detailed in Lapage et al. (1970). They were stored on Nutrient Agar (Oxoid) slopes (Lapage, Shelton and Mitchell, 1970) and refrigerated at 4°C after growth at 37°C for 18h. Each slope culture was opened only four times and was discarded after four weeks. When required for inoculation a loopful of the appropriate culture was streaked onto Plate Count Agar (PCA, Oxoid) and incubated at 37°C for 24h. A single colony was then transferred to 10ml Nutrient Broth No. 2 (Oxoid) and incubated at 37°C for 18h.

Serial dilutions were made of each overnight culture using 9ml quantities of 0.1% (w/v) peptone water (Oxoid) and 1.0ml from the appropriate dilution was used as the inoculum.

Purity of the cultures was checked at the beginning and end of each experiment, samples being streaked onto appropriate selective media and Gram stained films prepared.

Enteral feeds

The feeds used were Clinifeed 400, a UHT processed, milk-based, nutritionally complete liquid feed (Roussel Laboratories Ltd; preliminary experiment only); Clinifeed ISO, a UHT processed, isocaloric, isotonic, milk-based nutritionally complete liquid feed (Roussel Laboratories Ltd.); Triosorbon, a dried low residue, whole protein, lactose-free feed (E. Merck Ltd.); Vivonex Standard, a dried, elemental, minimal residue preparation (Eaton Laboratories Ltd.); and Vivonex HN, a dried, elemental, high nitrogen, minimal residue preparation (Eaton Laboratories Ltd).

Cans and packets of feed were opened aseptically, as described in Ch. 3.2. Dried feeds were rehydrated according to the manufacturers' instructions but using sterile distilled water.

In order to minimise variation in the composition of the feed samples used for different bacteria, bulk samples of all the feeds were thoroughly mixed in separate sterile 2000ml conical flasks for each experiment, following which a 20ml sample was removed and the pH measured. Portions of feeds (100ml) were then transferred to sterile 250ml Erlenmeyer flasks. The composition, pH and osmolarity of each of the feeds are shown in Table 14.

Procedure

A preliminary experiment was carried out to compare the growth of E. coli (NCTC 8007) in a range of concentrations of Clinifeed 400.

TABLE 14 : Composition, pH and osmolarity of enteral feeds diluted to strengths suitable for administration to patients*

Feed	Composition (g 100 ml^{-1})				m osm l^{-1}	pH	Preparation of Feed
	Protein	Amino acids	Carbohydrate	Fat			
Clinifeed ISO	2.8	-	13.1	4.1	270	6.8	Liquid, no dilution
Triosorbon	4.0	-	11.9	4.0	238	6.9	1 sachet powder and water to volume of 400 ml
Vivonex Standard (half-strength)	-	1.03	11.5	0.08	265	5.4	1 sachet powder and water to volume of 600 ml
Vivonex Standard (full-strength)	-	2.06	23.0	0.15	550	5.4	1 sachet powder and water to volume of 300 ml
Vivonex HN	-	4.44	21.10	0.09	800	4.4	1 sachet powder and water to volume of 300 ml

* a) Values given are those quoted by the manufacturer except for pH which was determined experimentally.
b) Osmolarity of human serum = 289 m osm l^{-1}

Four concentrations of feed were prepared, namely, undiluted Clinifeed 400 (direct from can), full-strength Clinifeed 400 (prepared according to the manufacturers instructions; 75ml Clinifeed 400 plus 25ml water) and two further dilutions of Clinifeed 400 (50ml feed plus 50ml water and 25ml feed plus 75ml water).

Samples (100ml) of these feeds were transferred to sterile 250ml Erlenmeyer flasks and inoculated with E. coli to give an initial count of 10^2 - 10^3 cfu ml^{-1} feed (Bastow et al. 1982). The flasks were incubated at 25°C and samples taken for viable counts at 0, 2, 4, 8, 16 and 24h.

For the main experiments one ml of the appropriate dilution of each bacterial inoculum was transferred to each of 3 separate flasks containing 100ml of freshly prepared feed to give an initial count of 10^2 - 10^3 cfu ml^{-1} of feed (Bastow et al. 1982). After careful mixing a viable count was made on a sample of feed from each flask. Flasks were then incubated at 4, 25 or 37°C and samples taken for viable counts as described in Ch. 3.2 at 4, 8 and 24h. The experiment was repeated three times. Control experiments with uninoculated feeds were done simultaneously.

5.1.3. RESULTS

Effect of feed concentration on the growth of E. coli

The rate of growth of E. coli in a range of concentrations of Clinifeed 400 at 25°C is shown in Fig. 7. It can be seen that the rate of growth at all four concentrations tested was very similar. Numbers increased from an initial count of 10^2 organisms ml^{-1} to $10^{4.5}$ organisms ml^{-1} after 8h, 10^7-10^8 organisms ml^{-1} after 16h and 10^8-10^9 organisms ml^{-1} after 24h.

Growth of bacteria in a range of enteral feeds

Both strains of each of the bacterial species used, namely Staph. aureus, Ps aeruginosa, Ent. cloacae, E. coli and K. aerogenes grew at similar rates in all the experiments and the results given are the average counts obtained. The results for all the feeds except Triosorbon are presented in Figures 8-10. No organisms were detected in the control flasks for Clinifeed ISO, Vivonex Standard (full and half strength) or Vivonex HN. For each of the three experiments the control flasks of Triosorbon were found to be contaminated with aerobic sporeforming bacilli; the counts increased over 8h from $< 10^0$ to 10^1 at 25°C and 10^2 cfu ml^{-1} at 37°C. After 24h counts had risen to 10^7 and 10^9 cfu ml^{-1} at 25 and 37°C respectively.

The counts for all the test strains in all the feeds remained fairly constant at 10^2 - 10^3 ml^{-1} over 24h at 4°C (Fig. 8).

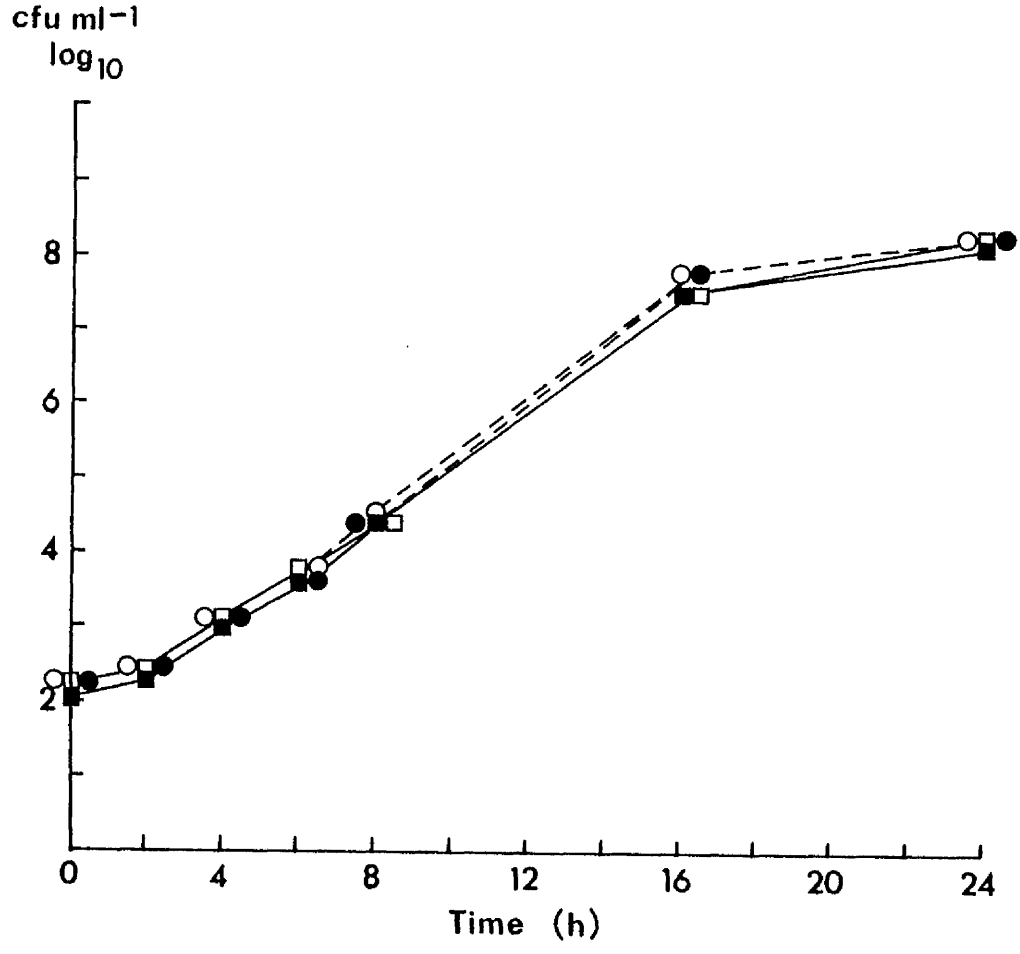


FIGURE 7 The growth of E. coli in a range of concentrations of Clinifeed 400 at 25°C (diluent = sterile water).

KEY

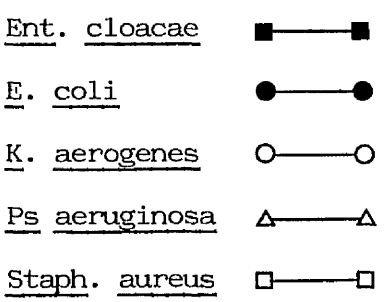
100% Clinifeed 400 ■——■

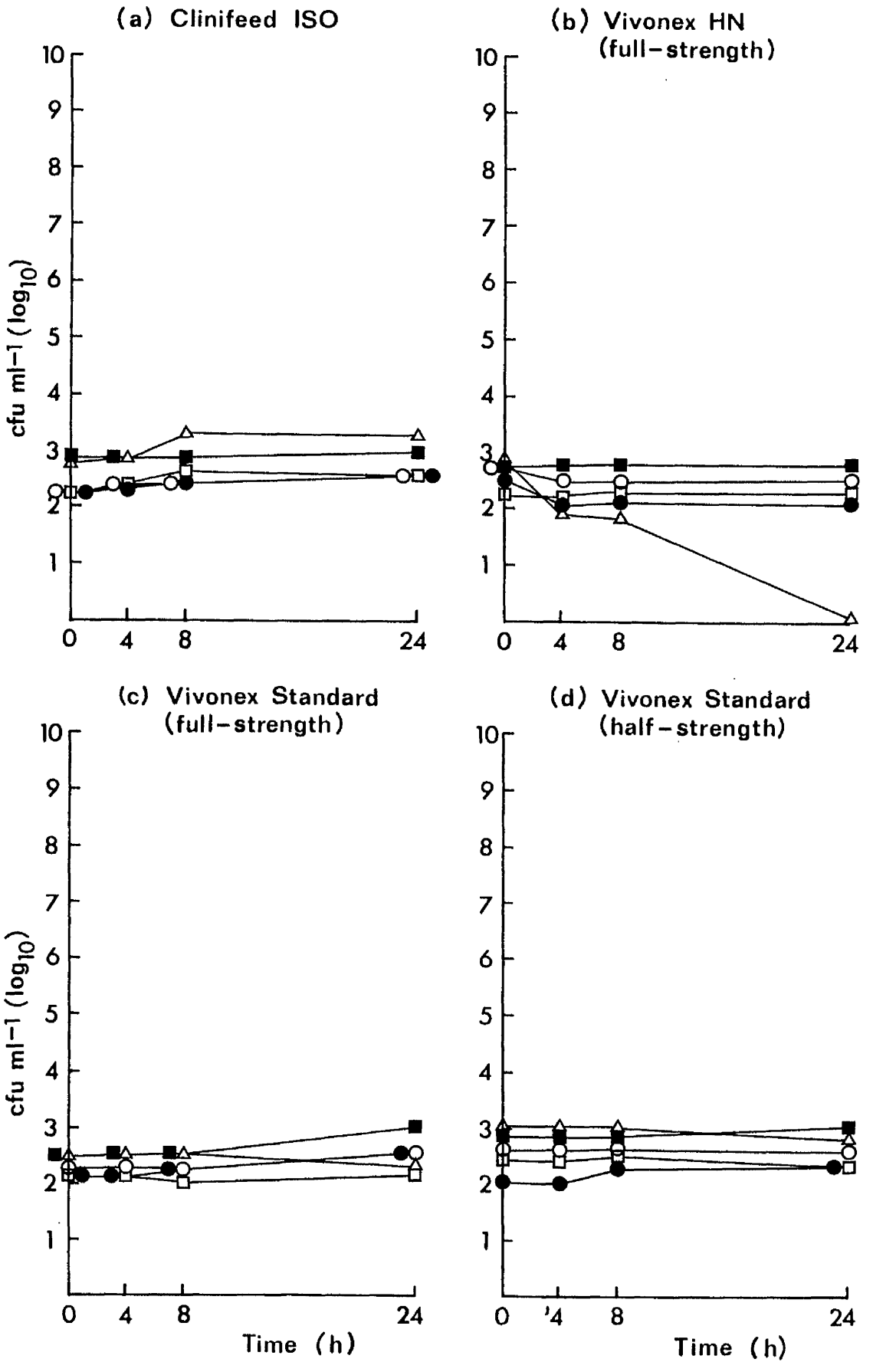
75% " " □——□

50% " " O- -O

25% " " ●- -●

FIGURE 8 Growth of various species of bacteria in Clinifeed ISO, Vivonex Standard and Vivonex HN at 4°C.




(a) Clinifeed ISO
(b) Vivonex HN
(full-strength)
(c) Vivonex Standard
(full-strength)
(d) Vivonex Standard
(half-strength)
cfu ml-1 (log10)
Time (h)
0
4
8
24
1
2
3
4
5
6
7
8
9
10

All test organisms multiplied rapidly in both Clinifeed ISO and Triosorbon at 25 and 37°C. The counts increased over 8h from 10^2 -10^3 to 10^4 - 10^5 at 25°C and to 10^5 - 10^6 cfu ml^{-1} at 37°C (Figs 9 and 10). It was difficult to make accurate counts of the numbers of test organisms in Triosorbon due to the growth of contaminants. After 24h, counts in Clinifeed ISO were 10^7 - 10^9 and 10^8 - 10^{10} cfu ml^{-1} at 25 and 37°C respectively; 24h viable counts for the test organisms were not made for Triosorbon due to the rapid growth of contaminants. A comparison of the effect of temperature on the rate of growth of all the test strains in Clinifeed ISO is presented in Fig. 11.

At 37°C the rate of growth of both strains of Ent. cloacae and K. aerogenes in half-strength Vivonex Standard was similar to that in Clinifeed ISO (Fig. 10). Growth of both strains of E. coli and Ps aeruginosa in half-strength Vivonex was slightly inhibited initially, only reaching 10^4 cfu ml^{-1} after 8h. However, growth was then more rapid, reaching 10^8 and 10^9 cfu ml^{-1} respectively after 24h. Half strength Vivonex inhibited the growth of both strains of Staph. aureus at both 25 and 37°C, there being very little increase in numbers after 8h and the final count at both temperatures being only 10^3 cfu ml^{-1} (Figs 9 and 10). Growth of all the remaining test organisms was slower at 25°C in half-strength Vivonex than in Clinifeed ISO, counts after 8h ranging from 10^3 - 10^4 and those at 24h from 10^6 - 10^8 cfu ml^{-1} (Fig. 9).

FIGURE 9 Growth of various species of bacteria in Clinifeed ISO, Vivonex Standard and Vivonex HN at 25°C.

For Key to symbols see Fig. 8 (page 76b)

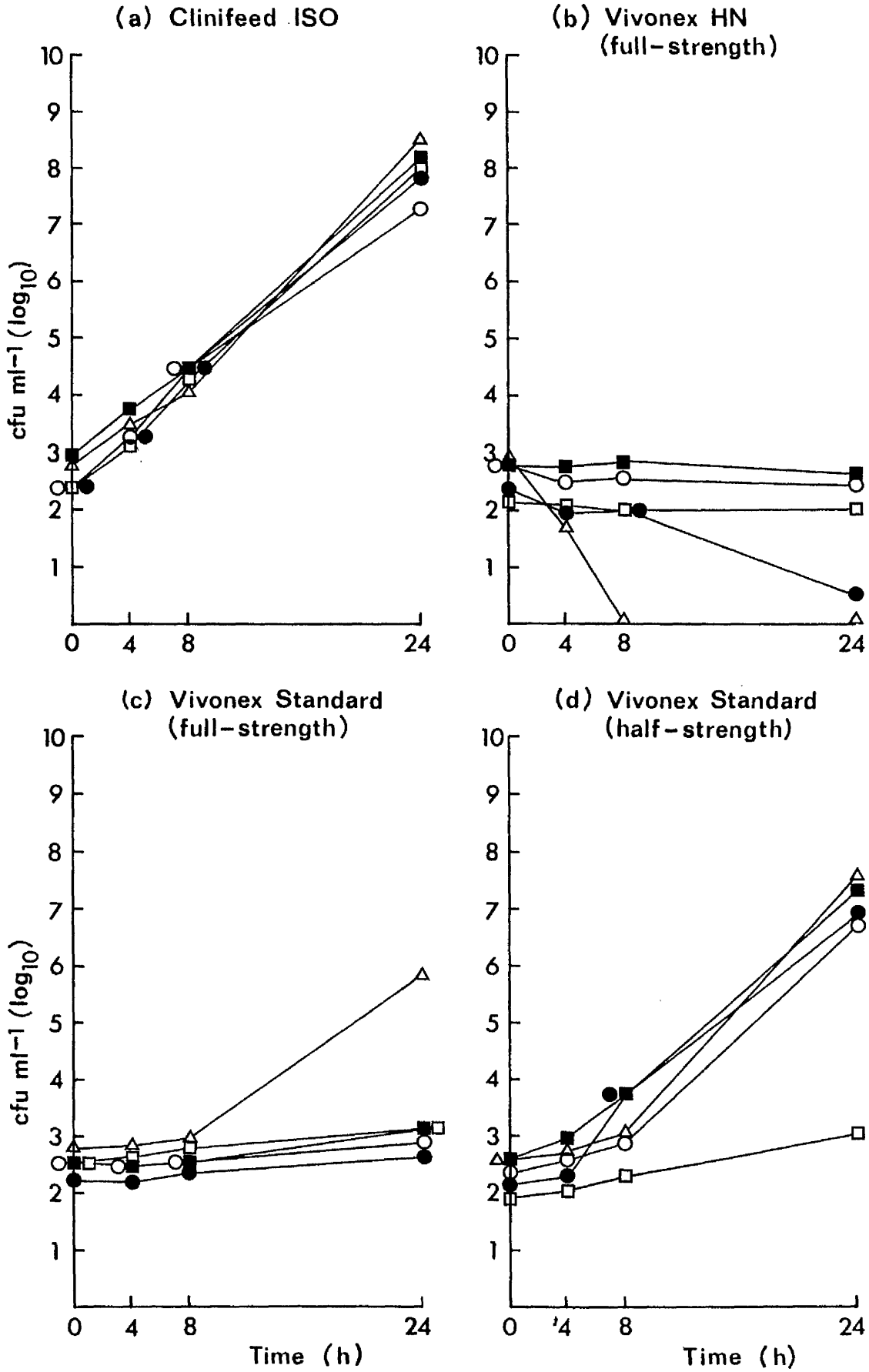
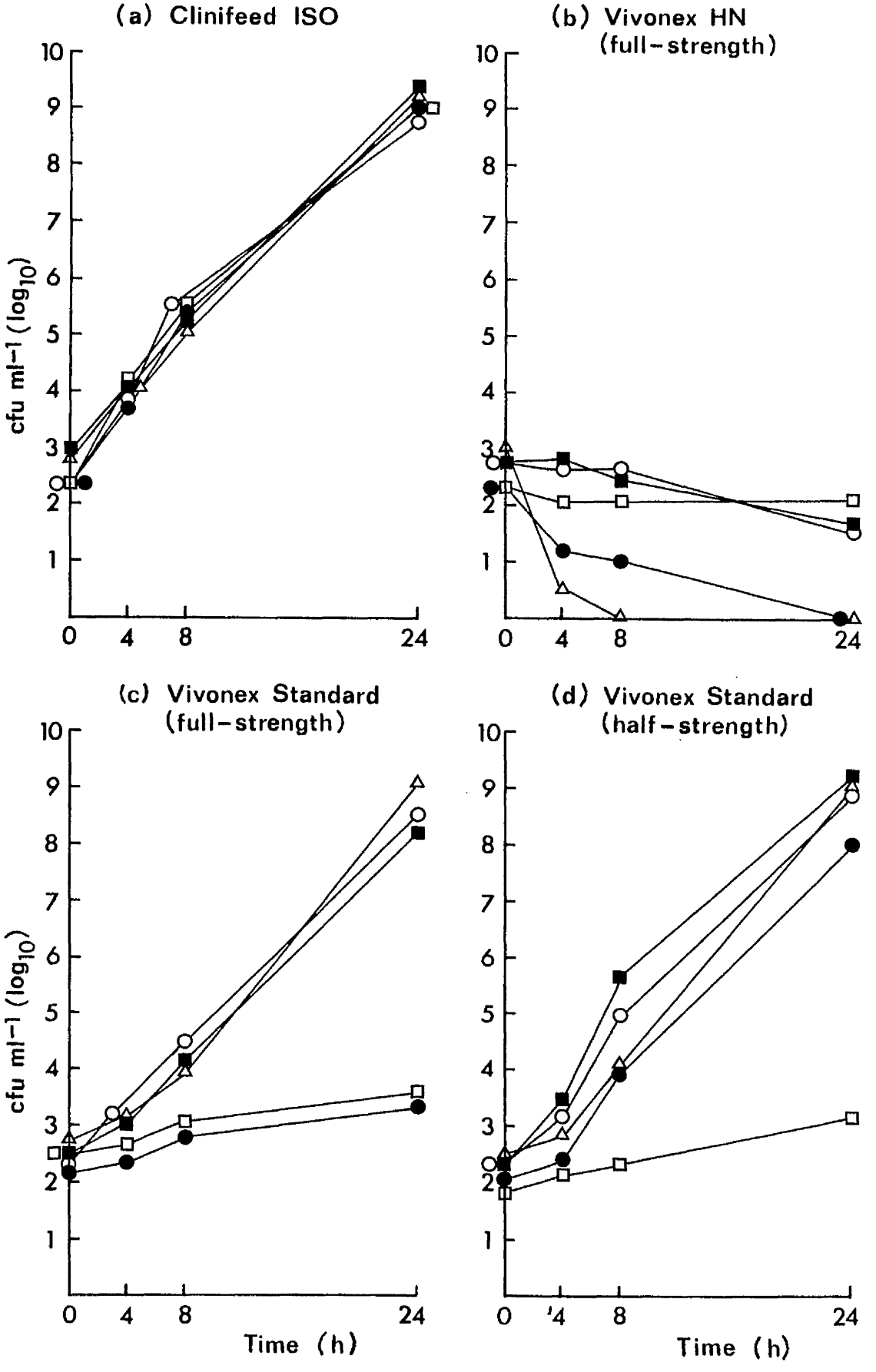

(a) Clinifeed ISO
(b) Vivonex HN
(full-strength)
(c) Vivonex Standard
(full-strength)
(d) Vivonex Standard
(half-strength)
cfu ml-1 (log10)
Time (h)
0
4
8
24
1
2
3
4
5
6
7
8
9
10

FIGURE 10 Growth of various species of bacteria in Clinifeed ISO, Vivonex Standard, and Vivonex HN at 37°C.

For Key to symbols see Fig. 8 (page 76b).


(a) Clinifeed ISO
(b) Vivonex HN
(full-strength)
(c) Vivonex Standard
(full-strength)
(d) Vivonex Standard
(half-strength)
cfu ml⁻¹ (log10)
Time (h)
0
4
8
24
1
2
3
4
5
6
7
8
9
10

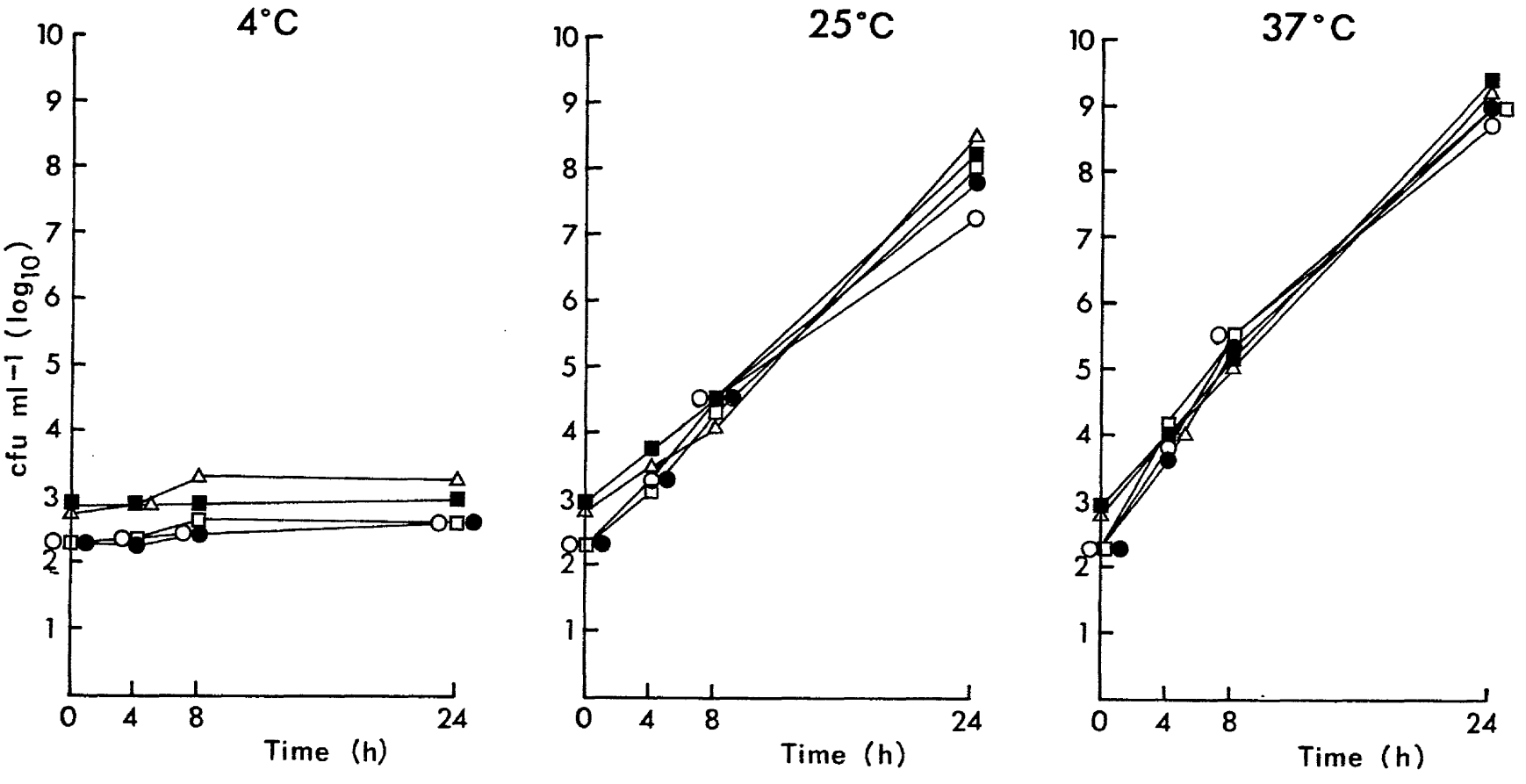


FIGURE 11 Effect of temperature on the rate of growth of various species of bacteria in Clinifeed ISO. For Key to symbols see Fig. 8 (page 76b).

In full-strength Vivonex Standard the growth of all the test organisms was inhibited at 25°C (Fig. 9), there being no increase in numbers after 8h or 24h with the exception of Ps aeruginosa which showed an increase in numbers from 10^3 to 10^6 cfu ml^{-1} between 8 and 24h. Both Staph. aureus and E. coli were also inhibited at 37°C, numbers only increasing from 10^2 to 10^3 - 10^5 cfu ml^{-1} in 24h (Fig. 10). However, the remaining organisms, Ps aeruginosa, K. aerogenes and Ent. cloacae multiplied to give counts of 10^4 - 10^5 after 8h and 10^8 -10^9 cfu ml^{-1} after 24h.

None of the test organisms multiplied in full strength Vivonex HN (Figs 8, 9 and 10). Counts for both strains of Ps aeruginosa decreased from 10^2 to 10^1 cfu ml^{-1} at all three temperatures after 4h. Counts remained at this level after 8h at 4°C but after 8h at 25 and 37°C and after 24h at 4°C all counts were $<10^0$ cfu ml^{-1}. There was no change in the numbers of Staph. aureus over 24h, counts remaining at 10^2 cfu ml^{-1} at 4, 25 and 37°C. E. coli survived for 24h at 4°C but showed a decrease from 10^2 to 10^1 after 8h and to 10^0 cfu ml^{-1} after 24h at 25 and 37°C. Strains of K. aerogenes and Ent. cloacae survived for 24h at 4° and 25°C, but decreased from 10^2 to 10^1 cfu ml^{-1} after 24h.

5.2 GROWTH OF MICRO-ORGANISMS IN ENTERAL FEEDS UNDER SIMULATED WARD CONDITIONS

5.2.1 INTRODUCTION

In a preliminary study of the growth of bacteria in enteral feeds, White *et al.* (1979) inoculated a commercial feed with approximately one *Staph. aureus* organism ml^{-1} and obtained, after 24h at 37°C, a viable count of 8.2×10^5 organisms ml^{-1}. Simmons (1981) inoculated 20ml portions of a hospital-prepared feed with 1×10^3 *Staph. aureus* or *E. coli* ml^{-1} and reported that the counts exceeded 1×10^5 ml^{-1} after 8h at 23°C. Although it is evident that bacteria grow in enteral feeds there appears to be little published information on the actual rate of growth of potential food poisoning organisms and/or opportunistic pathogens under hospital ward conditions except for the report of Bastow *et al.* (1982) that after 24h at ward temperature the number of organisms in hospital prepared feeds had increased from $10^2 - 10^3$ to $10^8 - 10^{10}$ ml^{-1}.

The aim of this part of the study was, therefore, to examine the growth of *E. coli* and *Staph. aureus* in feeding systems under simulated ward conditions including a comparison of the growth of the test organisms in Clinifeed ISO and Nutrient Broth No.2 and investigations into the effects of refilling or replacing laboratory contaminated systems over a period of 24h.

5.2.2 MATERIALS AND METHODS

Strains and inocula

Staph. aureus strain S-6 (NCTC 10657) and *E. coli* (NCTC 8007) were used. Inocula were prepared from stock cultures as described in Ch. 5.1.2 and 1.0ml volumes of the appropriate dilution aseptically added to 1000ml sterile enteral feed or bacteriological growth medium in two types of nutrient container, a sterile Clinifeeding System 3 reservoir (Roussel Laboratories Ltd., London) or a sterile Winchester bottle (D.H.S.S. Code 4169) to yield an initial viable count of approximately 10^2 cfu ml^{-1}

Enteral feed and bacteriological growth medium

The enteral feed used was Clinifeed ISO. The top of each can of Clinifeed ISO was swabbed with 70% ethanol, flamed prior to opening and the contents aseptically transferred to the sterile Clinifeeding System 3 reservoir or Winchester bottle.

The bacteriological growth medium used was Nutrient Broth No. 2 (Oxoid). One litre portions were dispensed in the Winchester bottles and autoclaved at 121°C for 15min. The Clinifeeding System 3 reservoirs were filled aseptically with 1l of sterile Nutrient Broth No.2.

Apparatus

Two types of enteral feeding system were used : System (a) the complete sterile Clinifeeding System 3 (Roussel) and System (b) a Winchester bottle connected to a Clinifeeding System 2.

The experimental feeding systems were suspended in a 25°C incubator room and the giving sets were passed to the adjacent laboratory through narrow portholes in the wall. After removal of the sterile cover from the female luer connector, each giving set was connected to the male connector of a fine-bore naso-gastric tube (Clinifeeding System 1, Roussel) with its outlet in a sterile 1500ml flask held in a 37°C covered water bath (Fig. 12). This experimental arrangement was designed to simulate the temperature conditions of the nutrient container in the ward and the naso-gastric tube in the patient (Maki 1976). The flow rate of the feed or medium was approximately 125ml h^{-1}, equivalent to that found under ward conditions (Bastow et al. 1982).

Comparison of growth of bacteria in an enteral feed and a bacteriological growth medium in two types of enteral feeding system

Three reservoirs of System (a) containing Clinifeed ISO were inoculated with known quantities of either E. coli or Staph. aureus or left uninoculated to act as controls. Viable counts were made on samples of Clinifeed ISO taken immediately after flow commenced and at regular intervals during the 8h experiment. The experiment was repeated with System (a) plus Nutrient Broth No.2 and then the whole experiment repeated using System (b). Sampling points for System (a) are shown in Fig. 12, namely the Clinifeeding System 3 reservoir, the outlet of the fine-bore naso-gastric tube and the flask. Reservoir samples were obtained with 1.0ml sterile disposable syringes and

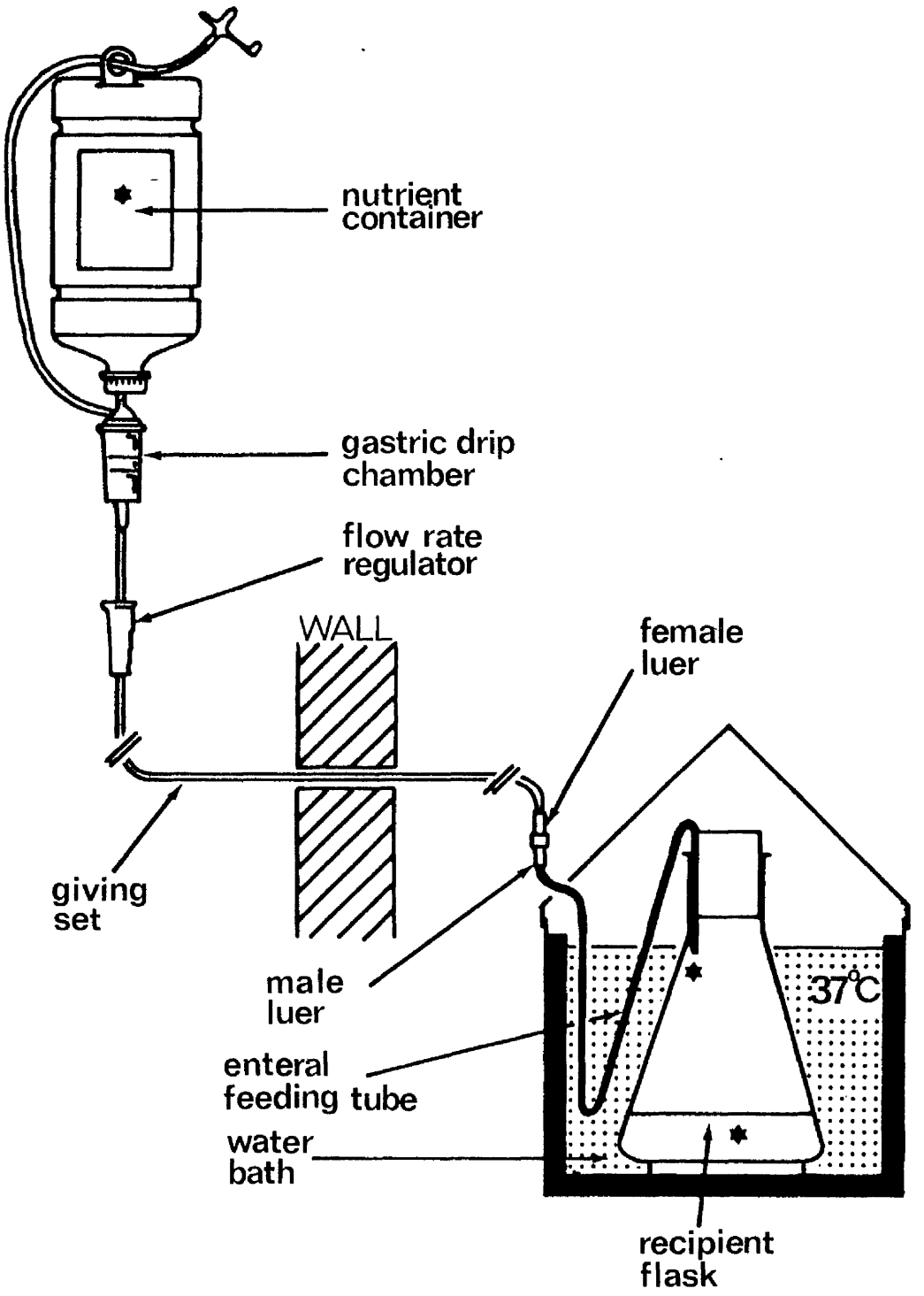


FIGURE 12 The experimental apparatus used to measure growth of Staph. aureus and E. coli in enteral feeds. The asterisks mark the sampling points.

needles, the surface of the reservoir being swabbed with 70% ethanol prior to insertion of the needle and the puncture hole covered with sterile zinc oxide tape. Sample points for System (b) were the outlet of the fine-bore naso-gastric tube and the flask. To prevent further possible contamination, the Winchester bottle was not disconnected. This and each of the following experiments were done three times.

Effect of handling procedures

For each of the following experiments, the enteral feeding system used was System (a). The enteral feed used was Clinifeed ISO and the organisms were Staph. aureus (NCTC 10657) and E. coli (NCTC 8007). One ml of the appropriate dilution of each bacterial inoculum was added to each sterile Clinifeeding System 3 reservoir, and 1000ml Clinifeed ISO added to give an initial count of 10^2 - 10^3 cfu ml^{-1}. Samples for viable counts were taken immediately after the flow commenced and at regular intervals throughout the experiment. In the following experiments the naso-gastric tube (Clinifeeding System 1) was replaced with the naso-enteric tube (Clinifeeding System 1/85).

a) Changing the nutrient container

In order to determine the influence of a change of nutrient container under ward conditions, the contaminated Clinifeeding System 3 reservoir was replaced with a sterile reservoir containing 1l of sterile Clinifeed ISO at 8 and 16h. At each reservoir change the recipient flask was also replaced. Viable counts were made as before.

b) Refilling the nutrient container

It sometimes happens on a ward that the nutrient container is not replaced but is refilled twice over a 24h period. To imitate this, the empty Clinifeeding System 3 reservoir was removed at 8 and 16h and a sterile cap was placed temporarily over the connector spike. One litre of sterile Clinifeed ISO was aseptically transferred to the reservoir which was immediately refitted to the system. Viable counts were made as before.

c) Changing the nutrient container and giving set

The recommended practice is to disconnect the empty nutrient container and giving set leaving only the enteral feeding tube in situ. This procedure was adopted and the initial contaminated Clinifeeding System 3 reservoir and giving set were replaced at 8 and 16h. Viable counts were made as before.

d) Stopping the flow of feed through the system

An experiment was conducted to simulate the effect of flow of feed through the system being stopped for a short period of time. The organism used was Staph. aureus (NCTC 10657). There was only one change of reservoir (at 8h) and at 12h the enteral feeding pump was switched off and the flow of feed stopped with feed still in the system. The flow was started again at 14h and viable counts were made at 14 and 16h.

e) Varying the initial level of contamination

Clinifeeding System 3 reservoirs were experimentally contaminated with either E. coli (NCTC 8007) or Staph. aureus (NCTC 10657) to give initial counts ranging from 10^1 to 10^5 cfu l^{-1} (10^{-2} - 10^2 cfu ml^{-1}). Viable counts were made on the original inoculum and 1, 0.5 and 0.25 ml amounts of the appropriate serial tenfold dilution were added to 1000ml amounts of sterile Clinifeed ISO in the reservoirs of System (a). For Staph. aureus the approximate number of colony-forming units in the 1000ml of Clinifeed ISO in the reservoir at the start of each experiment were :

i)	1 x 10^5	(i.e.	100	cfu ml^{-1})
ii)	5 x 10^4	(i.e.	50	cfu ml^{-1})
iii)	2.5 x 10^4	(i.e.	25	cfu ml^{-1})
iv)	5 x 10^3	(i.e.	5	cfu ml^{-1})
v)	1 x 10^3	(i.e.	1	cfu ml^{-1})
vi)	1 x 10^2	(i.e.	0.1	cfu ml^{-1})
vii)	1 x 10^1	(i.e.	0.01	cfu ml^{-1})

For E. coli the numbers were

i)	1 x 10^5	(i.e.	100	cfu ml^{-1})
ii)	1 x 10^4	(i.e.	10	cfu ml^{-1})
iii)	1 x 10^3	(i.e.	1	cfu ml^{-1})
iv)	1 x 10^2	(i.e.	0.1	cfu ml^{-1})

The systems were run for 16h, the contaminated reservoir being replaced with a sterile reservoir containing 1000ml sterile Clinifeed ISO at 8h. Viable counts were made every two hours from 0 to 16h.

5.2.3 RESULTS

Comparison of the growth of E. coli and Staph. aureus in Clinifeed ISO and Nutrient Broth No.2 in two types of enteral feeding system

Results of the experiments are presented in Figs 13 and 14. No organisms were detected in any of the control experiments. Both E. coli and Staph. aureus multiplied rapidly in both feeding systems and in both Clinifeed ISO and Nutrient Broth No.2. Counts for both organisms increased over 8h from 10^2 to 10^4 -10^5 cfu ml^{-1} in the System (a) reservoirs (Clinifeeding System 3) and from 10^2 to 10^7 -10^8 cfu ml^{-1} in the recipient flasks. The similarity between the growth curves for both E. coli and Staph. aureus in Clinifeed ISO and Nutrient Broth No.2 show that Clinifeed ISO is an excellent culture medium for bacteria.

The multiplication of both E. coli and Staph. aureus in the nutrient containers of both System (a) and System (b) led to the recipient flasks ('patients') receiving a steadily increasing dose of bacteria in the feed. Over the 8h of this experiment it was found that counts on samples from the reservoir and the end of the fine-bore tube of System (a) were very similar (Figs 13 and 14).

Effect of handling procedures

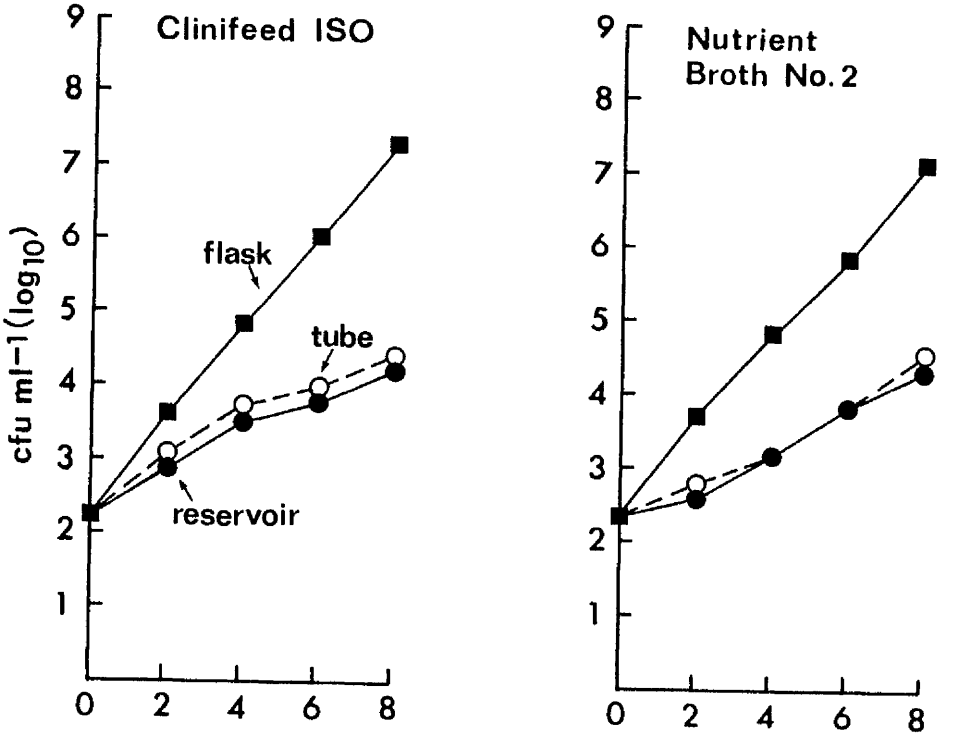
a) Changing the nutrient container

Results of the experiments are presented in Figs 15, 16 and Appendix E (Figs E1 and E2). No organisms were detected in any of the control experiments.

FIGURE 13 Comparison of the growth of Staph. aureus and E. coli in Clinifeed ISO and Nutrient Broth No. 2 in System (a) over 8h.

Samples were taken from the recipient flask (■———■), the Clinifeeding System 3 reservoir (nutrient container) (●———●) and the enteral feeding tube (O- - -O).

Staph. aureus



E. coli

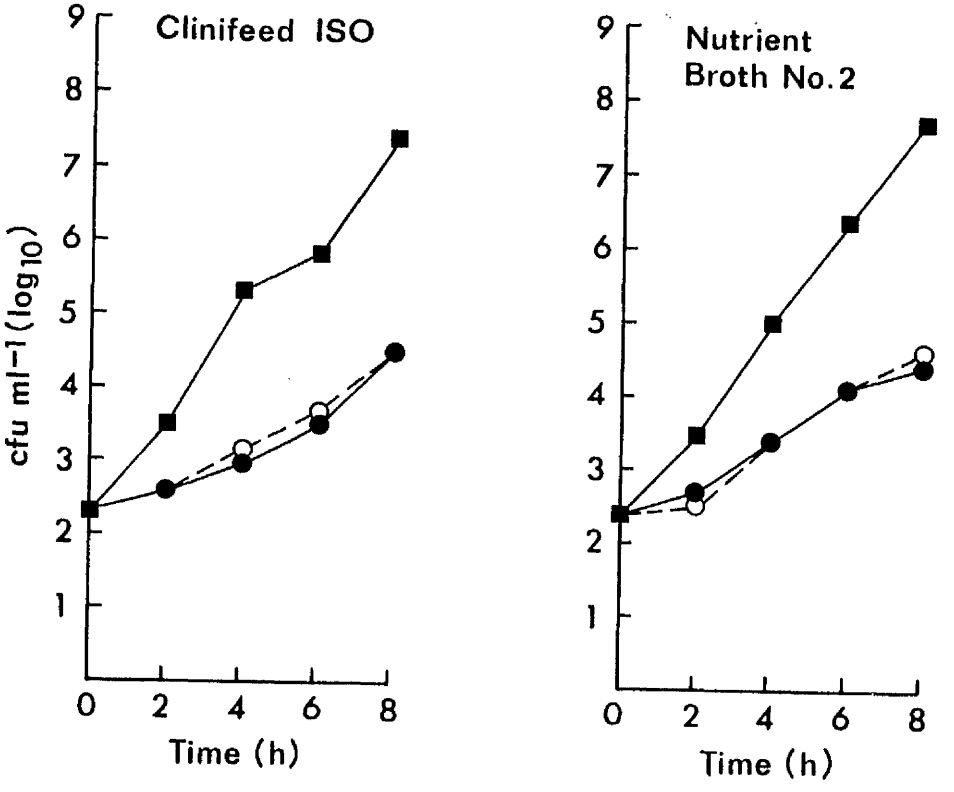
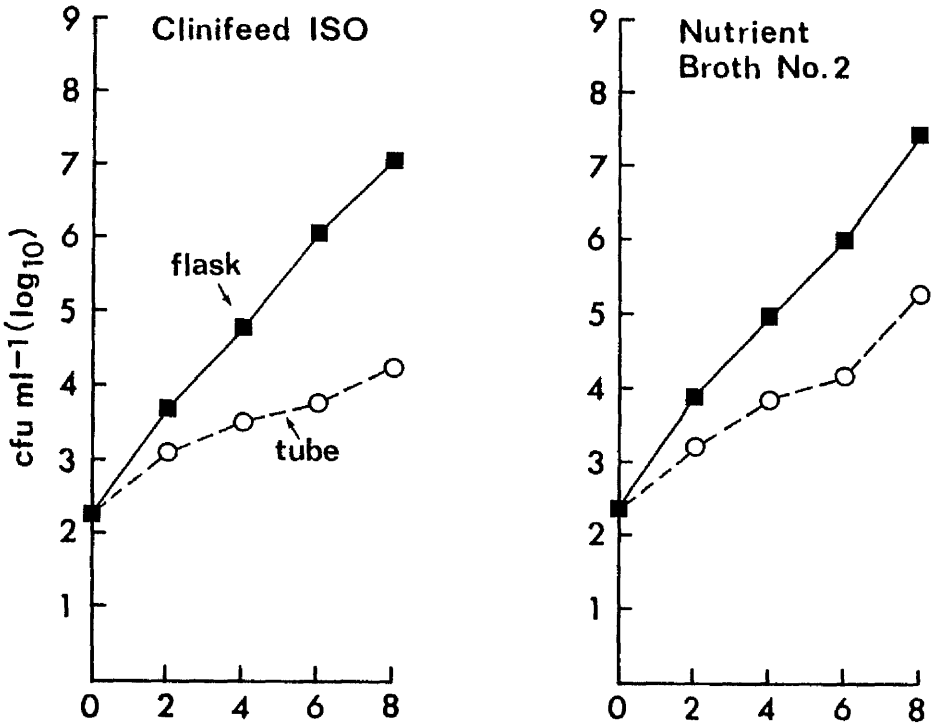


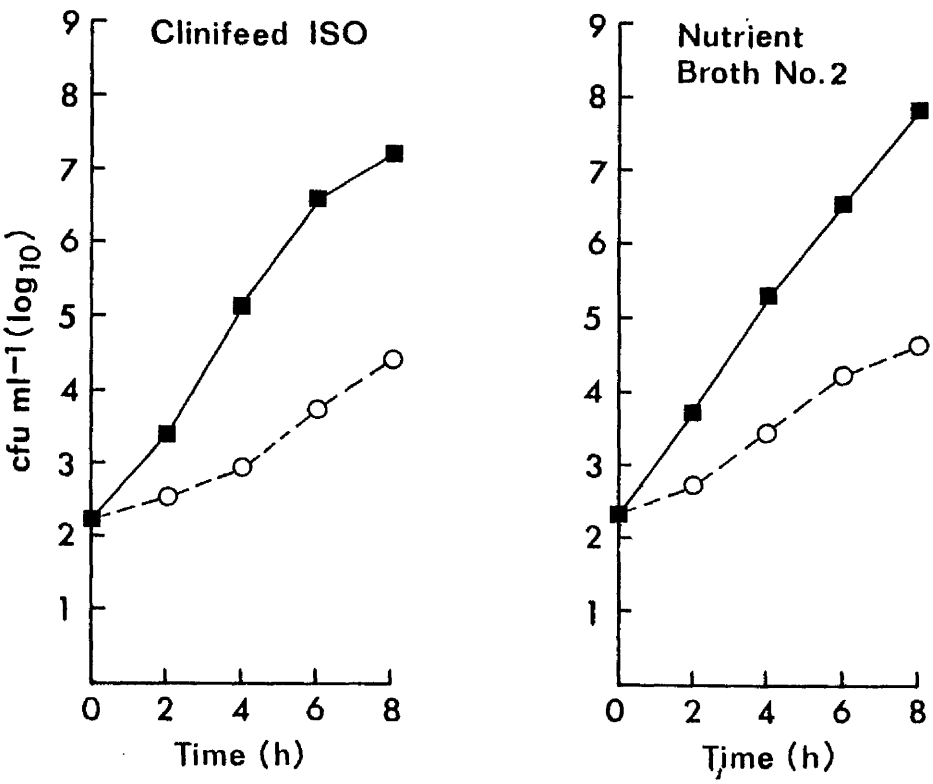
FIGURE 14 Comparison of the growth of Staph. aureus and E. coli in Clinifeed ISO and Nutrient Broth No. 2 in System (b) over 8h.

Samples were taken from the recipient flask (■———■) and the enteral feeding tube (O– – –O).

Staph. aureus



E. coli



Samples of feed withdrawn from the 8h replacement Clinifeeding System 3 reservoir immediately after it had been connected to the system yielded viable counts of approximately 10^1 cfu ml^{-1} for E. coli (Fig. 15) and $10^{0.5}$ cfu ml^{-1} for Staph. aureus (Fig. 16). After another 8h these counts had risen to 10^4 cfu ml^{-1} for E. coli and 10^3 cfu ml^{-1} for Staph. aureus. Replacement of this second reservoir with a new sterile reservoir at 16h resulted in immediate counts of $< 10^0$ cfu ml^{-1} in all samples of feed. However, 8h later (i.e. 24h from the original start of the experiment) multiplication of these organisms resulted in counts of 10^2 - 10^3 cfu ml^{-1} E. coli or Staph. aureus in the feed in the reservoir.

The number of organisms entering the "patient's" stomach or intestine is illustrated by the counts made on the feed collected from the end of the fine-bore tube. Over the first 8h the counts were similar to those obtained for the reservoir. However, for the samples taken from the fine-bore tubes immediately after the reservoir had been replaced at 8h and 16h, the counts were often higher than those obtained from samples taken prior to the change of reservoir. Since the reservoir and giving set were always empty at the time of changeover, the increase in bacterial numbers could be interpreted as being due to residual organisms in the giving set and fine-bore tube being washed through by the initial flow of liquid. This is further demonstrated by the fact that the counts for the next set of samples taken 2h after the reservoirs had been changed were always lower. A comparison of the counts in the samples of feed taken from the end of

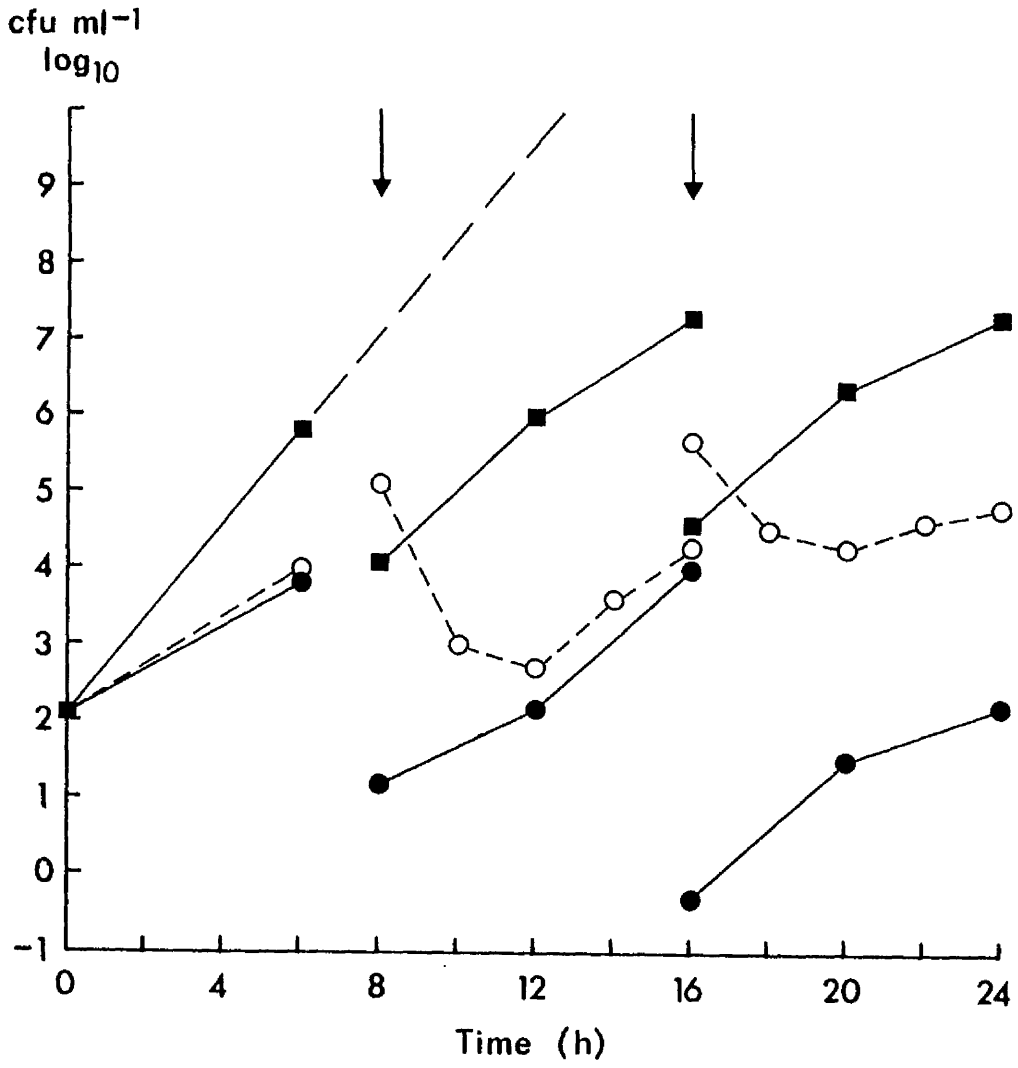


FIGURE 15 The effect of replacing the contaminated Clinifeeding System 3 reservoir (nutrient container) with a new reservoir containing sterile feed (Clinifeed ISO), on the growth of E. coli in the system.

The reservoir was changed at 8 and 16h (↓).

Samples were taken from the recipient flask (■———■), the reservoir (●———●) and the enteral feeding tube (O- - -O). The extended hatched line for the recipient flask indicates the predicted cumulative bacterial biomass.

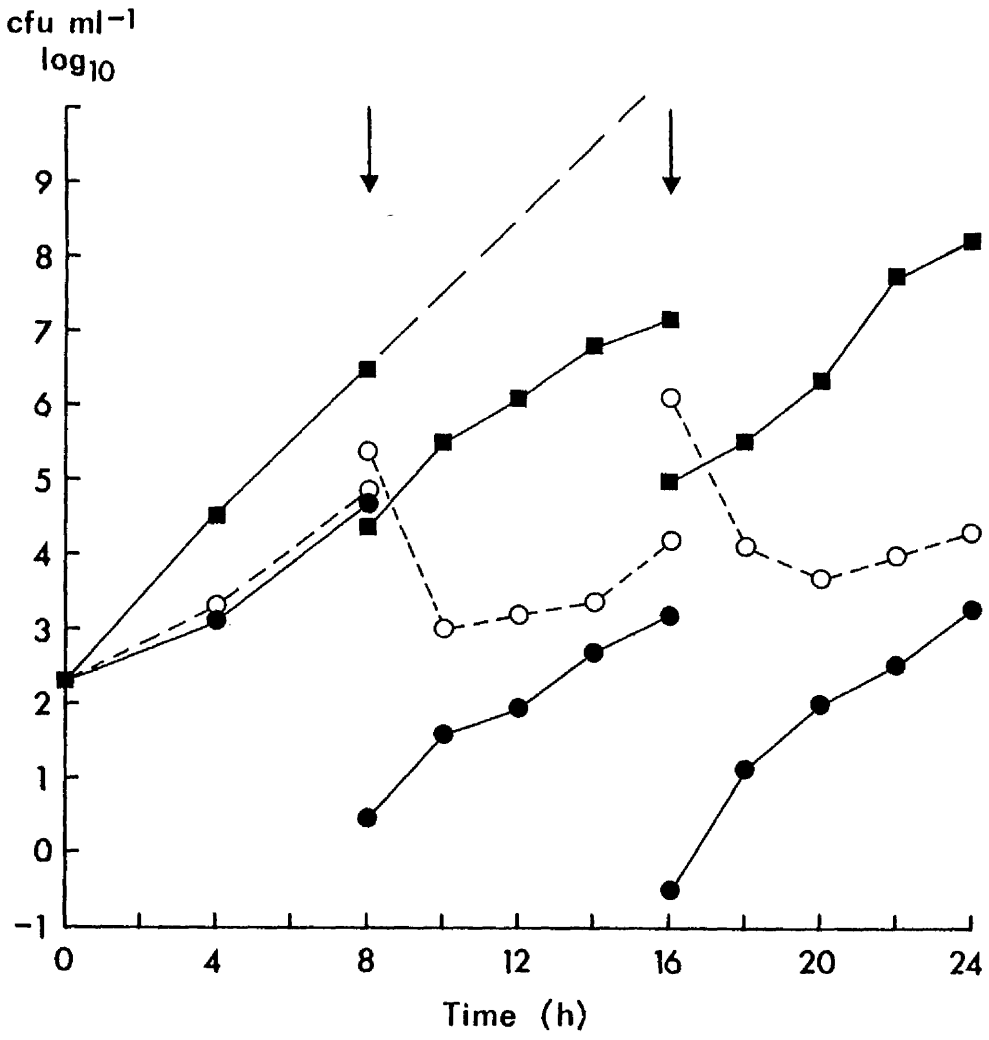


FIGURE 16 The effect of replacing the contaminated Clinifeeding System 3 reservoir (nutrient container) with a new reservoir containing sterile feed (Clinifeed ISO), on the growth of Staph. aureus in the system.

The reservoir was changed at 8 and 16h (↓).

For Key to symbols see Fig. 15 (page 86a).

the fine-bore tube and in the samples of feed from the reservoir also suggests that possibly organisms were multiplying in the fine-bore tube.

The samples of feed collected from the end of the fine-bore tube were contaminated with $\geqslant 10^2$ cfu ml^{-1} from 8h onwards, numbers reaching 10^4 ml^{-1} and $10^{4.5}$ ml^{-1} prior to replacement of the reservoirs at 16h and 24h for both E. coli and Staph. aureus.

The recipient flask was renewed at the time of changeover to avoid self-limitation of growth by large bacterial numbers, however, it should be noted that for both E. coli and Staph. aureus after 16h and 24h there were still $\geqslant 10^7$ cfu ml^{-1} in the feed in the flask.

b) Refilling the nutrient container

Results of these experiments are presented in Figs 17, 18 and Appendix E (Figs E3 and E4). No organisms were detected in the control experiments.

As in the previous experiments the counts for E. coli and Staph. aureus in the Clinifeeding System 3 reservoir and flask increased from 10^2 to 10^4 and 10^2 to 10^7 cfu ml^{-1} in the first 8h. However, the samples taken immediately after refilling the reservoir at 8h demonstrate that the residue of contaminated feed in the reservoir resulted in initial viable counts of 10^3 - 10^4 cfu ml^{-1} for both E. coli and Staph. aureus, the count increasing to 10^6 - 10^7 cfu ml^{-1} at 16h. Similarly, at 16h the initial viable count of the feed in the

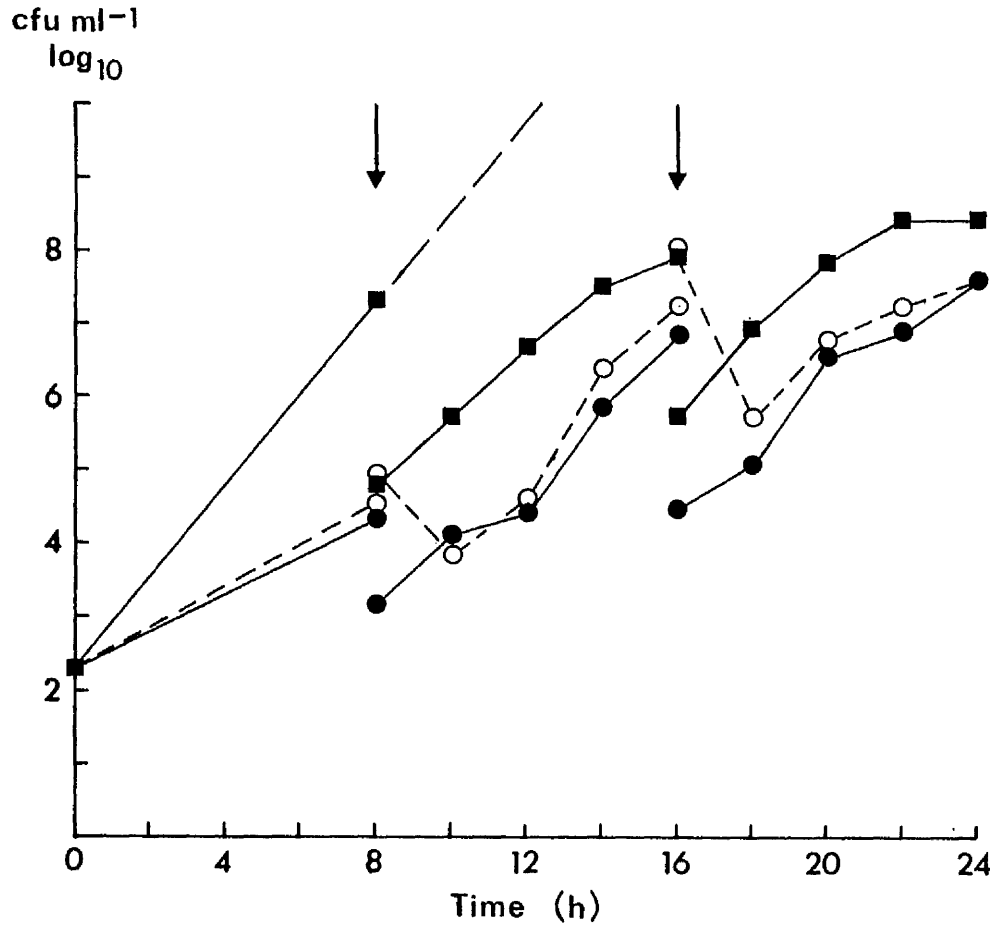


FIGURE 17 The effect of refilling the contaminated Clinifeeding System 3 reservoir (nutrient container) with sterile feed (Clinifeed ISO) on the growth of E. coli in the system. The reservoir was refilled at 8 and 16h (↓).

For Key to symbols see Fig. 15 (page 86a).

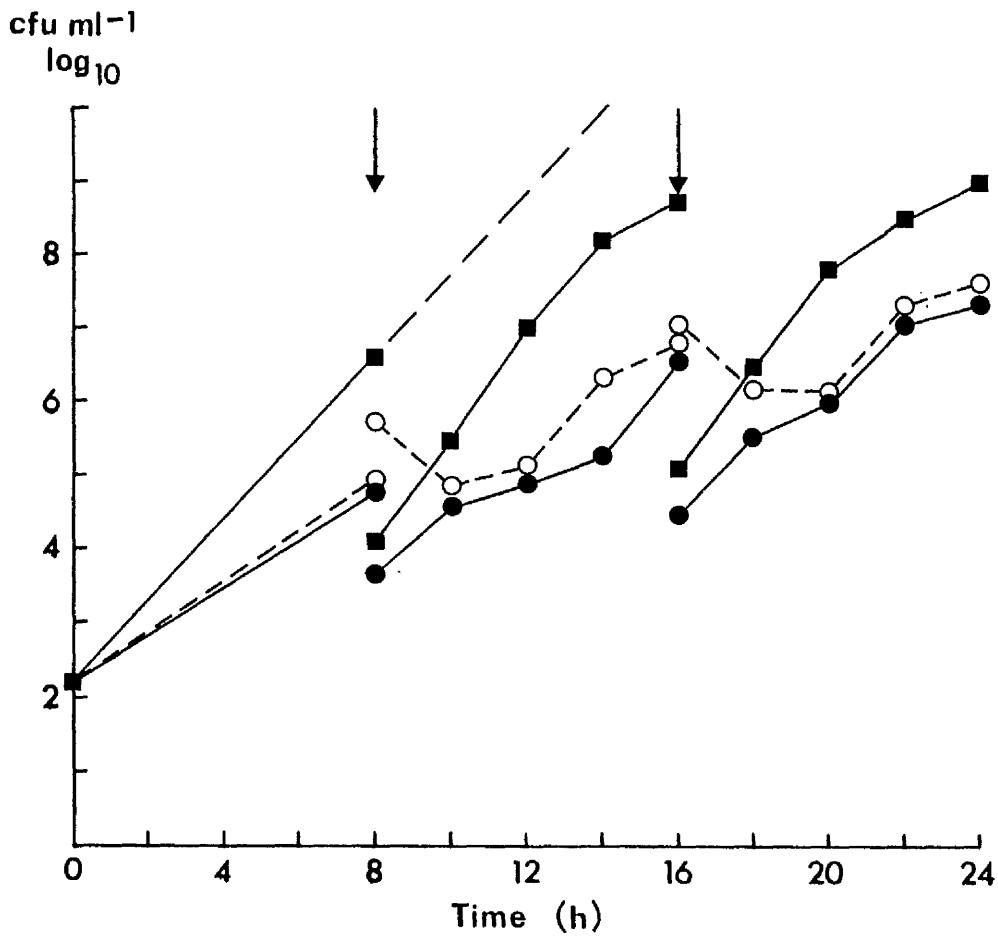


FIGURE 18 The effect of refilling the contaminated Clinifeeding System 3 reservoir (nutrient container) with sterile feed (Clinifeed ISO) on the growth of Staph. aureus in the system. The reservoir was refilled at 8 and 16h (↓).

For Key to symbols see Fig. 15 (page 86a).

refilled reservoir was 10^4 -10^5 cfu ml^{-1} for E. coli and Staph. aureus, increasing to $\geqslant 10^7$ cfu ml^{-1} for both organisms at 24h.

The feed collected from the end of the fine-bore tube was heavily contaminated from 8h onwards, and the initial wash through of residual organisms was again obvious. It is important to note that after 8h the viable count of the feed entering the patient did not fall much below 10^5 cfu ml^{-1} for either organism and after 24h the counts were in excess of 10^7 cfu ml^{-1}. Counts in the recipient flask were also at a high level from 8h onwards.

c) Changing the nutrient container and giving set

Results of the experiments are presented in Figs 19, 20 and Appendix E (Figs E5 and E6). No organisms were detected in the control experiments and there was no contamination of the new sterile nutrient container.

The results show that when the Clinifeeding System 3 reservoir and giving set were replaced at 8 and 16h viable organisms continued to be detected in the feed flowing from the end of the fine-bore tube. Once again there was an initial wash-through of organisms at 8 and 16h when the system was changed. This was followed by a fall in numbers at 10 and 18h, but subsequent to this there was a gradual increase in numbers to 10^2 -10^3 cfu ml^{-1} for both E. coli and Staph. aureus at 24h.

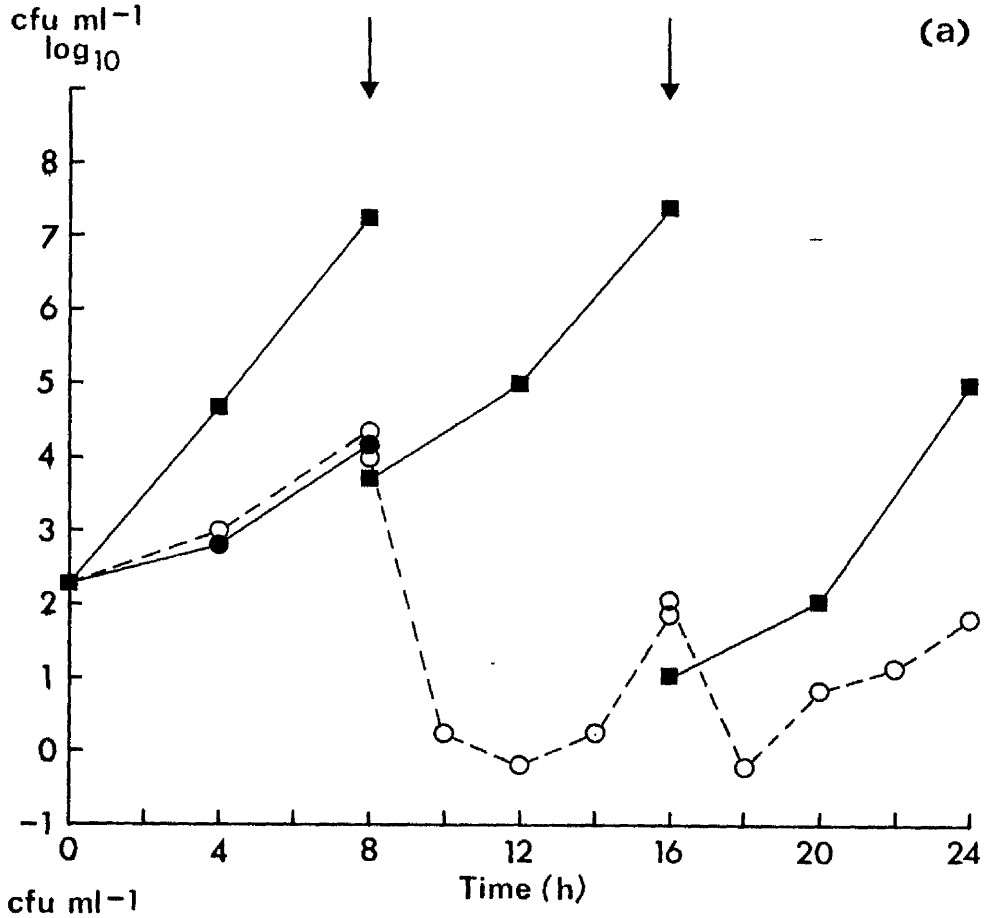
FIGURE 19a The effect of replacing the Clinifeeding System 3 reservoir (nutrient container), the feed and the giving set, on the growth of E. coli in the system.

The reservoir and giving set were changed at 8 and 16h (↓).

No organisms were detected in the reservoir after 8h.

For Key to symbols see Fig. 15 (page 86a).

FIGURE 19b As above except that after 8h samples were only collected from the enteral feeding tube to minimize the risk of contamination due to handling procedures.


cfu ml⁻¹
log10
(a)
8
7
6
5
4
3
2
1
0
-1
0
4
8
12
16
20
24
Time (h)

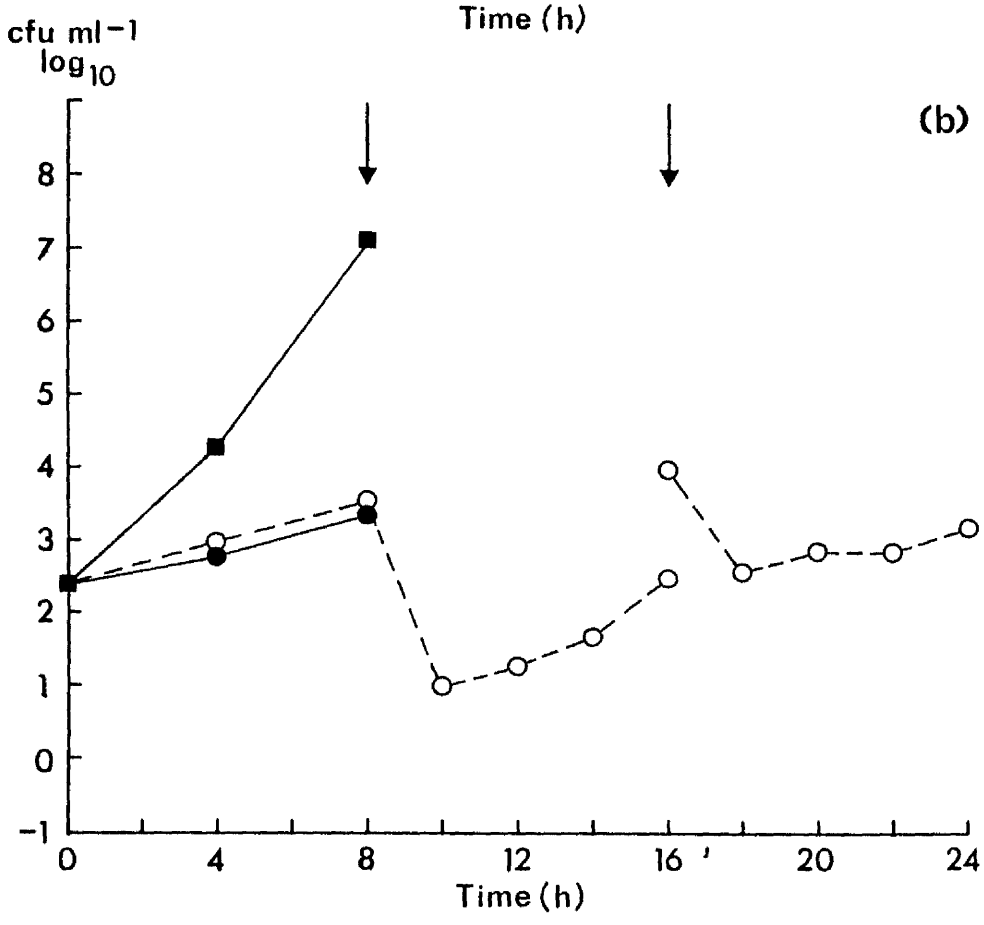
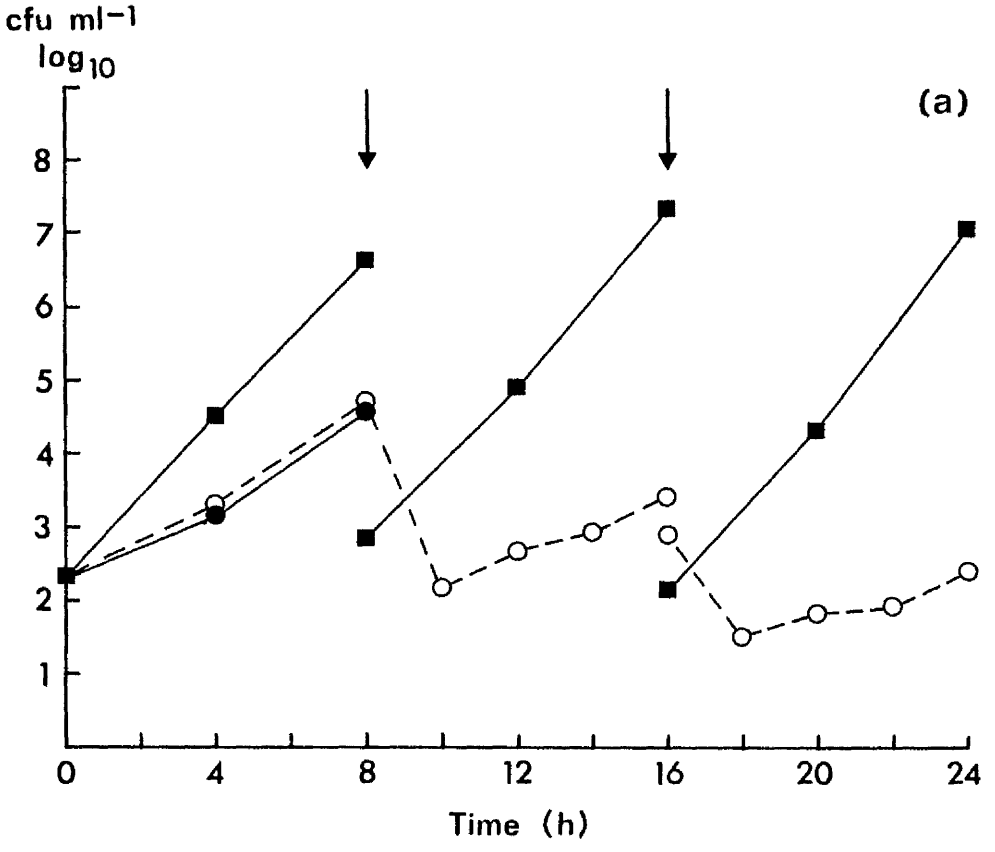

cfu ml⁻¹
log10
(b)
8
7
6
5
4
3
2
1
0
-1
0
4
8
12
16
20
24
Time (h)

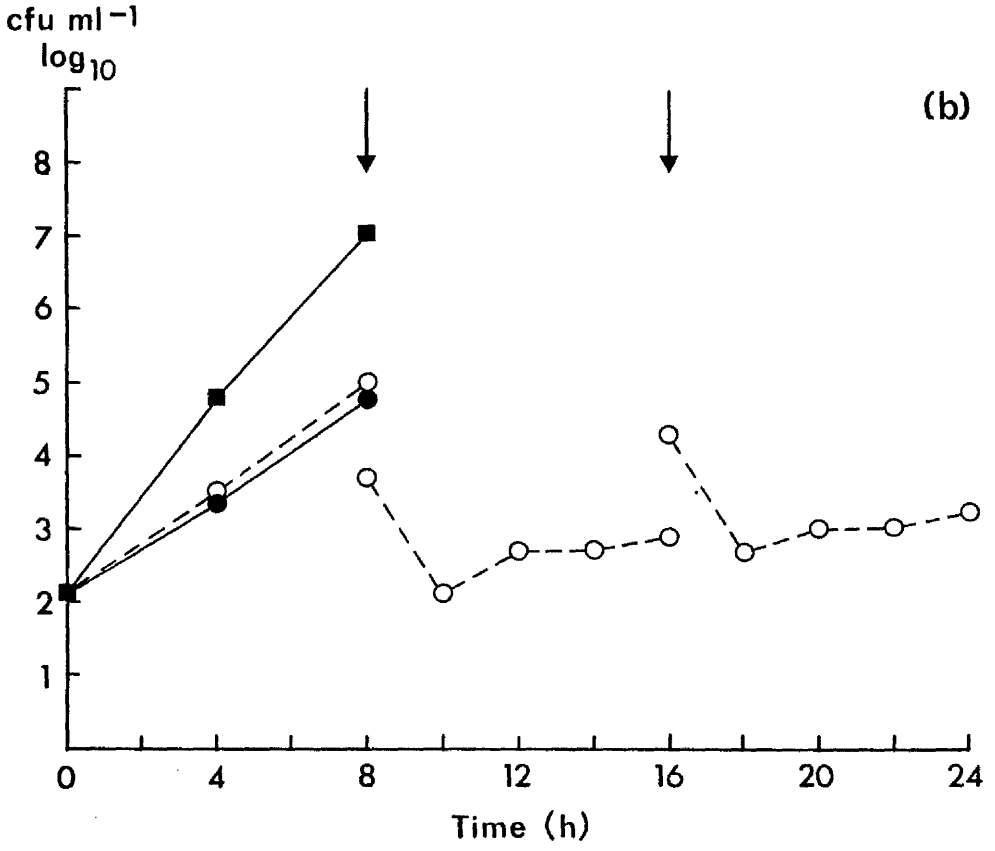
FIGURE 20a The effect of replacing the Clinifeeding System 3 reservoir (nutrient container), the feed and the giving set, on the growth of Staph. aureus in the system.

The reservoir and giving set were changed at 8 and 16h (↓).

For Key to symbols see Fig. 15 (page 86a). No organisms were detected in the reservoir after 8h.

FIGURE 20b As above except that after 8h samples were only collected from the enteral feeding tube to minimize the risk of contamination due to handling procedures.


cfu ml⁻¹
log10
(a)
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
Time (h)


cfu ml⁻¹
log10
(b)
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
Time (h)

The effect of the initial flushing out of the tube was to provide an inoculum for the feed flowing into the recipient flask, so that after 16h the counts were 10^7 cfu ml^{-1} for E. coli and Staph. aureus and after 24h they were 10^5 cfu ml^{-1} for E. coli and $\geqslant 10^7$ cfu ml^{-1} for Staph. aureus.

In order to minimize the risk of any contamination being introduced due to handling procedures, experiments were also carried out for both E. coli and Staph. aureus where the only samples taken after 8h were those from the end of the fine-bore tube. The results are presented in Figs 19b and 20b and indicate that the handling procedures employed were not a source of contamination.

d) Stopping the flow of feed through the system

The results are presented in Fig. 21. No organisms were detected in the control experiments.

Figure 21 shows that stopping the flow of feed through the system for 2h resulted in rapid multiplication of the organisms in the fine-bore tube. Counts increased from 10^3 cfu ml^{-1} at 12h to approximately $10^{4.5}$ cfu ml^{-1} at 14h, whereas in a similar experiment where flow was not stopped, counts increased from 10^3 to $10^{3.5}$ cfu ml^{-1} over the same time (Fig. 16).

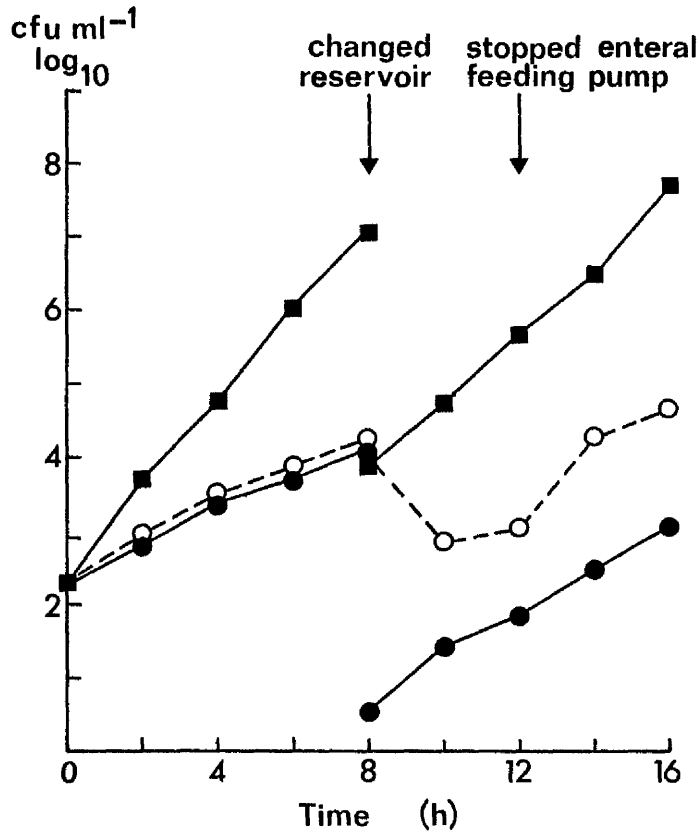


FIGURE 21 The effect of stopping flow of feed through the system for 2h. The reservoir was changed at 8h. The enteral feeding pump was turned off at 12h and restarted at 14h.

For Key to symbols see Fig. 15 (page 86a).

Viable counts made on samples from the reservoir over the same time period only increased from 10^2 at 12h to $10^{2.5}$ at 14h demonstrating that the increase in numbers of organisms in the fine-bore tube was due to it being held at 37°C (patient's body temperature) rather than 25°C (ambient ward temperature).

e) Varying the initial level of contamination

The results for Staph. aureus are shown in Figs 22-24 and for E. coli in Figs 25-27. The figures presented for viable counts of ≤ 10 cfu ml^{-1} at 0h have been calculated from actual values obtained from viable counts on the original inoculum.

Results for Staph. aureus and E. coli were similar. Over the first 8h of the study the growth of the test micro-organisms for all inocula were virtually parallel for each of the three sampling sites. The rate of multiplication in the reservoir and the fine-bore tube over this time was similar (Figs 23 and 24 for Staph. aureus and Figs 26 and 27 for E. coli), there being an average increase in numbers of 2 log cycles (mean generation time = 1.2h). The rate of multiplication in the flask (held at body temperature, viz 37°C) was more rapid, with an average increase of 4.5 log cycles (mean generation time = 0.5h).

After the reservoir had been replaced the growth curves of the range of inocula for both the reservoir and the flask were again

FIGURE 22 The effect of varying the initial level of contamination in the Clinifeeding System 3 reservoir (nutrient container) on the growth of Staph. aureus in the recipient flask. The reservoir was changed at 8h.

The approximate number of colony forming units in the 1000 ml of Clinifeed ISO in the reservoir at the start of each experiment was :

1×10^5 (i.e. 100 cfu ml^{-1}) ▲——▲

5×10^4 (i.e. 50 cfu ml^{-1}) ✸——✸

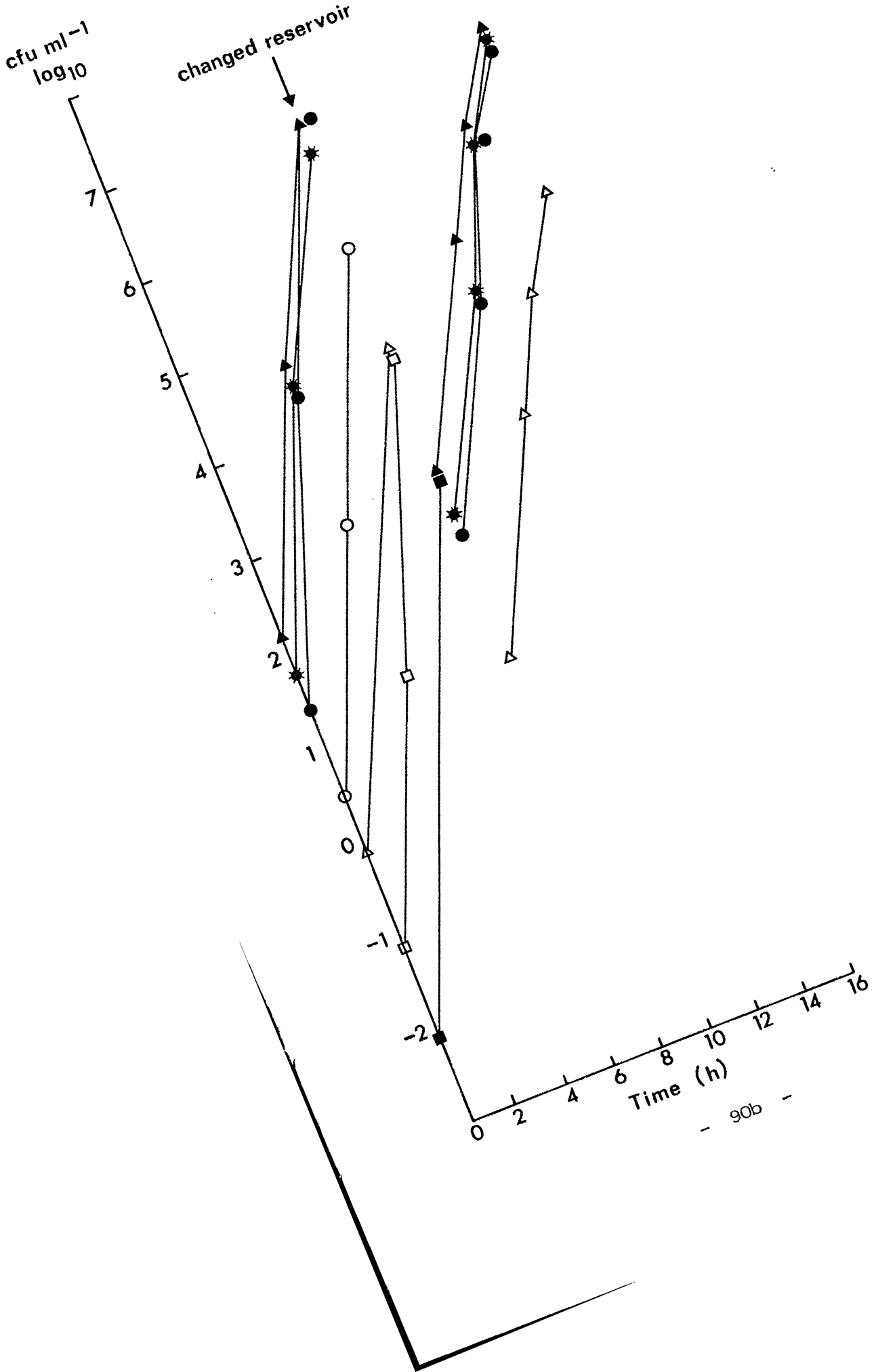
2.5×10^4 (i.e. 25 cfu ml^{-1}) ●——●

5×10^3 (i.e. 5 cfu ml^{-1}) ○——○

1×10^3 (i.e. 1 cfu ml^{-1}) △——△

1×10^2 (i.e. 0.1 cfu ml^{-1}) □——□

1×10^1 (i.e. 0.01 cfu ml^{-1}) ■——■


cfu ml⁻¹
log10
changed reservoir
7
6
5
4
3
2
1
0
-1
-2
0
2
4
6
8
10
12
14
16
Time (h)

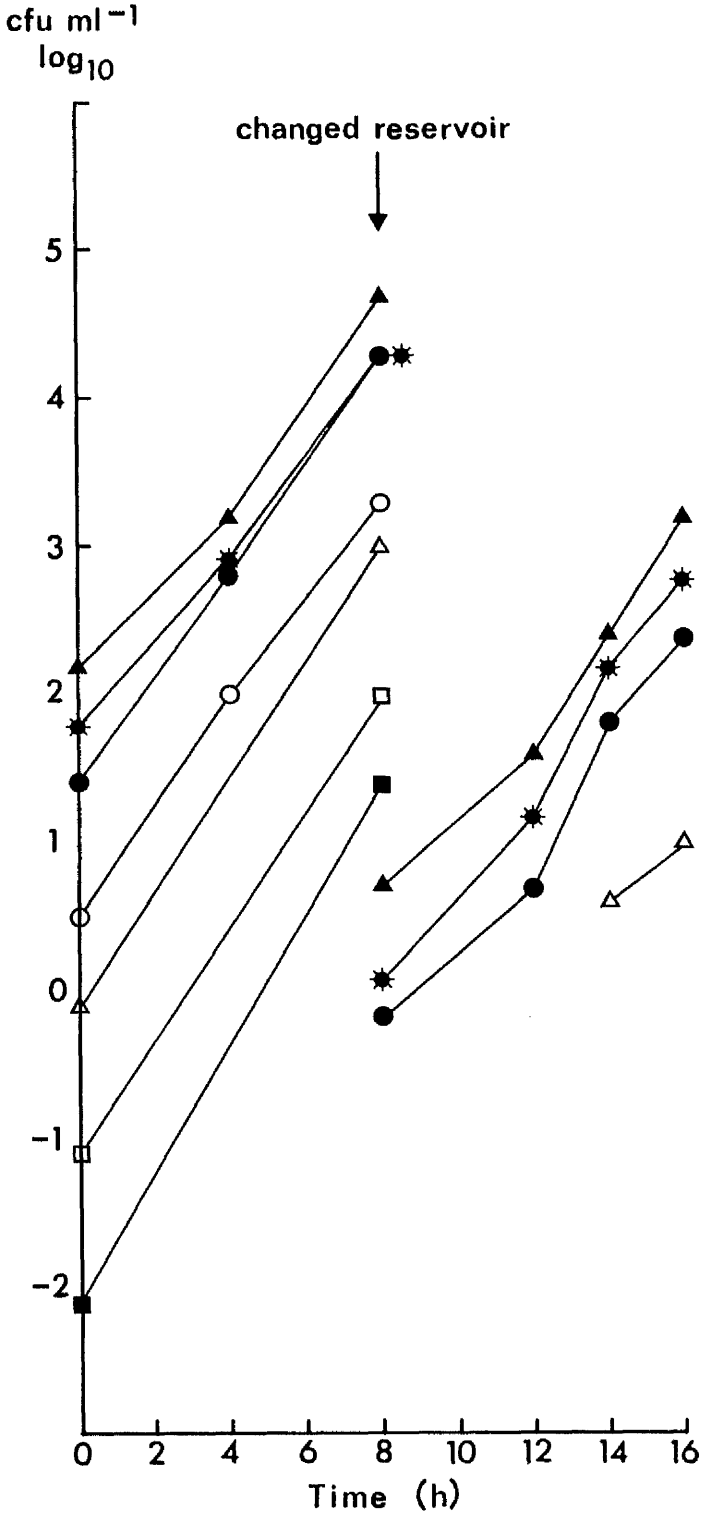


FIGURE 23 The effect of varying the initial level of contamination in the Clinifeeding System 3 reservoir (nutrient container) on the growth of Staph. aureus in the reservoir.

For Key to symbols see Fig. 22.

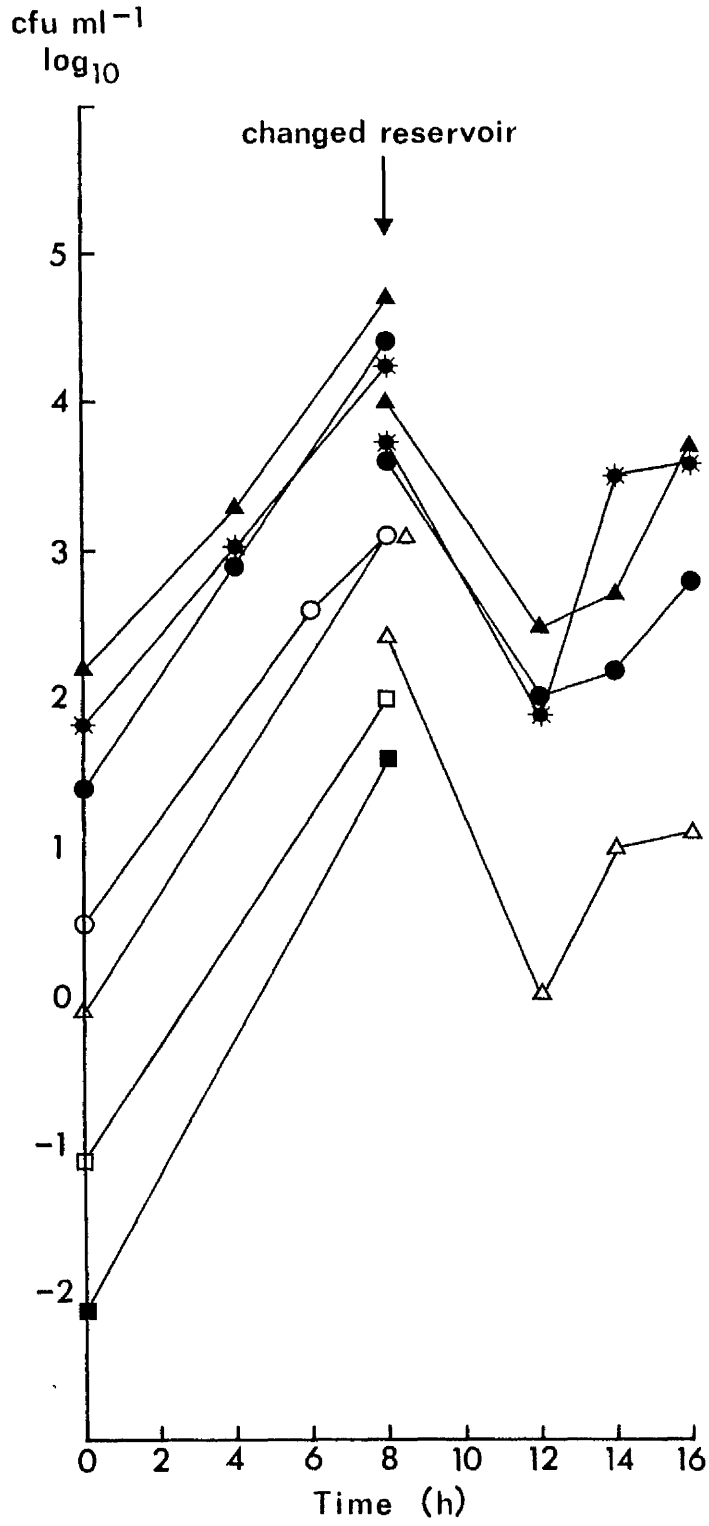


FIGURE 24 The effect of varying the initial level of contamination in the Clinifeeding System 3 reservoir (nutrient container) on the growth of Staph. aureus in the enteral feeding tube.

For Key to symbols see Fig. 22.

FIGURE 25 The effect of varying the initial level of contamination in the Clinifeeding System 3 reservoir (nutrient container) on the growth of E. coli in the recipient flask.

The reservoir was changed at 8h.

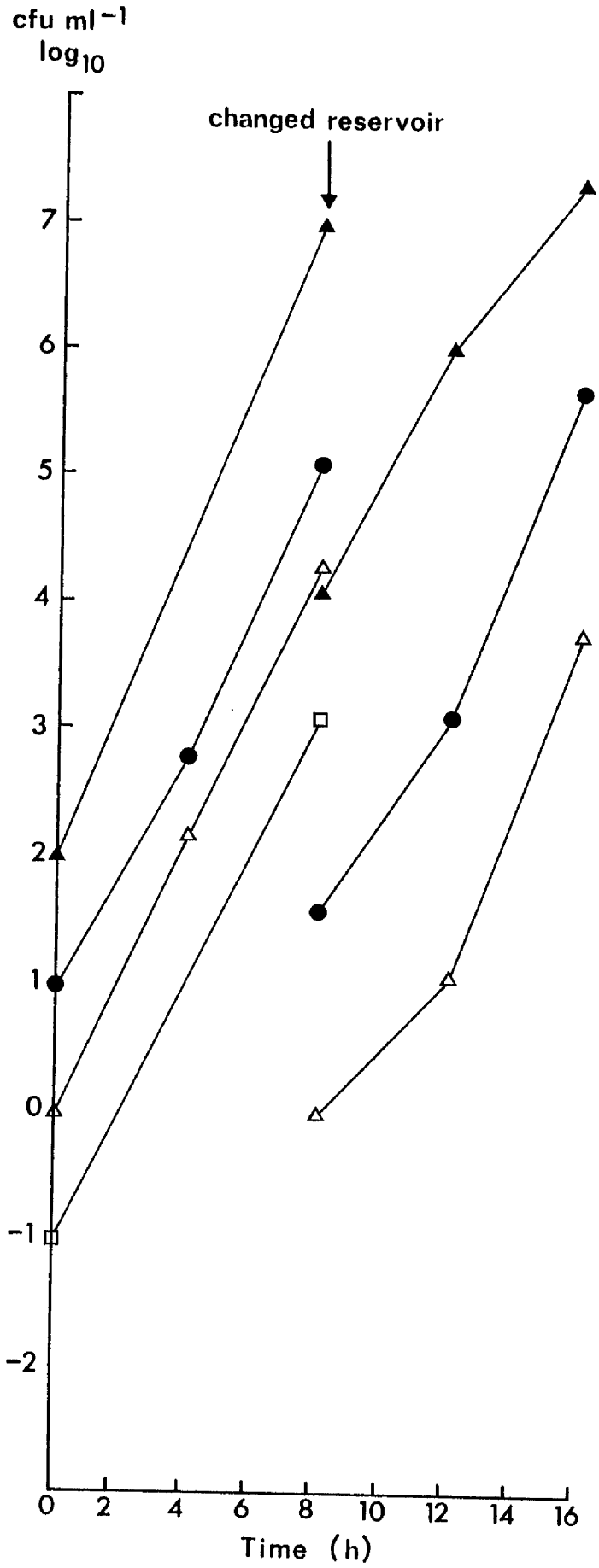
The approximate number of colony forming units in the 1000 ml of Clinifeed ISO in the reservoir at the start of each experiment was :

1×10^5 (i.e. 100 cfu ml^{-1}) ▲——▲

1×10^4 (i.e. 10 cfu ml^{-1}) ●——●

1×10^3 (i.e. 1 cfu ml^{-1}) △——△

1×10^2 (i.e. 0.1 cfu ml^{-1}) □——□


cfu ml⁻¹
log10
changed reservoir
7
6
5
4
3
2
1
0
-1
-2
0
2
4
6
8
10
12
14
16
Time (h)

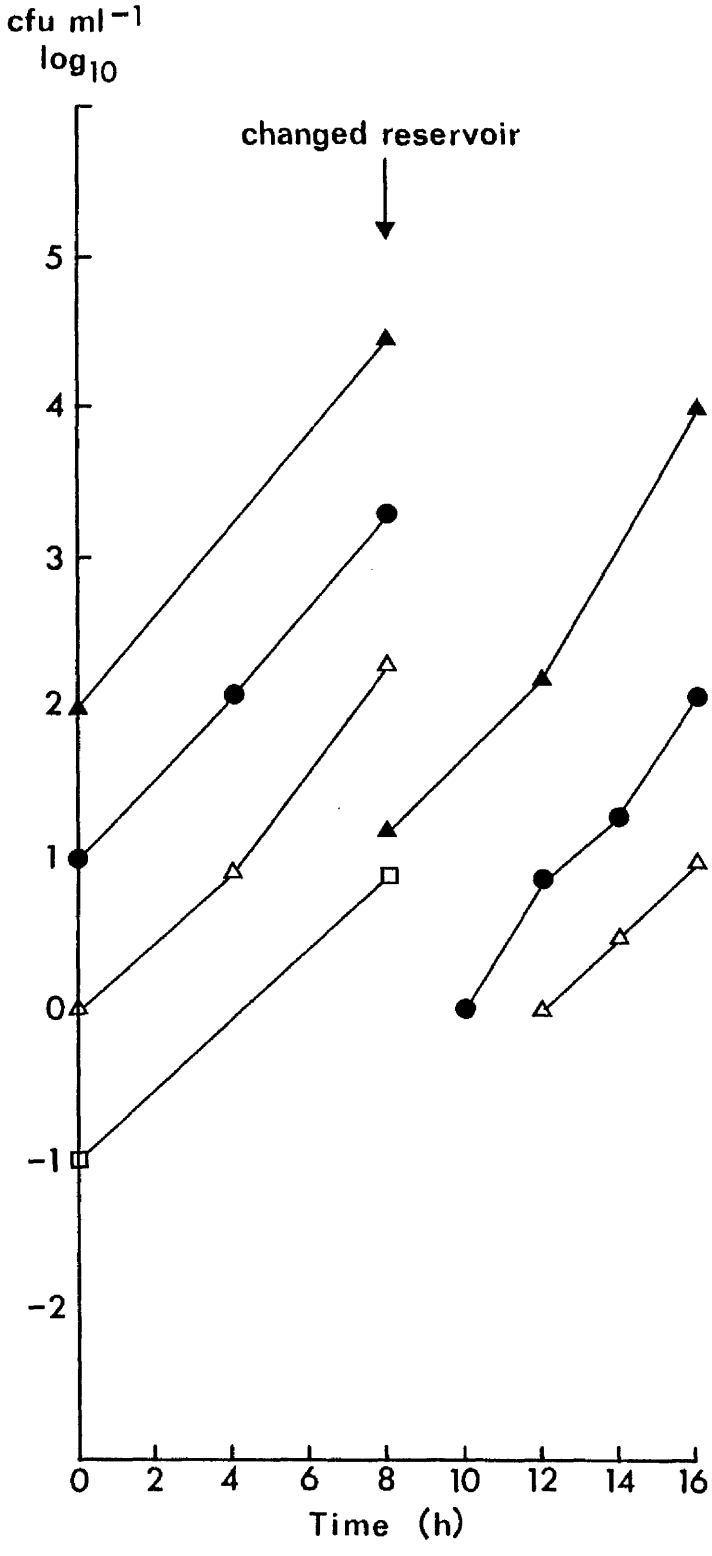


FIGURE 26 The effect of varying the initial level of contamination in the Clinifeeding System 3 reservoir (nutrient container) on the growth of E. coli in the reservoir.

For Key to symbols see Fig. 25.

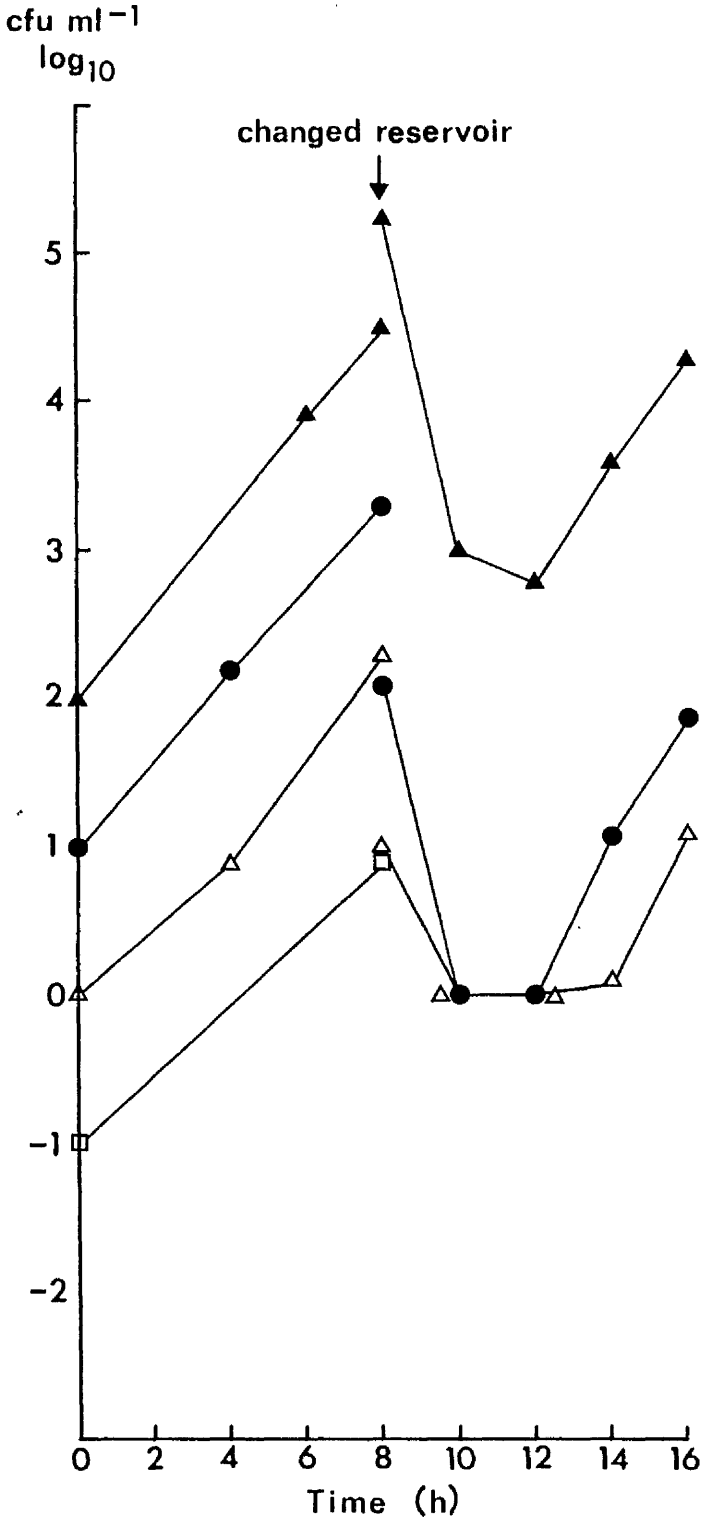


FIGURE 27 The effect of varying the initial level of contamination in the Clinifeeding System 3 reservoir (nutrient container) on the growth of E. coli in the enteral feeding tube.

For Key to symbols see Fig. 25.

parallel (Figs 22 and 23 for Staph. aureus and Figs 25 and 26 for E. coli). The initial count in the new reservoir at 8h was consistently lower than at 0h, the number of bacteria introduced into the reservoir being less than the original contaminating inoculum. Conversely, the counts in the new flask at 8h were usually higher than at 0h (Figs 22 and 25), this can be explained by the wash-through of residual organisms in the giving set and fine-bore tube, as demonstrated in previous experiments.

The numbers of viable organisms in the feed collected from the fine-bore tube were again high immediately after changing the reservoir, falling over the next 2 to 4h after which numbers increased steadily (Figs 24 and 27).

It is interesting to note that after 16h the viable counts obtained from the feed in the reservoir were usually less than those obtained from the feed collected from the end of the fine-bore tube. This indicates that organisms were multiplying in the fine-bore tube and it is possible that they were also colonizing the lumen of the tube.

5.3 ASSESSMENT OF THE SUITABILITY OF FOOD COLOURING MATERIALS AS INDICATORS OF MICROBIAL CONTAMINATION OF ENTERAL FEEDS

5.3.1 INTRODUCTION

Colour-coding of enteral feeds has been widely used in the U.S.A. to prevent the inadvertent intravenous infusion of enteral products (Fagerman and Dean 1983). These authors reported colour changes in enteral feeds to which red food colouring had been added and stated that bacterial contamination was common in colour-coded feeds in which colour changes occurred. Micro-organisms isolated included *E. coli* and *Bacillus* spp.

The reduction of water-soluble azo-dyes by intestinal bacteria has been reported by a number of authors (Roxon *et al.* 1967, Larsen *et al.* 1976, Chung *et al.* 1978, Watabe *et al.* 1980).

A study was therefore made of the feasibility of adding food colouring materials including a range of azo-dyes to enteral feeds to act as indicators of microbial contamination.

5.3.2 MATERIALS AND METHODS

Strains and inocula

The organism selected was *E. coli* (NCTC 8007). Inocula were prepared from stock cultures as described in Ch. 5.1.2.

Enteral feeds

The feeds used were Clinifeed ISO, Triosorbon and Vivonex Standard. Cans and packets of feed were opened and reconstituted aseptically.

Food colouring materials

Details of the five water-soluble sulphonated azo-dyes and the xanthene dye selected for this experiment are given in Table 15 and Appendix B. Filter-sterilized 10% (w/v) aqueous solutions of these dyes were prepared.

Procedure

One ml of the appropriate dilution of the bacterial inoculum was transferred to each of three separate sterile 250 ml Erlenmeyer flasks containing 100 ml of freshly prepared feed to give an initial count of 10^2 - 10^3 cfu ml^{-1} feed. One tenth of a ml of a 10% (w/v) filter sterilized aqueous solution of carmoisine was added to each flask. After careful mixing a viable count was made on a sample of a feed from each flask. Flasks were then incubated at 4, 25 or 37°C and samples taken for viable counts at 4, 8 and 24h. The flasks were examined at 3, 6, 12, 18, 24, 48 and 72h for any visible change in colour of the feeds. Control experiments with uninoculated coloured feeds were carried out simultaneously.

The procedure was then repeated with each of the five remaining food colouring materials.

TABLE 15 : Food colouring materials assessed for their suitability as indicators of bacterial contamination of enteral feeds

Common name*	E.E.C. Serial No.	Principal chemical constituent	Colour
Azo dyes			
Amaranth	E123	Trisodium 3-hydroxy-4-(4-sulpho-1-naphthylazo)-naphthalene - 2,7 - disulphonate	red-purple
Carmoisine (azorubine)	E122	Disodium 4-hydroxy-3-(4-sulpho-1-naphthylazo) naphthalene-1-sulphonate	red-purple
Ponceau 4R (new coccine)	E124	Trisodium-7-hydroxy-8-(4-sulpho-1-naphthylazo)-naphthalene-1,3-disulphonate	red
Sunset Yellow FCF	E110	Disodium 6-hydroxy-5-(4-sulphophenylazo)-naphthalene-2-sulphonate	orange
Tartrazine	E102	Trisodium 5-hydroxy-1-(4-sulpho-phenyl)-4-(4-sulphophenylazo)-pyrazole-3-carboxylate	yellow
Xanthene dye			
Erythrosine BS	E127	Disodium -2,4,5,7 - tetraiodofluorescein	red

* for details of manufacturers see Appendix B

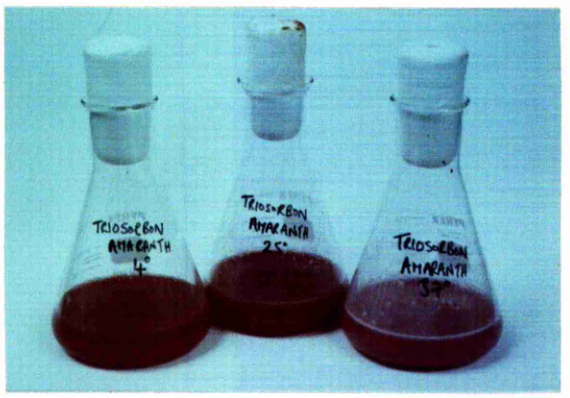
5.3.3 RESULTS

There was no change in colour and no increase in numbers of micro-organisms in any of the samples over 72h at 4°C.

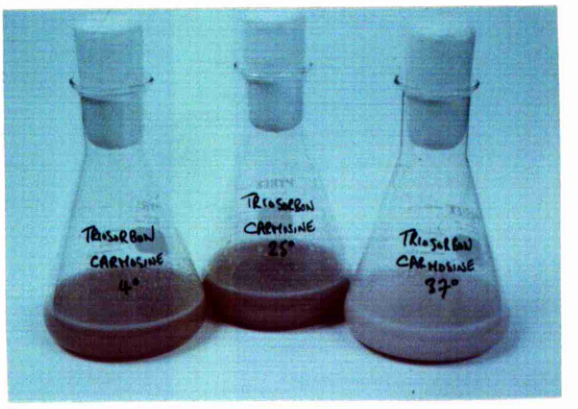
In the flasks containing Clinifeed ISO plus food colouring materials, Clinifeed ISO plus E. coli plus food colouring materials, half-strength Vivonex Standard plus food colouring materials and half-strength Vivonex Standard plus E. coli plus food colouring materials no change in colour was observed over 72h at either 25°C or 37°C even though viable counts had increased from 10^0 - 10^2 organisms ml^{-1} at 0h to 10^7 - 10^8 and 10^8 -10^9 organisms ml^{-1} at 25 and 37°C respectively after 24h, and remained at this level after 72h.

No colour changes were observed in the flasks containing Triosorbon plus food colouring materials or Triosorbon plus E. coli plus food colouring materials after 3, 6 or 12h incubation at 25 or 37°C. However, Fig. 28 illustrates that, with the exception of the flask containing tartrazine, all the flasks containing Triosorbon plus food colouring materials that had been incubated at 37°C for 18h were slightly paler in colour than the control flasks. After 18h incubation at 25°C there was no change in colour in any of the flasks containing Triosorbon plus food colouring materials.

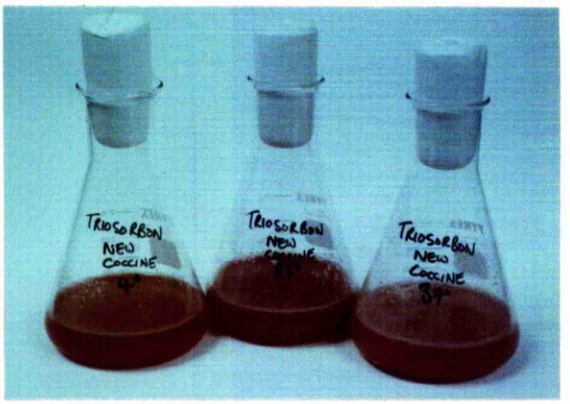
The colour changes in the flasks containing Triosorbon plus colouring materials after 48h incubation at 25 or 37°C are illustrated



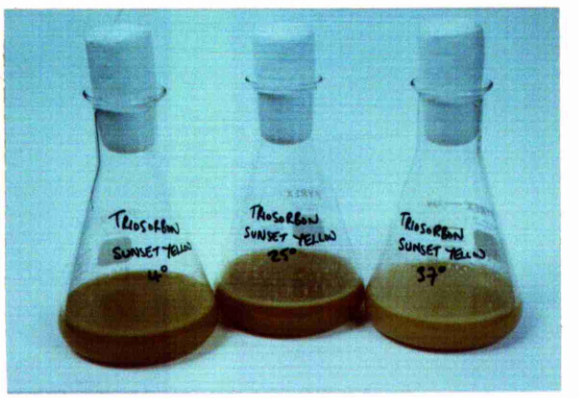
amaranth



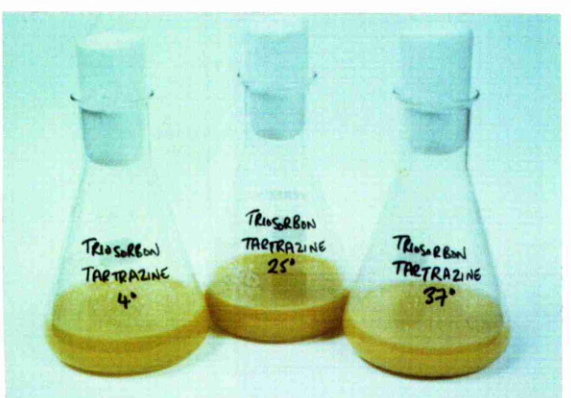
carmoisine



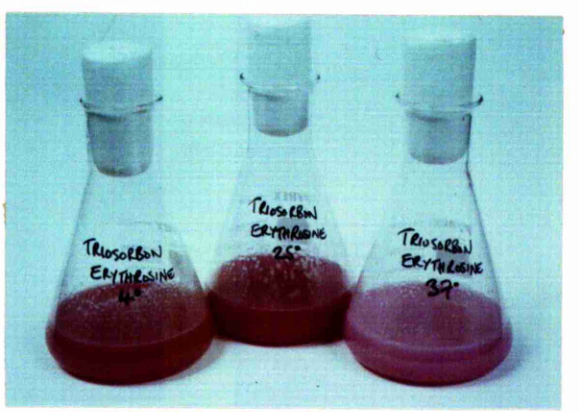
new coccine



sunset yellow



tartrazine



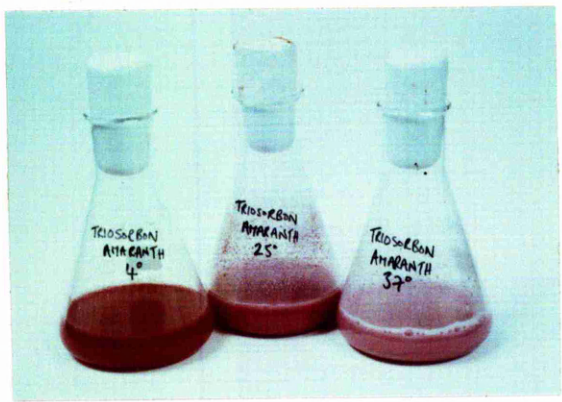
erythrosine

FIGURE 28 Colour changes observed in flasks containing Triosorbon plus food colouring materials after 18h incubation at 4, 25 and 37°C.

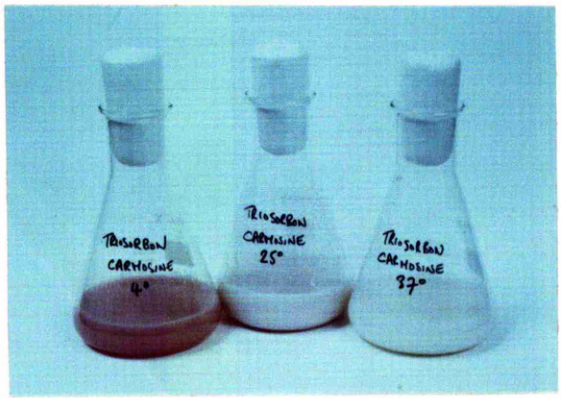
in Fig. 29. After 48h at 25°C there was very little change in colour in the flasks containing new coccine, sunset yellow, tartrazine or erythrosine but the contents of the flasks containing amaranth and carmoisine were slightly paler than the controls. Similar colour changes were observed after 48h incubation at 25°C of the flasks containing Triosorbon plus colouring materials plus E. coli (Fig. 30).

After 48h at 37°C the contents of the flasks containing Triosorbon plus carmoisine, sunset yellow or tartrazine were completely decolourized and those flasks containing Triosorbon plus amaranth, new coccine or erythrosine were all noticeably paler than the control flasks (Fig. 29). After 72h at 25 or 37°C no more samples had been decolourized, however, the contents of all the flasks were paler in colour than after 48h, with the exception of the flasks containing Triosorbon plus erythrosine where there had been no further change in colour.

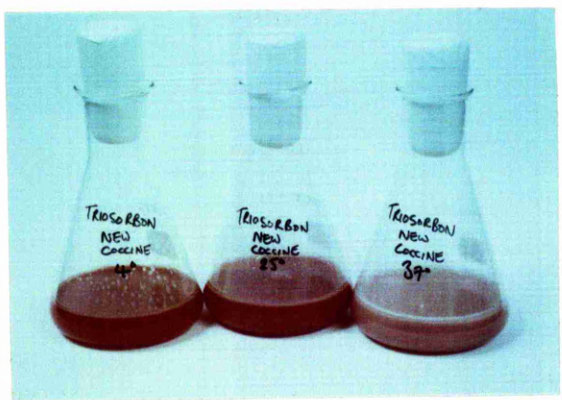
At 37°C the rate of loss of colour in the flasks of Triosorbon to which E. coli had been added was more rapid than in those containing Triosorbon alone. This is illustrated in Fig. 30 where it can be seen that after 48h at 37°C the contents of the flask containing Triosorbon and amaranth were slightly paler than the control whereas the contents of the flask containing Triosorbon and amaranth plus E. coli had lost their colour completely.



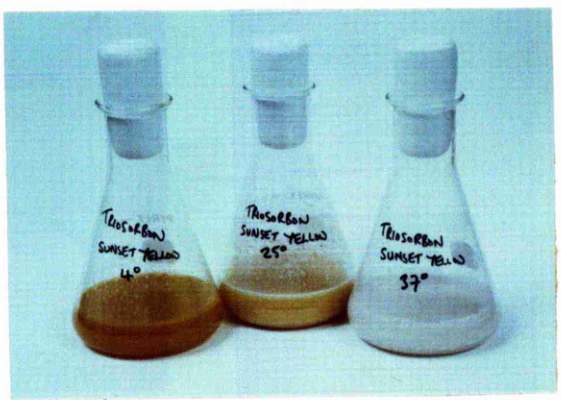
amaranth



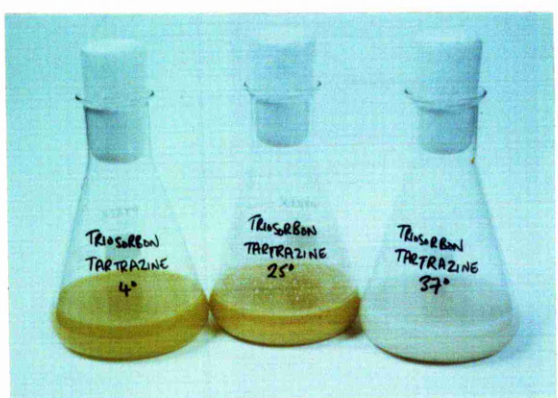
carmoisine



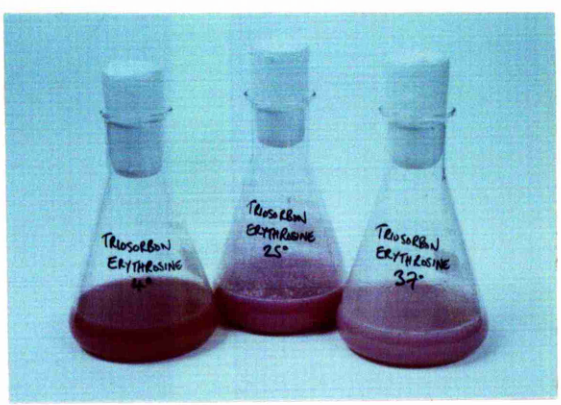
new coccine



sunset yellow

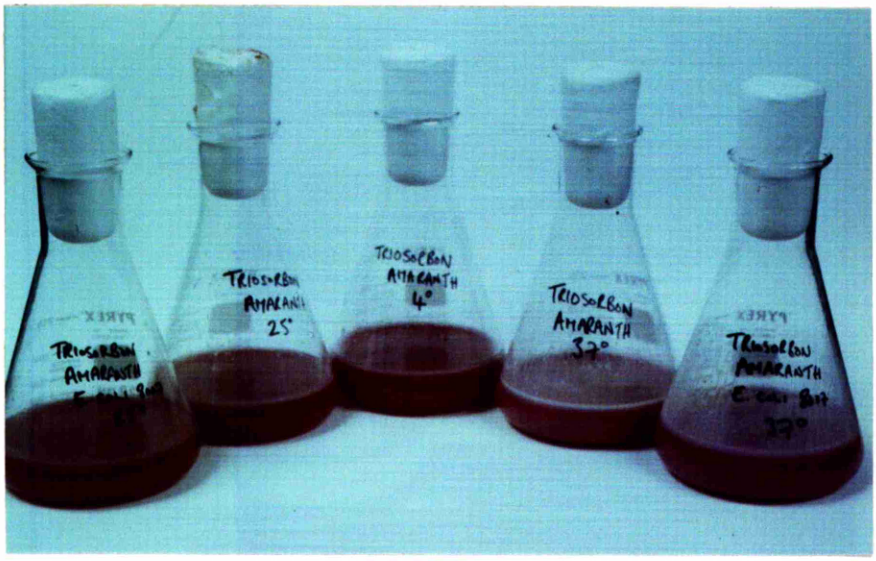


tartrazine

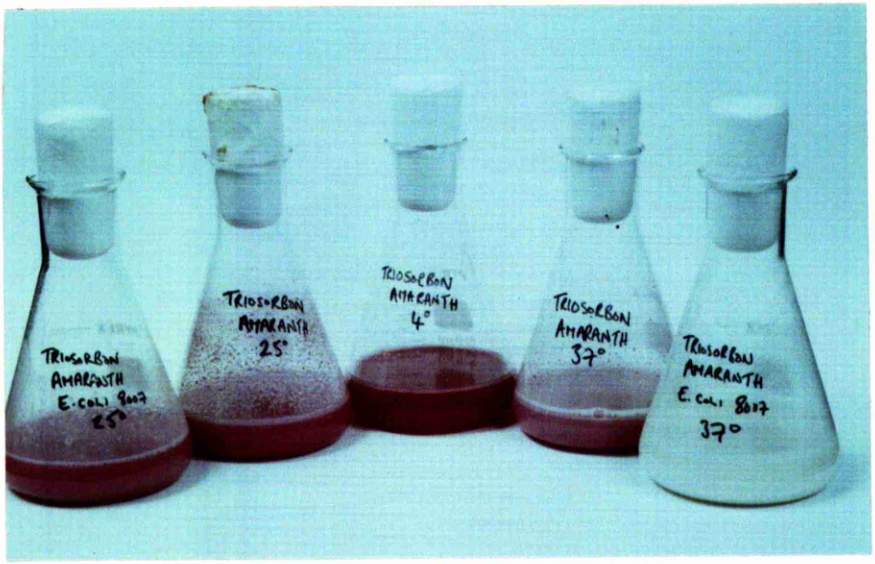


erythrosine

FIGURE 29 Colour changes observed in flasks containing Triosorbon plus food colouring materials after 48h incubation at 4, 25 and 37°C.



a



b

FIGURE 30 Comparison of the rate of loss of colour in flasks containing Triosorbon plus amaranth with those containing Triosorbon plus amaranth plus E. coli after incubation at 4, 25 or 37°C for (a) 18h and (b) 48h.

Viable counts in all the flasks containing Triosorbon and colouring materials were similar to those in Triosorbon alone, rising from 10^{0} at 0h to 10^{8} and 10^{9} organisms ml^{-1} at 25 and 37°C after 24h. The main organisms isolated were aerobic sporeforming bacilli. Viable counts in the flasks containing Triosorbon and colouring materials plus E. coli increased from 10^{2} organisms ml^{-1} at 0h to 10^{7} and 10^{8} organisms ml^{-1} after 24h at 25 and 37°C respectively. The main organism isolated from these flasks after 24h was E. coli.

The aerobic spore-forming bacilli from Triosorbon were purified by streaking on PCA and incubated at 37°C for 24h. A single colony was then transferred to 10ml of Nutrient Broth No.2 (Oxoid) and incubated at 37°C for 18h. One ml aliquots of the appropriate dilution of this bacterial inoculum were transferred to sterile 250ml Erlenmeyer flasks containing 100ml portions of Clinifeed ISO plus food colouring materials as detailed in Ch. 5.3.2. After 18h incubation at 37°C the contents of all the flasks were paler than the controls and after 48h the flasks containing carmoisine, sunset yellow or tartrazine were decolourised. These results, which were similar to those obtained with Triosorbon plus food colouring suggested that it was the organism isolated from Triosorbon that was responsible for decolourising the food colouring materials.

CHAPTER 6

BACTERIAL ADHESION STUDIES ON EQUIPMENT USED FOR THE ADMINISTRATION OF ENTERAL FEEDS

6.1 INTRODUCTION

The possibility was considered that contaminating micro-organisms in enteral feeds might adhere to the surfaces of the enteral feeding systems. Residual feed and/or micro-organisms adhering to the inner surfaces of the nutrient container could contaminate fresh feed used to refill the container over a 24h period. Also the colonisation of enteral feeding tubes by adhering micro-organisms could result in patients being dosed with infecting micro-organisms and/or their toxic metabolites over an extended period of time.

The purpose of this part of the study was, to determine the number of bacteria adhering to the inner surfaces of laboratory contaminated nutrient containers , to examine the surface topography of the inner and outer walls of a range of unused enteral feeding tubes for surface irregularities in which micro-organisms could become trapped and to examine laboratory contaminated tubes for adherent particles of feed and bacteria.

6.2 MATERIALS AND METHODS

Recovery of bacteria from the inner surfaces of nutrient containers

The empty nutrient containers (Clinifeeding System 3 reservoirs) removed after the first 8h in the experiments described in Ch. 5.2.2 and which had contained an average of 10^4 - 10^5 viable organisms (*E. coli* or *Staph. aureus*) ml^{-1} of feed (Clinifeed ISO), were used to

investigate the adhesion of organisms to the plastic, which is polypropylene (A.J. Haskins 1982; pers. comm.).

Each reservoir was cut into strips (approximately 5 x 2 cm) with a sterile scalpel. The strips were then divided into four groups which were treated as follows (handled with sterile forceps at all times) :-

Group 1 : The contaminated surfaces of these strips were pressed gently onto the surface of either PCA (Oxoid) or MacConkey Agar (Oxoid), for reservoirs contaminated with Staph. aureus and E. coli respectively.

Group 2 : These strips were laid in an empty sterile petri dish (Sterilin), contaminated side upwards and 1.0ml of PCA (for Staph. aureus) or 1.0ml of MacConkey Agar (for E. coli) at 45-50°C pipetted carefully onto the surface.

Group 3 & 4 The strips were sequentially washed for a total of 75s by gentle manual agitation in 5 jars of sterile distilled water in order to dislodge all the loosely adherent cells and any food debris (Samaranayake and

MacFarlane 1980). Distilled water was used rather than saline in order to simulate the rinsing procedure that might occur on a hospital ward. The strips were then treated as described in (1) and (2) above.

All plates were covered and incubated at 37°C for 24h. The number of colonies per cm^2 were counted with a plate microscope, avoiding areas within 0.5cm of the edge of each strip as this area had been handled with the forceps.

Scanning electron microscope studies of enteral feeding tubes

a) Preparation of tubes for S.E.M. examination

The methods of preparation are summarised in Fig. 31. All tubes were sampled along the whole of their length. Segments, 1cm long, were sectioned with sterile razor blades and opened up longitudinally. Transverse sections ($<$1mm) were also prepared.

Experimentally contaminated samples were washed for a total of 75s by gentle manual agitation in 5 jars of sterile distilled water (Samaranayake and MacFarlane 1980) or left unwashed. Samples were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate-HCl buffer (pH 7.2). The samples were rinsed in buffer followed by a secondary fixation in 0.1% osmium tetroxide in 0.1M sodium cacodylate-HCl buffer (pH 7.2). Samples were dehydrated through a graded series of ethanol solutions (30%, 50%, 70%, 85%, 95% for 10min. each, and to absolute

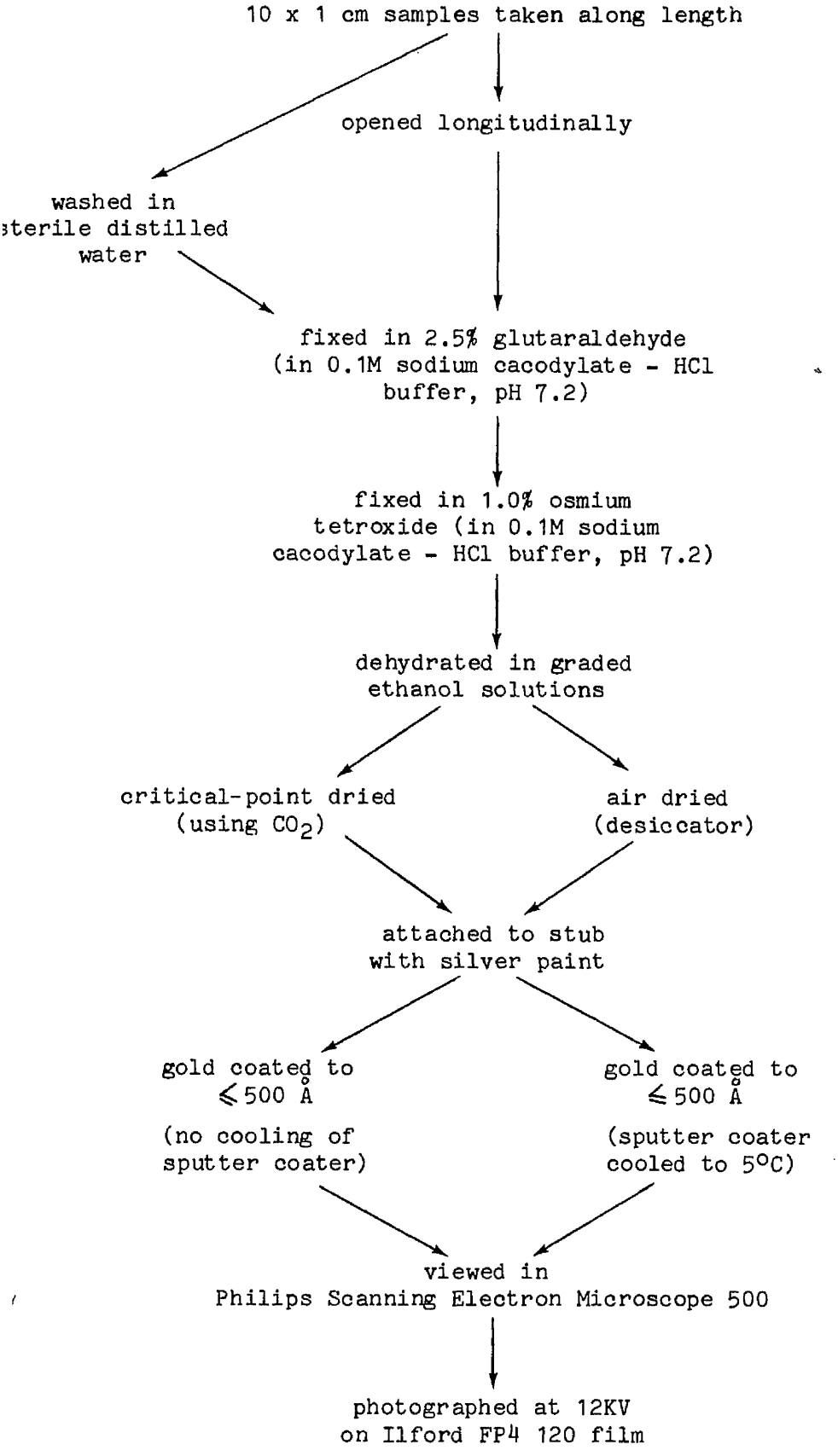


FIGURE 31 : Preparation of enteral feeding tubes for examination in the Scanning Electron Microscope

alcohol with several changes over 60min.) and either air dried in a desiccator or critical-point dried using liquid carbon dioxide. Unused tubes were also subjected to the same treatments in order to monitor the effects of these treatments on the tube materials.

The tube samples were attached to specimen stubs with a thin layer of silver conducting paint (Agar Aids) and gold coated to 200-500 Å thickness in a Polaron Sputter Coater (London, U.K.). The sputter coater was run at a low voltage and cooled to 5°C to minimise heating effects. Unfixed samples of unused tubes were similarly treated in order to minimise the introduction of artefacts as a result of preparation techniques. Specimens were viewed in a Phillips Scanning Electron Microscope 500 and photographed at 12 KV on Ilford FP4 120 film.

b) Surface characteristics of unused tubes

Details of the unused tubes examined are presented in Table 16. The range of tube sizes and materials were selected to be representative of the range of tubes on the market (Fig. 32). Both the internal and external surfaces of the tubes were examined.

c) Perfusion of tubes with contaminated feeds

The tubes selected for perfusion experiments were Clinifeed enteric tubes (Clinifeed 1/85, Roussel) which are composed of extruded polyvinylchoride (PVC) made radio-opaque with barium sulphate. Each

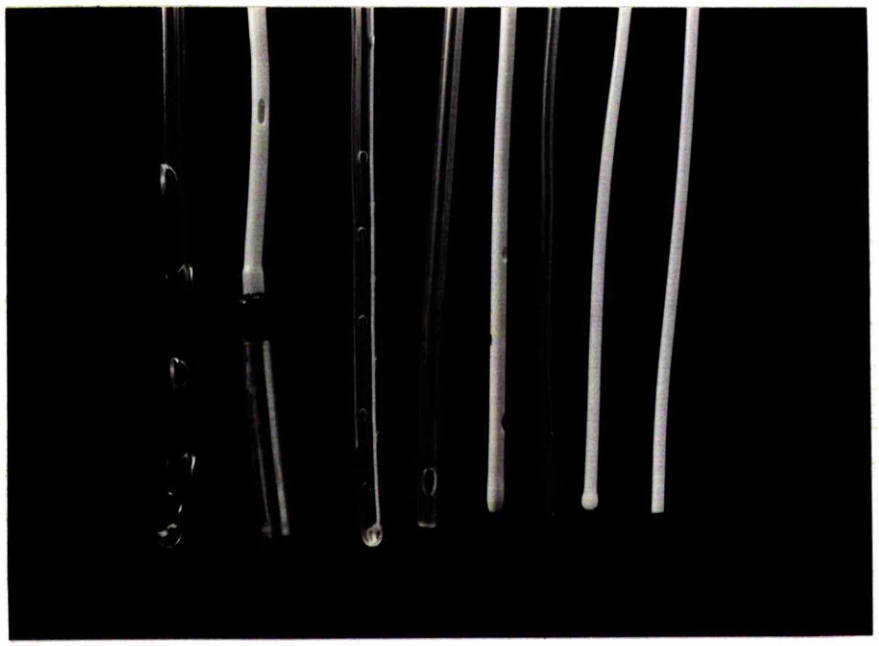


FIGURE 32 Gross appearance of enteral feeding tubes showing the variability in the tube ends and size of tubes.

The tubes shown are (from left to right) Aldon nasoduodenal Levin's tube, Viomedex enteral feeding tube, Argyle Scotsman, Vygon duodenal tube, Aldon feeding tube, Vygon paediatric XRO feeding tube for incubators, Entron nasogastric feeding tube, Clinifeeding System 1/85 (naso-enteric tube).

TABLE 16 : Details of enteral feeding tubes examined in the Scanning Electron Microscope

Enteral feeding tube	Material	Supplier*
'Aldon' Feeding tube	PVC (radio-opaque)	Aldington Laboratories Ltd.
'Aldon' Nasoduodenal (Levins) Tube	PVC	" "
'Aldon' Child's Feeding Tube	PVC	" "
Argyle Scotsman	PVC	Argyle
Entriflex	Polyurethane with internal coating of hydrophilic polymer (radio-opaque)	Franklin Medical
Entron	" "	Franklin Medical
Clinifeeding System 1 (naso-gastric tube)	PVC (radio-opaque)	Roussel Laboratories Ltd.
Clinifeeding System 1/85 (naso-enteric tube)	"	" "
Viomedex Enteral Feeding Tube (Dobbhoff)	Polyurethane (radio-opaque)	Viomedex Limited
Vygon Paediatric XRO Feeding Tube for Incubators	PVC (radio-opaque)	Vygon (U.K.) Limited
Vygon Paediatric Duodenal Tube	Silicone	" " "

* Addresses given in Appendix B.

tube was connected to a Clinifeeding System 3 (Roussel), the reservoir of which contained one of the following :

1. 1000ml of sterile Clinifeed ISO,
2. 1000ml of Clinifeed ISO which had been experimentally contaminated with Staph. aureus (NCTC 10657) at an initial level of approximately 10^2 cfu ml^{-1},
3. 1000ml of Clinifeed ISO which had been contaminated with E. coli (NCTC 8007),
4. 1000ml 0.1% (w/v) peptone water contaminated with Staph. aureus,
5. 1000ml 0.1% (w/v) peptone water contaminated with E. coli, and
6. 1000ml Triosorbon (reconstituted according to the manufacturer's instructions but using sterile distilled water).

Each of the six systems were prepared and run at 125ml h^{-1} as described in Ch. 5.2.2. The whole experiment was repeated three times. After 8h, when each system was empty, the fine-bore tube was lifted into a vertical position to empty out any residual feed. Each of the three fine-bore tubes from the six systems were prepared for examination in the scanning electron microscope. Tube samples were also obtained from systems containing Clinifeed ISO contaminated with Staph. aureus which had run for 24h, the reservoirs being refilled at 8 and 16h.

Various manufacturers (e.g. Biosearch Medical Products Inc.) recommend that the enteral feeding tubes are irrigated with water at regular intervals during the course of administration of the feed and therefore the effect of this procedure was examined. Reservoirs containing 1000ml Clinifeed ISO were experimentally contaminated with Staph. aureus (NCTC 10657) and the systems run for 8h at 125ml h^{-1}. Each fine bore tube was disconnected aseptically and rinsed through with 10ml sterile water using a sterile disposable syringe prior to preparing it for examination in the scanning electron microscope.

In a second study on the effect of irrigating the tube, the system was prepared as described above but in this case the system was run for 4h, the fine-bore tube rinsed, the system left static for 2h and then the experimentally contaminated feed allowed to run through at 125 ml h^{-1} for a further 2h. The fine-bore tube was then removed and prepared for examination in the scanning electron microscope.

d) Immersion of tubes in contaminated feeds

The tubes selected for immersion in laboratory contaminated feeds were the Clinifeeding System 1/85, the Entriflex Naso-gastric tube, the Aldon Naso-duodenal (Levin's) tube and the Vygon Paediatric Duodenal tube (Table 16).

Forty 1cm lengths of each tube were cut in two longitudinally with sterile razor blades. Ten of the resulting sections of each tube were then transferred to each of 8 empty sterile Universal bottles. Twenty ml quantities of the following enteral feeds were then added to two of each of these bottles, labelled 1h and 24h, respectively:-

1. Sterile Clinifeed ISO.
2. Clinifeed ISO experimentally contaminated with E. coli (NCTC 8007; 10^2 - 10^3 ml^{-1} feed).
3. Clinifeed ISO experimentally contaminated with Staph. aureus (NCTC 10657; 10^2 - 10^3 ml^{-1} feed).
4. Triosorbon (prepared according to the manufacturer's instructions but using sterile distilled water).

Each of the samples was thoroughly mixed on a Rotamixer (Hook and Tucker Instruments Ltd.) and then incubated at 37°C in an orbital incubator (Gallenkamp) at 120 rpm. After 1h and 24h the appropriate Universal bottles were removed from the incubator and each piece of tube was washed for a total of 75s by gentle manual agitation in 5 jars of sterile distilled water. The sections of tube from each bottle were then prepared for examination in the scanning electron microscope, samples being critical-point dried using carbon dioxide, and gold-coated to 200-500 Å in a sputter coater cooled to 5°C.

6.3 RESULTS

Recovery of E. coli and Staph. aureus from the inner surfaces nutrient containers

The results are shown below :

TABLE 17 Colonies per cm^2 after incubation of reservoir plastic with agar for 24h at 37°C (each figure is the average of six counts).

Organism	Treatment of strips	Washed (cfu per cm^2)	Unwashed (cfu per cm^2)
E. coli	pressed onto agar	37	534
	agar poured on	119	785
Staph. aureus	pressed onto agar	137	641
	agar poured on	415	927

The results show that both E. coli and Staph. aureus organisms adhered to the plastic. Washing the strips resulted in a substantial decrease (> 50%) in the number of colony forming units detected for both E. coli and Staph. aureus. The reduction in numbers of E. coli after washing (85-93%) was greater than that of Staph. aureus (56-79%). Higher counts (x1.5 for unwashed and x3 for washed strips) were obtained for both organisms when the agar was poured onto the plastic strip rather than the strip pressed onto the agar. This suggests that some organisms were lodged in irregularities on the surface of the plastic since pouring the agar onto the strip would result in better contact with an uneven surface than pressing the strip onto the agar surface.

Scanning electron microscope studies of enteral feeding tubes

a) Surface characteristics of unused tubes

The appearance of unfixed and fixed tubes was very similar, indicating that processing had not altered the surface characteristics of the tubes. Both the inner and outer surfaces of all the samples of unused feeding tubes were free from microbial contamination. However, a detailed study of the microtopography of these surfaces revealed many surface irregularities in which micro-organisms could become trapped if the tube comes into contact with contaminated materials. Figures 33 and 34 show the variation in appearance of the inner and outer surfaces of PVC, silicone, radio-opaque PVC and radio-opaque polyurethane tubes. Surfaces were ridged longitudinally, pitted, wave-like or reticulated. Figures 33 and 34 also illustrate that the outer surfaces of the tubes were less irregular than the inner surfaces.

Figures 35 and 36 show that there were noticeable differences between the appearance of the internal lumina of two tubes (Entriflex and Entron, Franklin Medical) both of which were made of Erythrothane^{R} (a specially formulated medical grade polyurethane which does not stiffen in the gastrointestinal tract). Both types of tube contain barium sulphate for radio-opacity and have the tube lumina coated with Hydromer^{R} (a hydrophilic polymer which is activated by water and provides a lubricant for passage of the guide-wire).

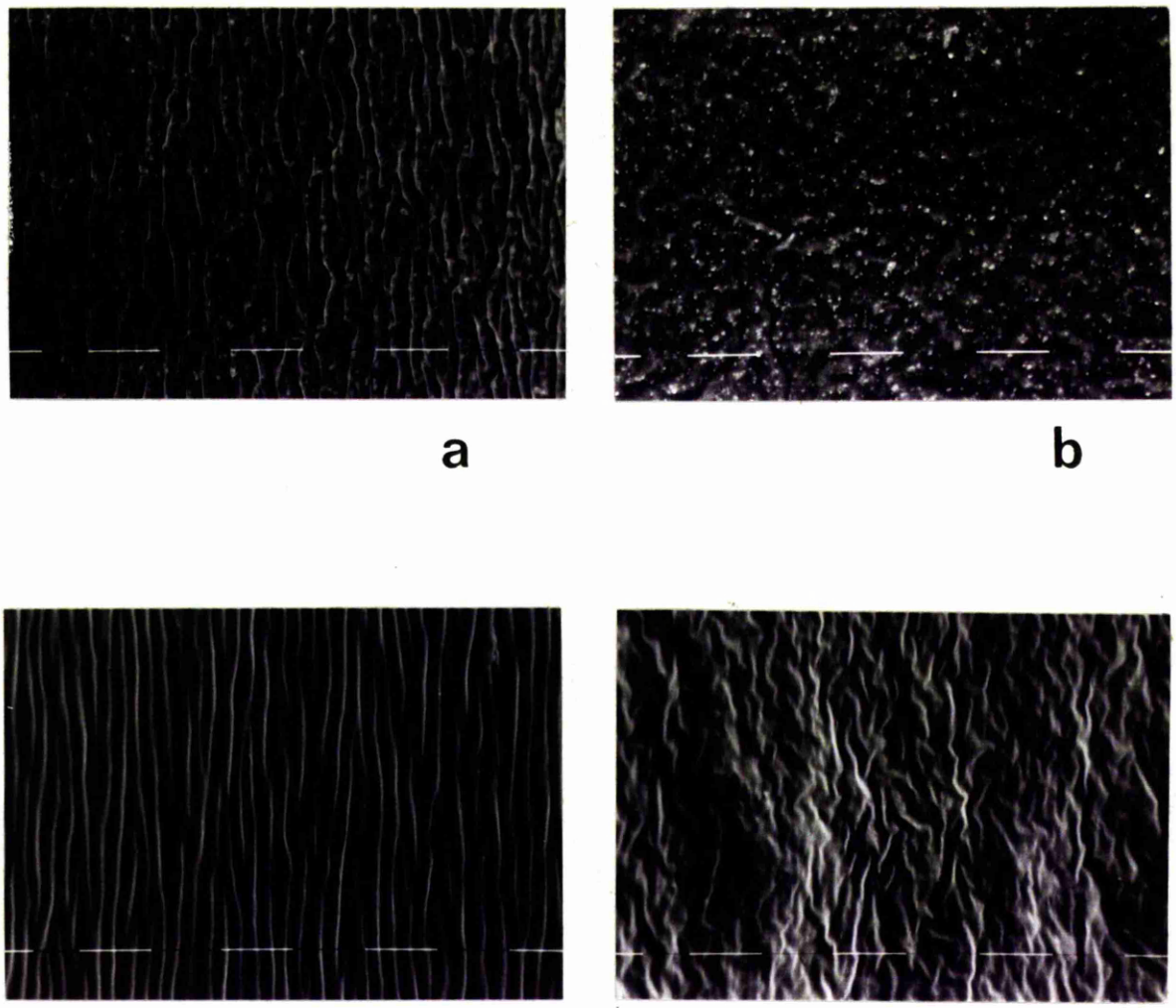


FIGURE 33 Microtopography of enteral feeding tubes.

Tube materials are : radio-opaque PVC (Clinifeed 1/85, Roussel (a) internal and (b) external surface; PVC (Aldon Nasoduodenal Levin's Tube, Aldington) (c) internal and (d) external surface.

Scale bar = 10 µm

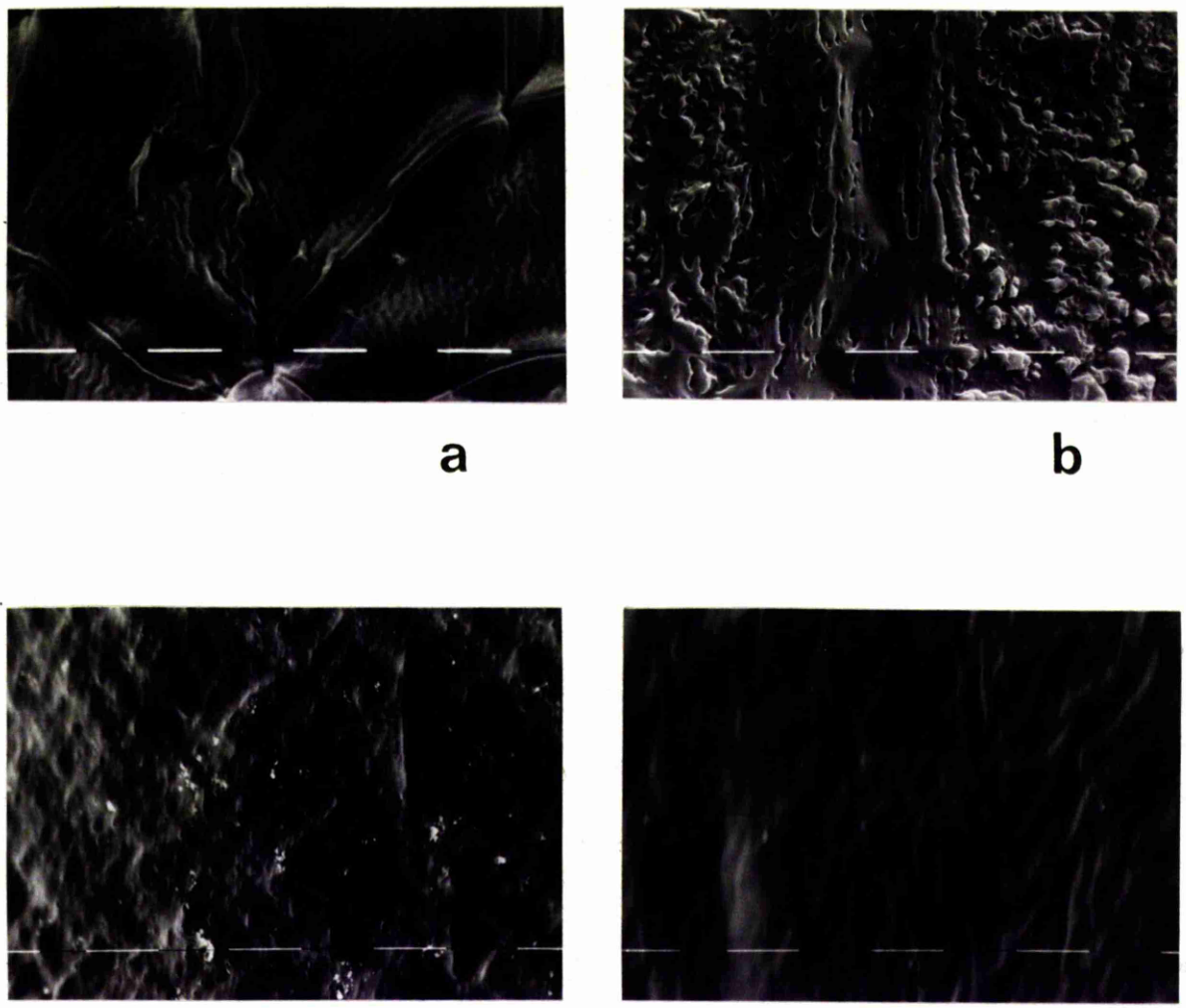
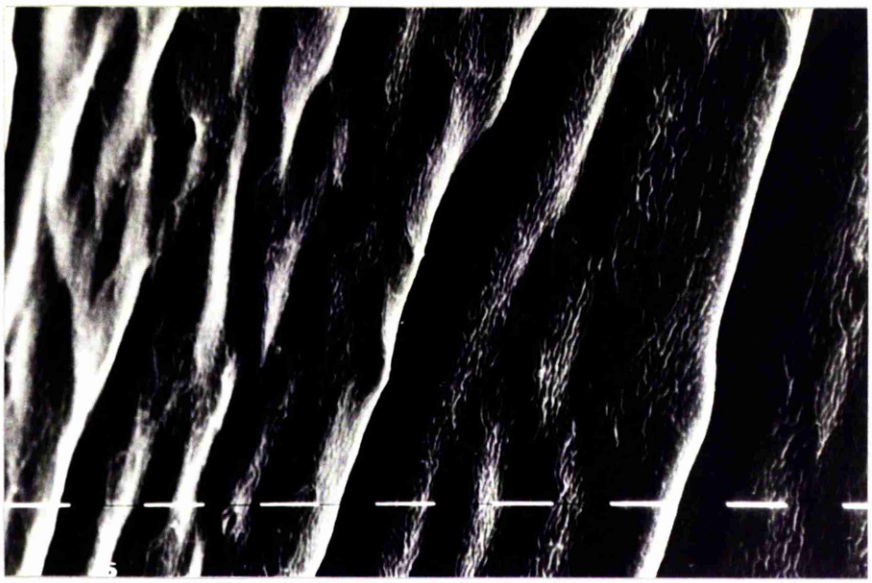


FIGURE 34 Microtopography of enteral feeding tubes.

Tube materials are : radio-opaque polyurethane (Entriflex, Franklin Medical) (a) internal and (b) external surface; silicone (Vygon paediatric duodenal tube, Vygon) (c) internal and (d) external surface.

Scale bar = 10 μm



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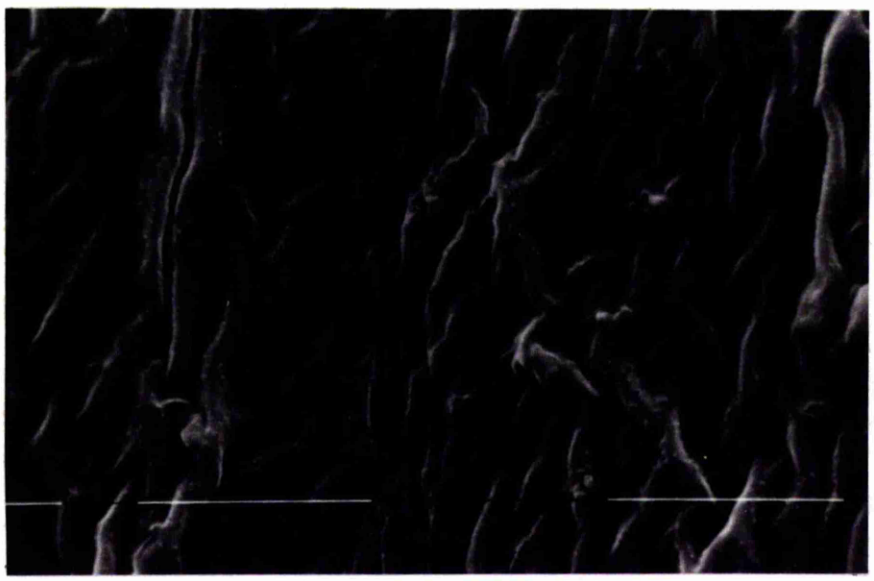


b

FIGURE 35 Appearance of the internal surface of radio-opaque polyurethane tube coated with hydrophilic polymer (Entron, Franklin Medical) (a) gross surface structure, (b) detail of surface structure.

Scale bar = 10 μm

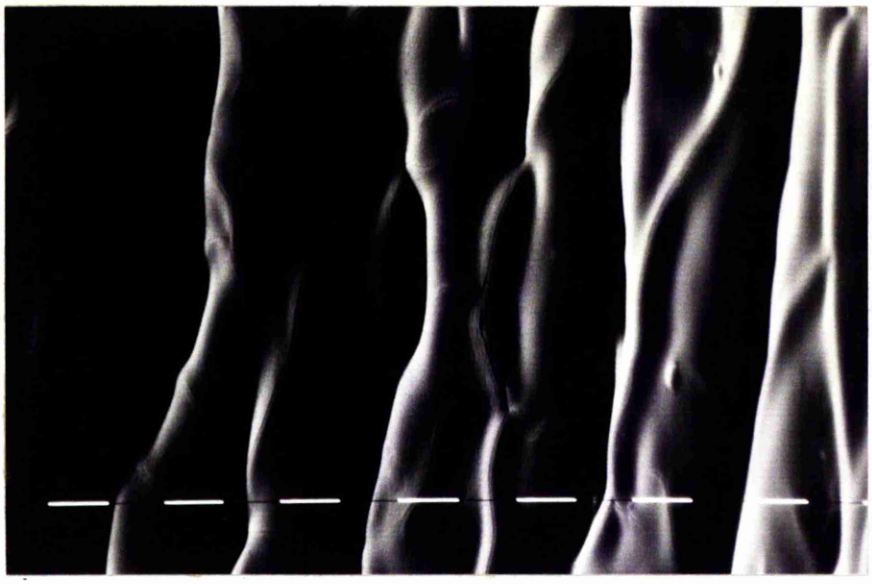
FIGURE 36 Appearance of the internal surface of radio-opaque polyurethane tube coated with hydrophilic polymer (Entriflex, Franklin Medical).

a) gross surface structure

b) details of surface structure

c) appearance of the internal surface as observed in an oblique view of a transverse section of tube.

Scale bar = 10 µm



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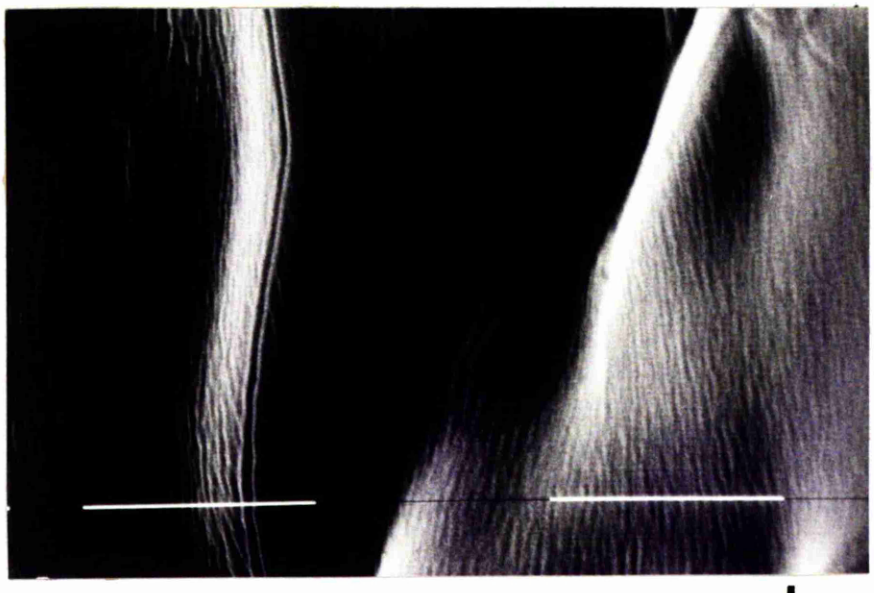

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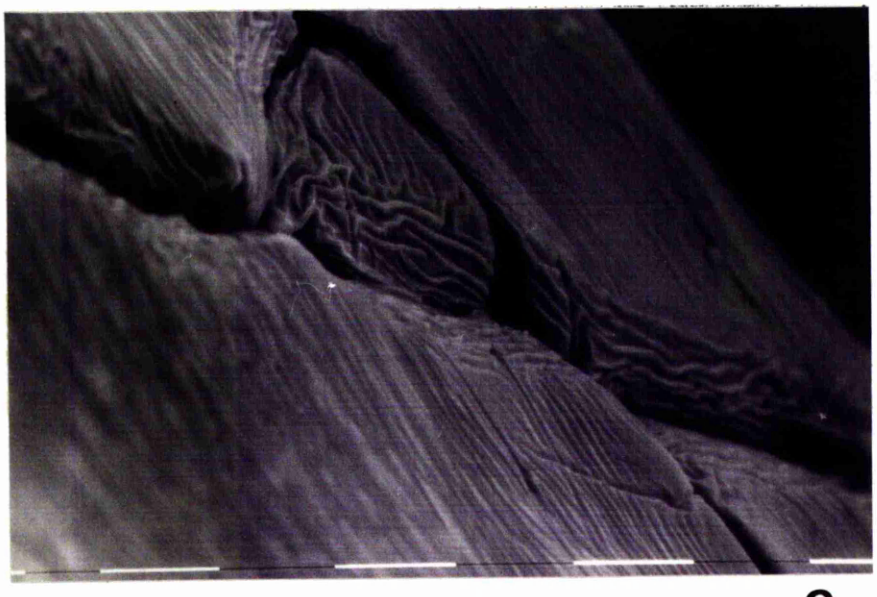


c

The gross surface structure of the lumina of the tubes was similar (Figs 35a and 36a), although on careful examination the surface of the Entron tube seemed to be slightly rougher than that of the Entriflex tube. This difference was highlighted at a higher magnification (Figs 35b and 36b) where the surface of the Entron tube (Fig. 35b) was observed to be deeply wrinkled whereas the surface of the Entriflex tube (Fig. 36b) was finely ridged. Fig. 36c shows a transverse section of an Entriflex tube and illustrates the deep fissures observed in the lumen of this type of tube.

Noticeable roughness was observed on the inner surfaces of radio-opaque tubes or tubes with radio-opaque areas (Figs 35b and 37). The distribution of the radio-opaque material varied in the different types of tube. In some tubes it was evenly distributed as small particles (Figs 35b and 37a), in some the distribution was patchy (Fig. 37b) and in some tubes angular chips of radio-opaque material were observed to be actually protruding from the tube surface (Fig. 37c).

Although, in general, the outer surfaces of the tubes appeared to be slightly smoother than the inner surfaces (Figs 33 and 34) some major defects were observed in the outer surfaces of the tubes, e.g. deep pits in the outer surface of radio-opaque PVC (Clinifeed 1/85, Roussel) and polyurethane (Entron, Franklin Medical) tubes (Fig. 38).

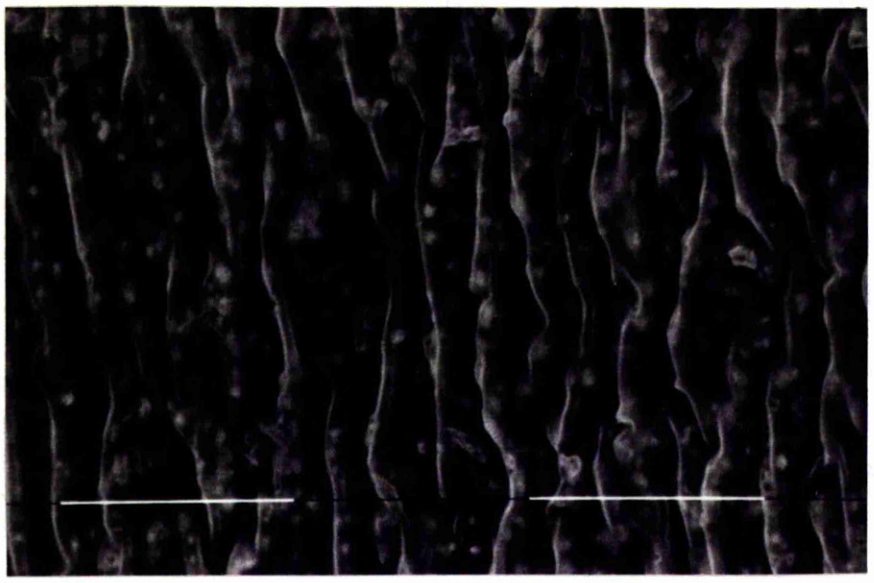
FIGURE 37 Surface irregularities due to the presence of radio-opaque material.

a) evenly distributed particles of radio-opaque material in a PVC tube.

b) patch of radio-opaque material in a PVC tube.

c) angular chips of radio-opaque material protruding from inner surface of a polyurethane tube.

Scale bar = 10 μm



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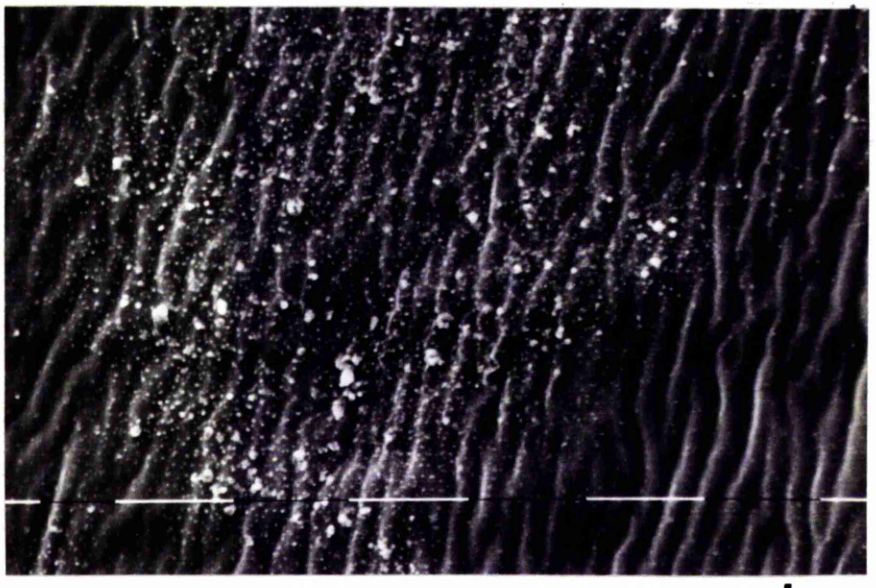

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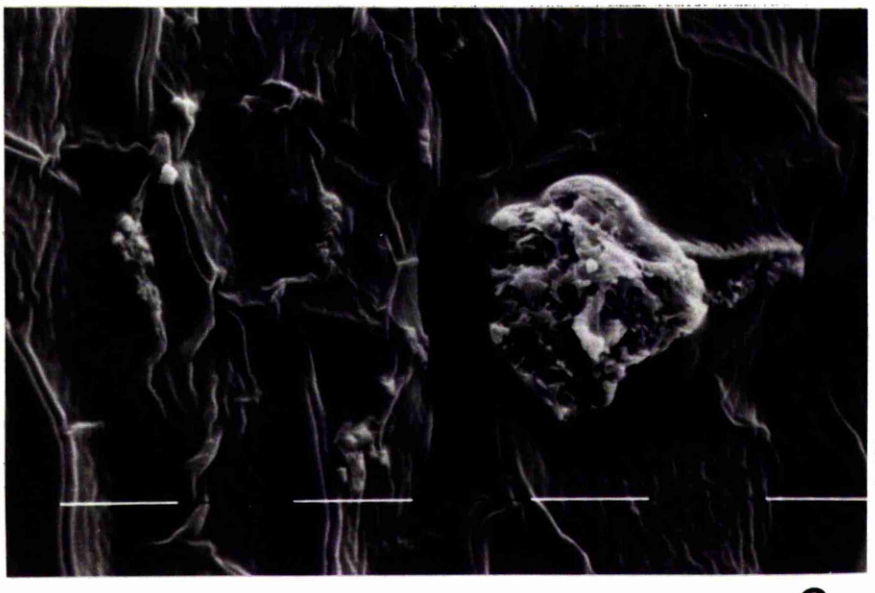

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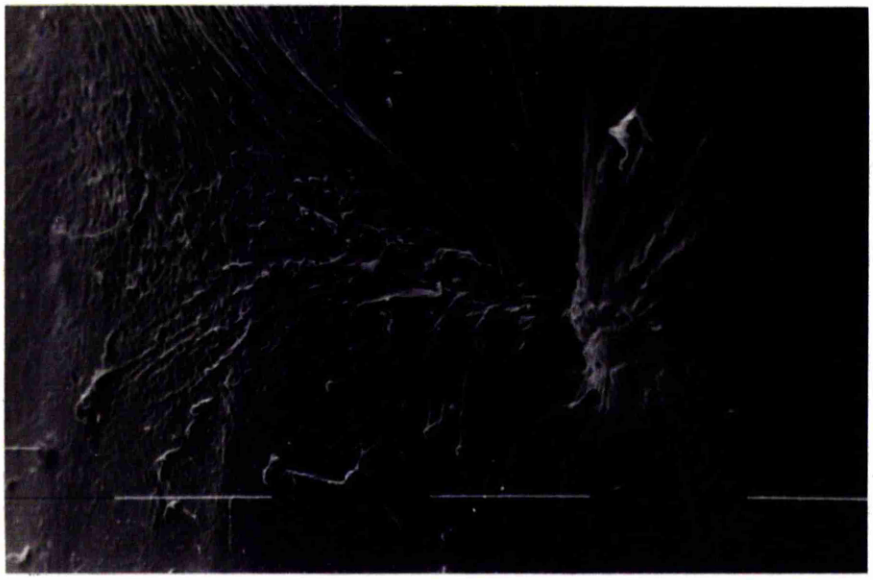

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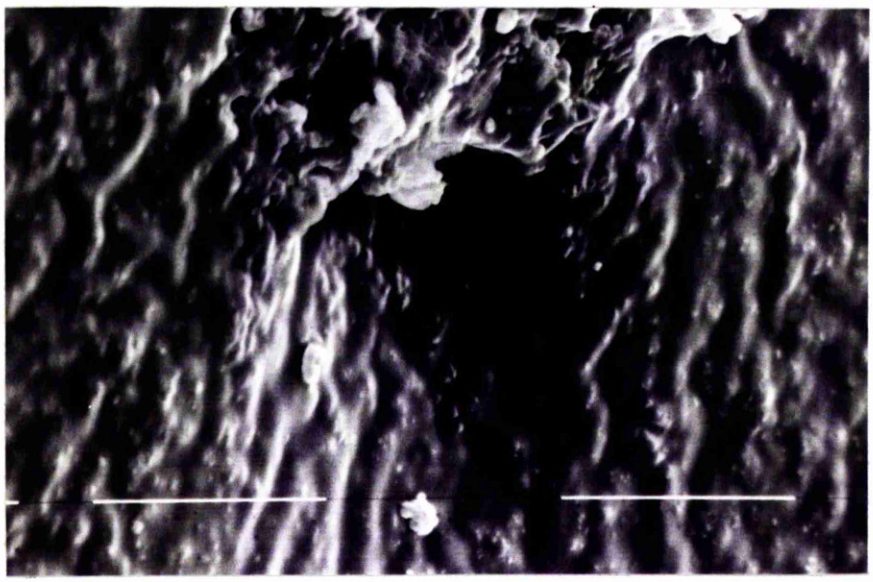


b

FIGURE 38 Deep pits in the outer surfaces of tubes, (a) polyurethane tube (Entron, Franklin Medical), (b) radio-opaque PVC tube (Clinifeed 1/85, Roussel).

Scale bar = 10 µm

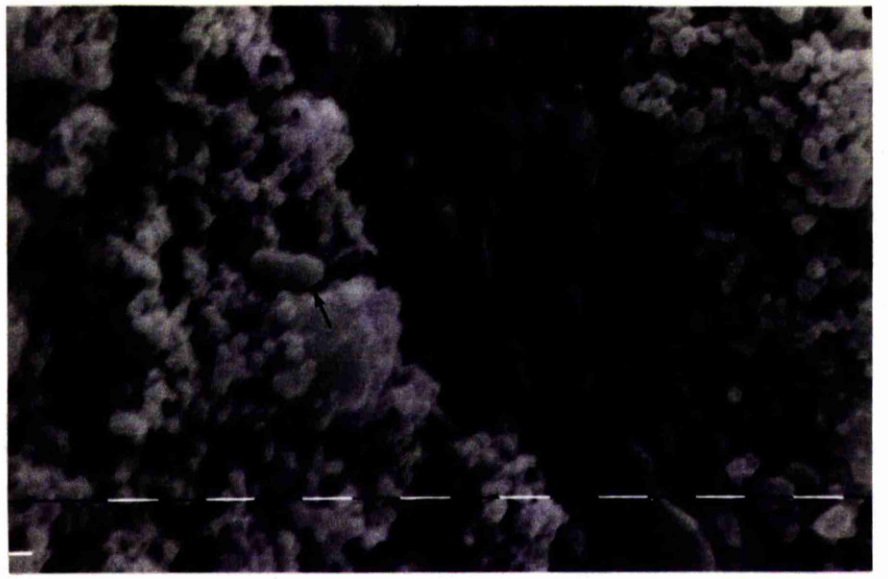
b) Perfusion of tubes with contaminated feeds

Fig. 39a shows the result of perfusing a tube with Clinifeed ISO contaminated with an initial inoculum of 10^2 Staph. aureus ml^{-1} at a rate of 125 ml h^{-1} for 8h. After 8h the feed contained over 10^4 cfu ml^{-1}. Particles of the feed could clearly be seen coating the lumen of the tube and dividing Staph. aureus cells were visible. Similar results were obtained for tubes perfused with Clinifeed ISO experimentally contaminated with E. coli. Also, particles of feed were observed trapped in grooves and other surface irregularities on the inner surface of tubes that had been perfused for 8h with sterile Clinifeed ISO.

Bacilli present as contaminants in the Triosorbon multiplied over the 8h of the experiment and can be seen amongst the particles of feed in Fig. 39b.

In the experiments where the tubes were perfused with 0.1% (w/v) peptone water contaminated with either Staph. aureus or E. coli the inner surfaces of the tubes were found to have patches of amorphous material deposited on them (Fig. 40).

Fig. 41 shows the large amount of material coating the inner surface of a tube that had been perfused for 24h at a rate of 125 ml h^{-1} with Clinifeed ISO contaminated with Staph. aureus. This tube was not rinsed in water prior to fixing in order to examine the total



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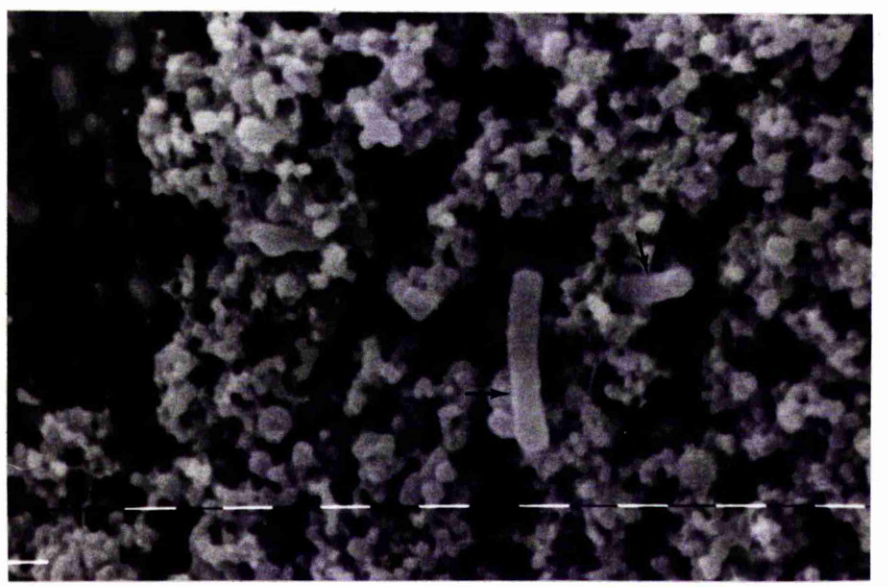

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FIGURE 39 Internal surfaces of tubes (radio-opaque PVC) following perfusion for 8h with (a) Clinifeed ISO experimentally contaminated with Staph. aureus and (b) Triosorbon, contaminated with bacilli.

Adherent particles of feed and bacterial cells (arrows) can be seen .

Scale bar = 1 µm

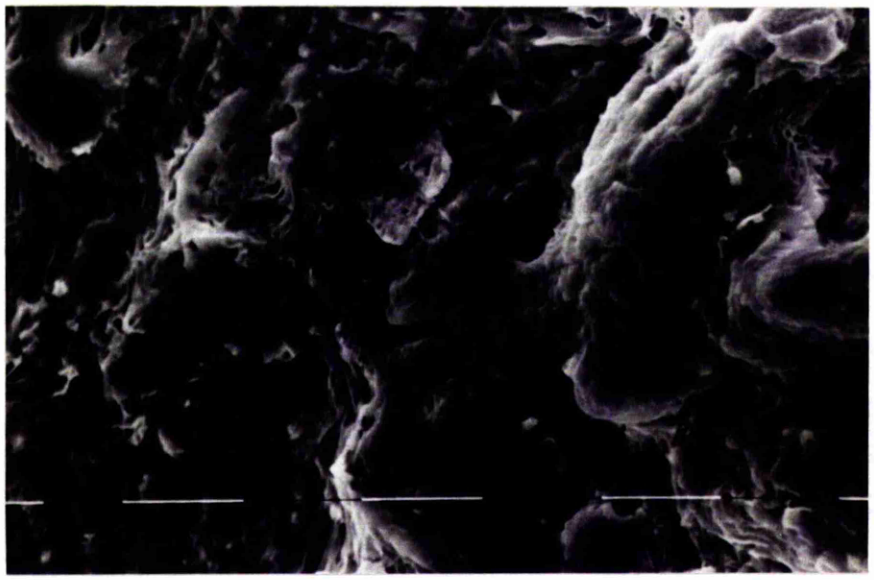


FIGURE 40 Amorphous deposits in tubes (radio-opaque PVC) perfused for 8h with 0.1% ($^{w}/_{v}$) peptone water contaminated with Staph. aureus.

Scale bar = 10 µm

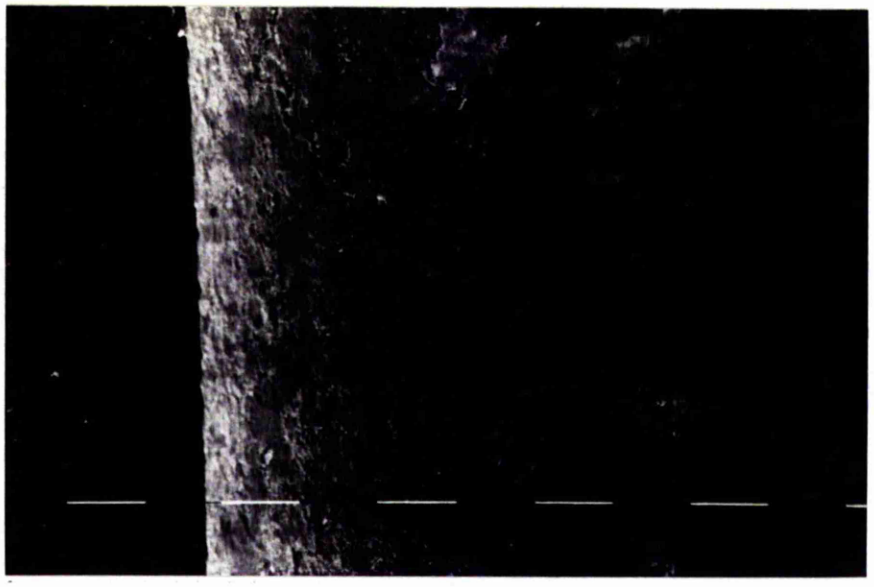
FIGURE 41 Deposits in a tube (radio-opaque PVC) perfused for 24h with Clinifeed ISO experimentally contaminated with Staph. aureus.

a) Overall view of inner surface of tube showing deposited material.

Scale bar = 100 μm

b) + (c) Enlarged views showing that the inner surface of the tube is virtually obscured by the deposited material.

Scale bar = 10 μm



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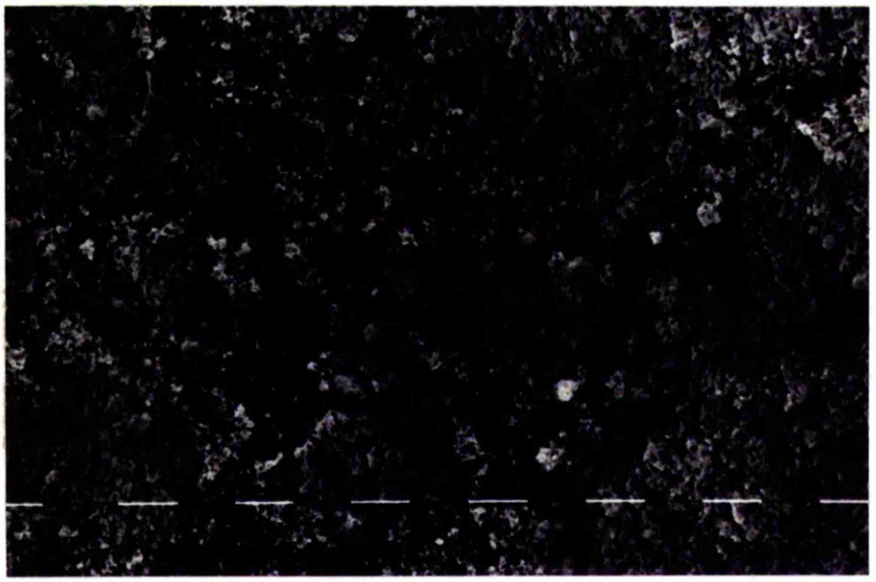

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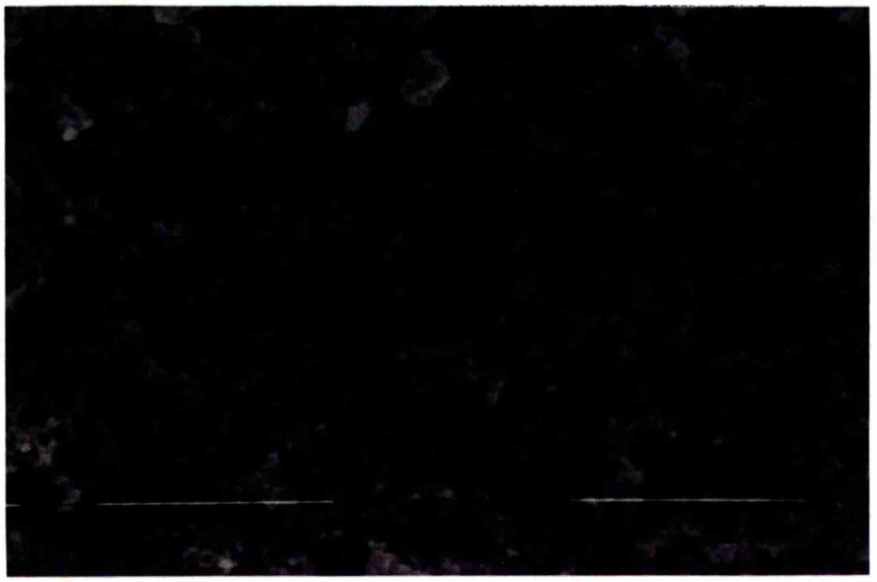


c

amount of residual material left in the tube after administration. An overall view of the inner surface of the tube is presented in Fig. 41a showing patches of material deposited over it. Increasing the magnification (Figs 41b and 41c) revealed that the inner surface of the tube was almost totally obscured by the deposited material.

The effect of irrigating the tubes with sterile water during the course of administration demonstrated that some of the deposited material could be removed by this method. Large areas of deposited feed and bacteria were again observed after tubes had been perfused for 8h with Clinifeed ISO contaminated with Staph. aureus. Irrigating the tubes with sterile water resulted in much of this material being removed. However, when tubes were perfused with contaminated feed after they had been irrigated with sterile water, deposits were once again observed on the inner surfaces of the tubes.

c) Immersion of tubes in contaminated feeds

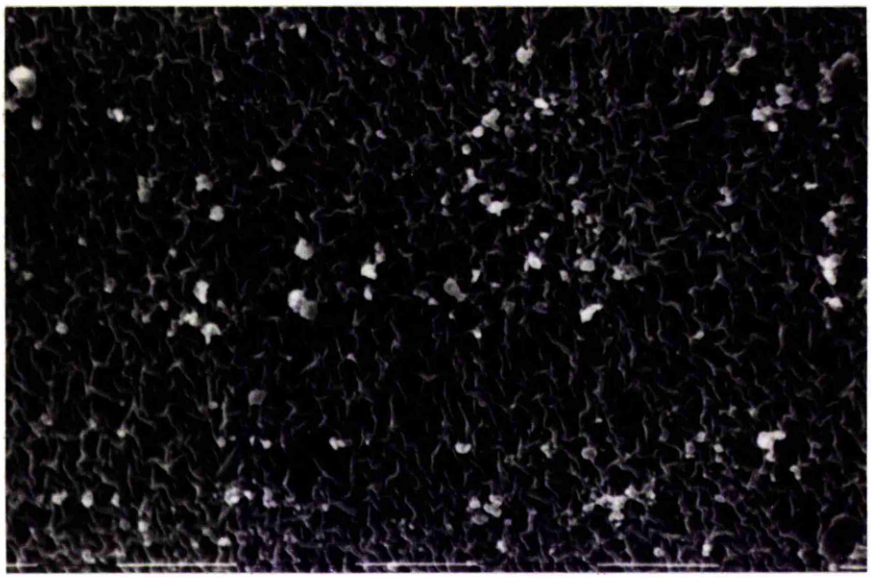
In all the experiments it was found that there was more material adhering to the sections of tube that had been incubated in contaminated feed for 24h than to those incubated for 1h.

Fig. 42 shows the appearance of the outer surface of sections of PVC tubes (Aldon Naso-Duodenal, Levins) that had been incubated for either 1h (Fig. 42a) or 24h (Fig. 42b) in Clinifeed ISO contaminated with Staph. aureus. The viable count was 10^3 and 10^9 cfu ml^{-1} after

FIGURE 42 Outer surface of PVC tube after immersion in feed experimentally contaminated with Staph. aureus, (a) after 1h immersion (b) and (c) after 24h immersion.

Bacterial cells and particles of feed can be seen on the reticulated surface (arrows).

Scale bar = 10 μm.



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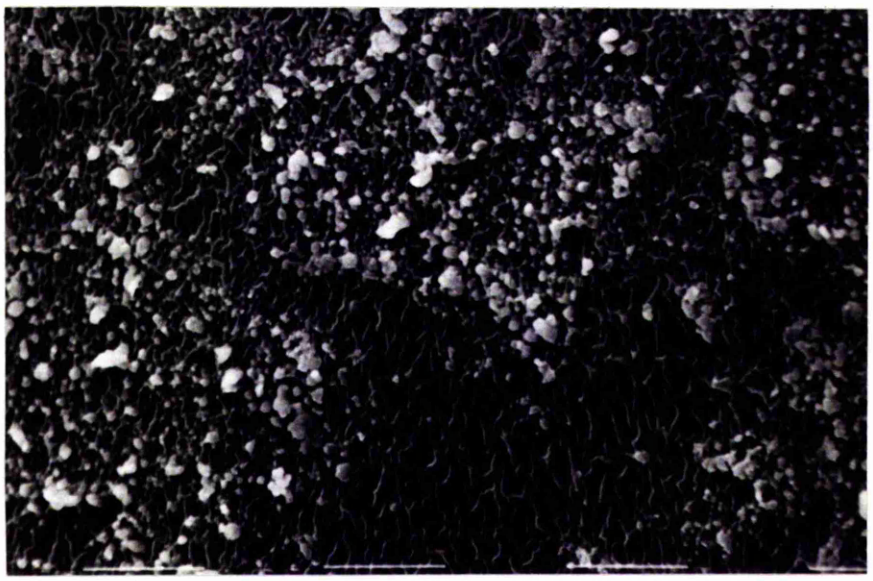

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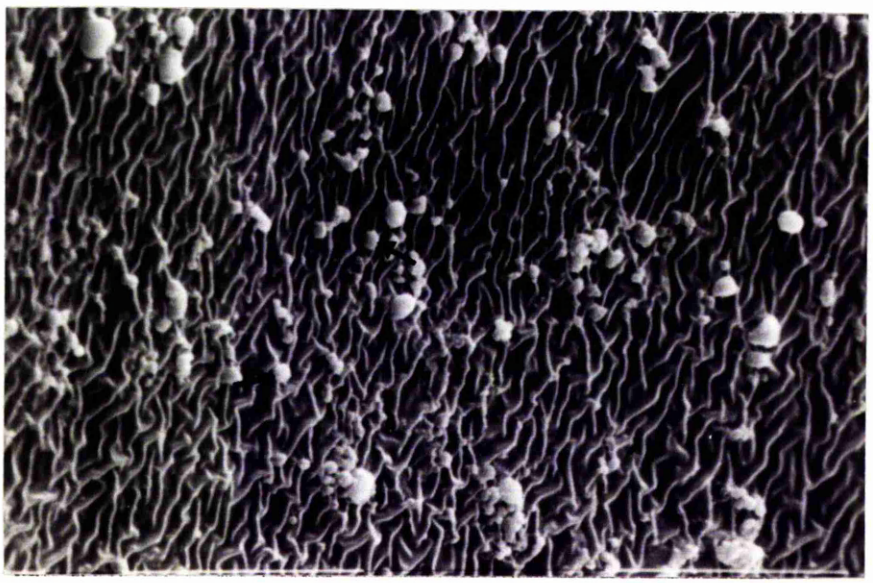
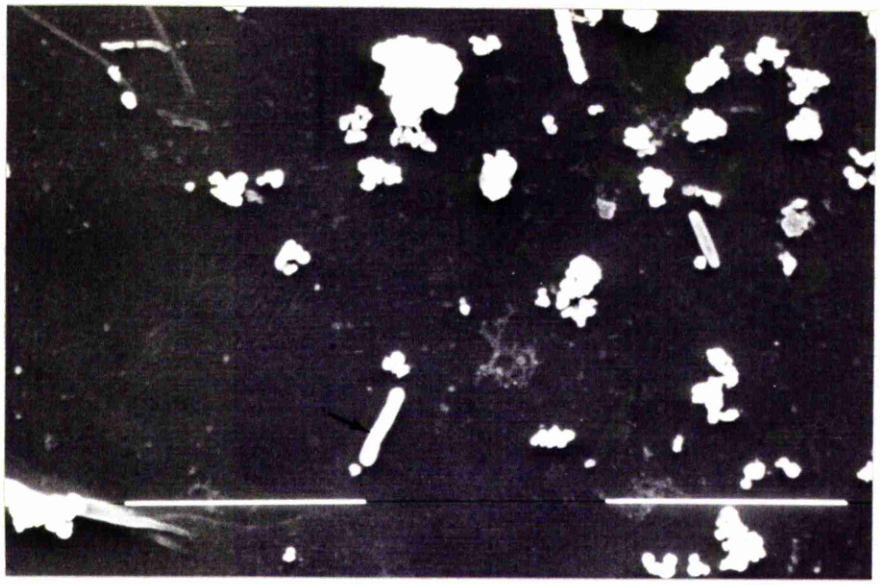


c

1h and 24h respectively. After 1h a few particles of feed were observed to have lodged in irregularities in the surface of the tube (Fig. 42a) whereas after 24h a greater proportion of the surface area was coated with feed particles (Fig. 42b) and some bacterial cells were visible (Fig. 42c). After 1h, the amount of material observed on the outer and inner surfaces of the sections of PVC tube was about the same. However, after 24h there appeared to be slightly more material on the inner surface of the tube than the outer surface (Fig. 43b). Similar results were obtained for sections of tube incubated in Triosorbon (Fig. 43a) and in Clinifeed ISO contaminated with E. coli.

After 24h incubation at 37°C in Clinifeed ISO contaminated with Staph. aureus numerous large patches of material were observed on the inner surface of the radio-opaque PVC tube (Clinifeed 1/85, Roussel) (Fig. 44a). Bacterial cells may have been present in this material but could not be seen clearly. There appeared to be less material on the outside of the tube, but there were a number of pits on the outer surface in which feed particles and bacterial cells were trapped (Fig. 44b). Similar results were obtained for radio-opaque PVC tubes incubated in Clinifeed ISO contaminated with E. coli and Fig. 44c shows one of a number of small colonies observed on the inner surface of a tube incubated for 24h.

An interesting phenomenon was observed on some of the sections of radio-opaque PVC tube that had been incubated for 24h in Triosorbon.



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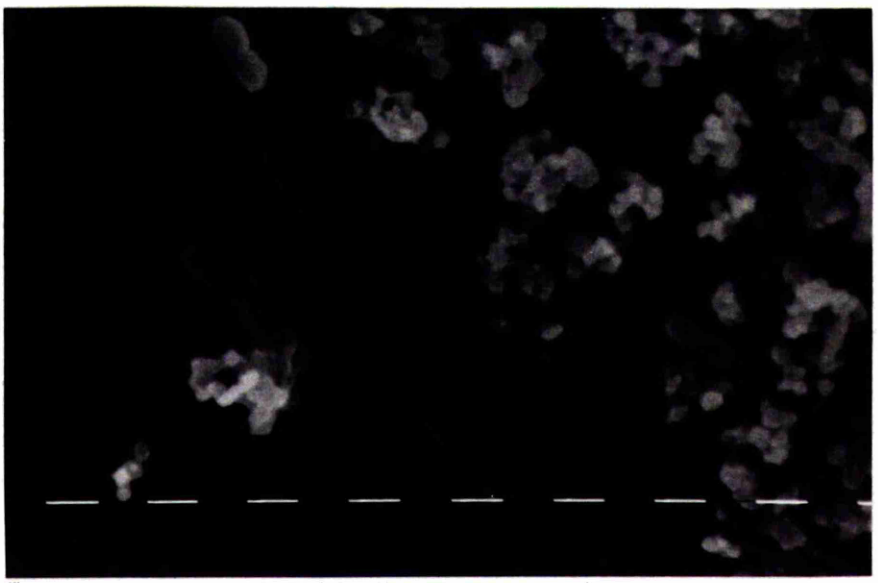

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FIGURE 43 Inner surface of PVC tube after immersion in feed at 37°C for 24h. (a) Triosorbon contaminated with bacilli and (b) Clinifeed ISO experimentally contaminated with Staph. aureus.

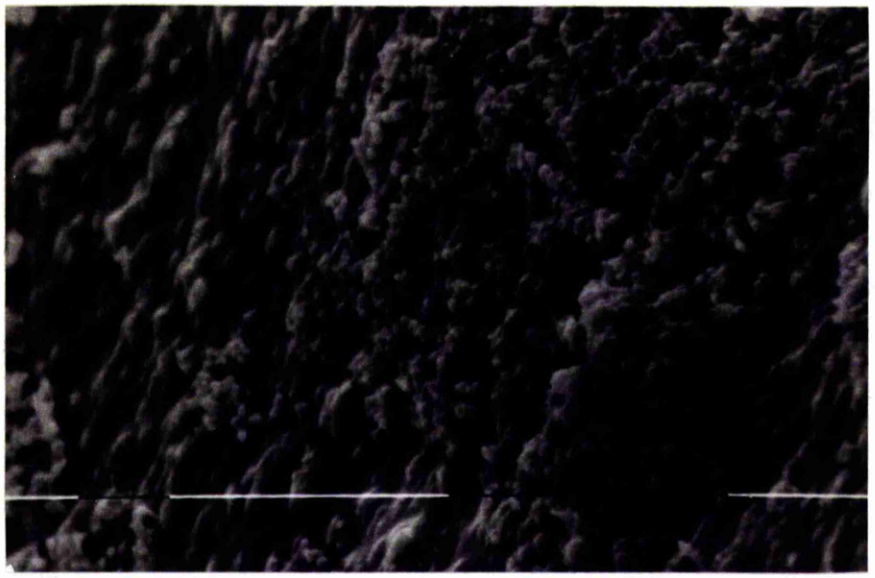
Bacterial cells can be seen (arrows).

a) Scale bar = 10 µm
b) Scale bar = 1 µm

FIGURE 44 (a) Inner and (b) Outer surfaces of radio-opaque PVC tube (Clinifeed 1/85) after immersion for 24h in Clinifeed ISO experimentally contaminated with Staph. aureus. Bacterial cells and particles of feed can be seen trapped in a pit on the outer surface of the tube (b, arrow).

(c) Inner surface of radio-opaque PVC tube (Clinifeed 1/85) after immersion for 24h in feed experimentally contaminated with E. coli. A clump of bacterial cells is clearly visible.

Scale bar = 10 μm



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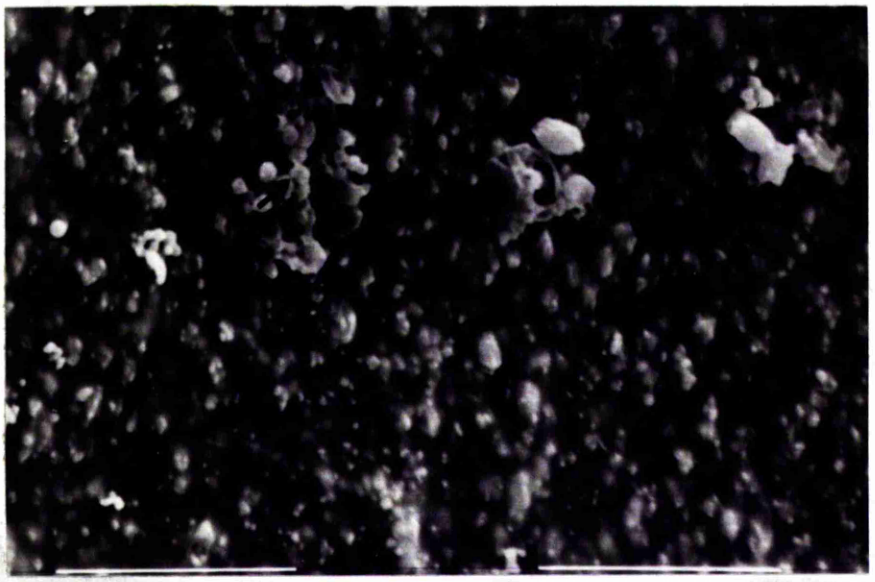

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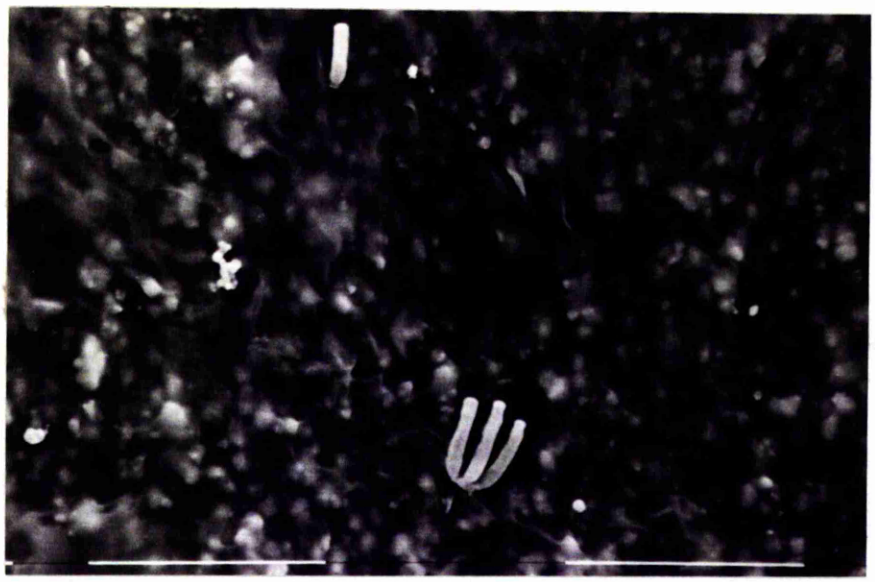
Patches of the inner surface of the tube were covered with a thin film of transparent material (Fig. 45a) and the rod-shaped bacteria present in the Triosorbon appeared to be 'cemented' to the tube surface (Fig. 45b).

Particles of feed and bacterial cells were present on both the inner and outer surfaces of the sections of radio-opaque polyurethane tube incubated in Clinifeed ISO plus Staph. aureus, Clinifeed ISO plus E. coli and Triosorbon. Slightly more material was present on the inner surfaces than the outer ones. In Fig. 46 it can be seen that bacterial cells (Staph. aureus) were not only trapped in surface fissures but also attached to the smooth surface of the tube.

The small amount of material adhering to the surfaces of the sections of silicone tube after 24h incubation with Clinifeed ISO plus Staph. aureus is illustrated in Fig. 47. Very little material was observed on either the inner or outer surfaces of the silicone tubes, but a few bacterial cells (Fig. 47b) appeared to be attached to the comparatively smooth surfaces of these tubes.



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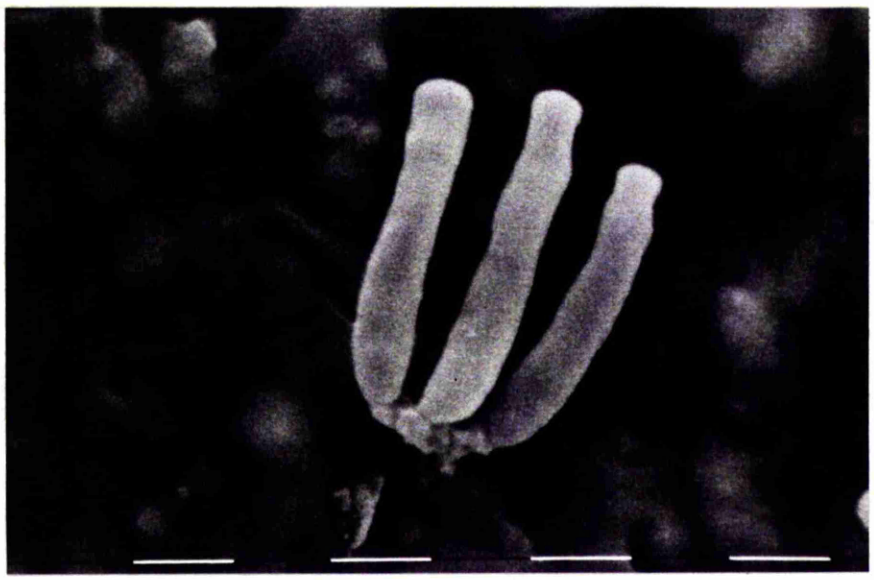


b

FIGURE 45 Inner surface of radio-opaque PVC tube after 24h immersion in Triosorbon, showing a layer of amorphous material coating the surface. Adherent bacilli are also visible.

(a) Scale bar = 10 μm

(b) Scale bar = 1 μm

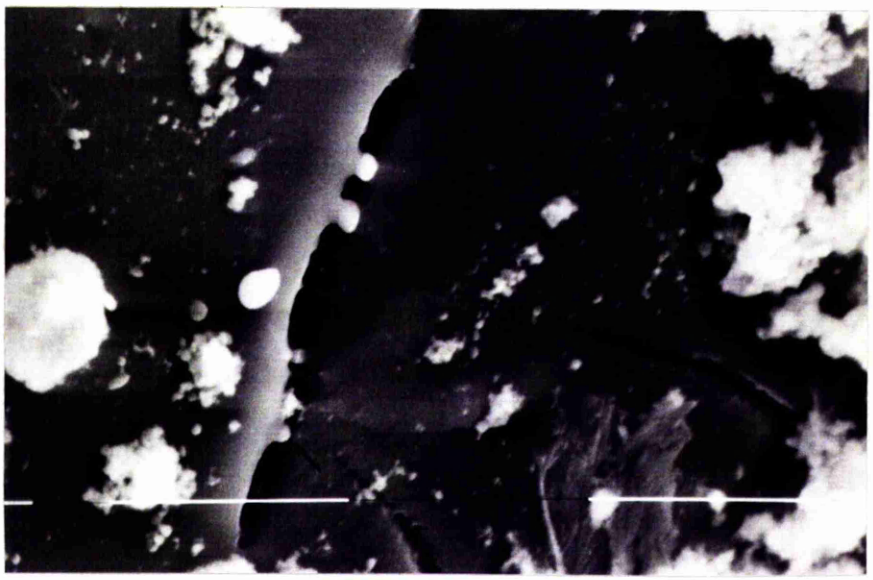
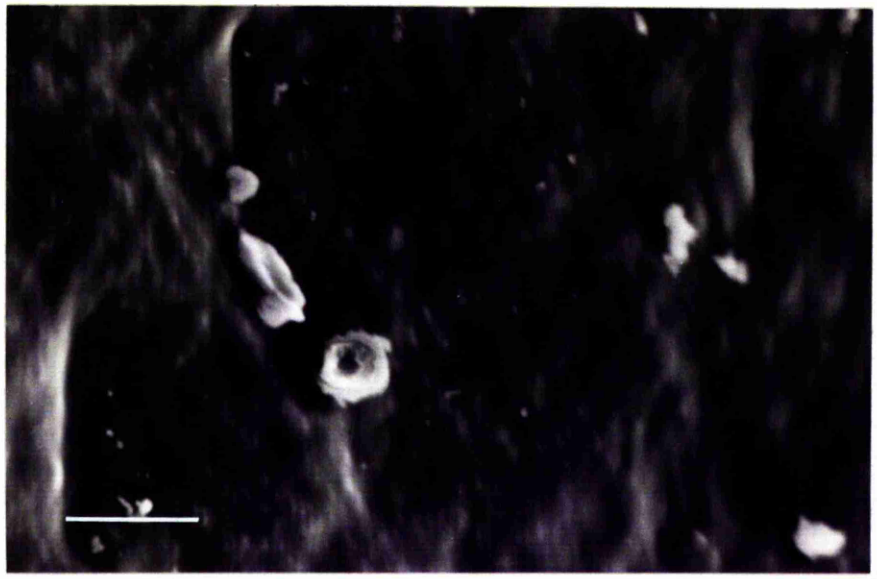


FIGURE 46 Inner surface of radio-opaque polyurethane tube after immersion for 24h in feed experimentally contaminated with Staph. aureus. Particles of feed and bacterial cells (arrows) can be seen.

Scale bar = 10 μm



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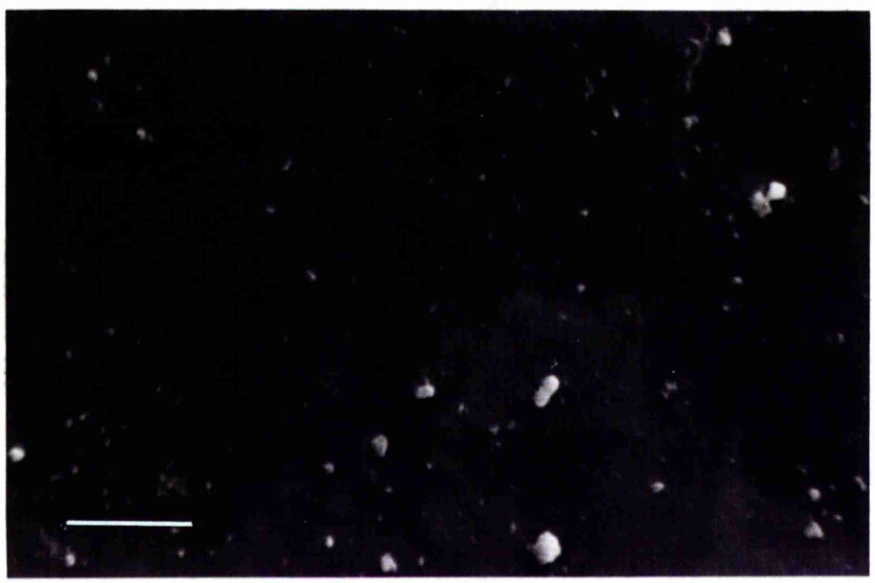


b

FIGURE 47 (a) Inner and (b) outer surfaces of silicone tube after 24h immersion in Clinifeed ISO experimentally contaminated with Staph. aureus. Dividing bacterial cells can be seen (arrow).

Scale bar = 10 μm

CHAPTER 7

DISCUSSION

7.1 THE USE OF ENTERAL FEEDS IN HOSPITALS : A SURVEY OF HANDLING PRACTICE

The surveys carried out during 1982 - 1984 revealed a variety of practices in the preparation and administration of enteral feeds. Over the three years it was found that the feeds most frequently used were the ready-to-use commercial formulae ($\geqslant$70% of the feeds). This result differs from that of Tredger *et al*. (1981) who reported that in a survey of 72 hospitals made in 1979 the type of tube feed most frequently selected in 38 (51%) of the hospitals was hospital-prepared feed. This apparent increase in the use of ready-to-use commercial feeds may be explained by the improvements there have been in the range of commercial formulae available during recent years and a greater awareness of the potential hazards of microbial contamination of enteral feeds.

Ingredients used in hospital-prepared feeds included pasteurised milk, dried skim milk, Complan and raw egg. Tredger *et al*. (1981) also reported the use of Complan in hospital-prepared feeds. Complan and raw eggs have both been cited as sources of contamination in enteral feeds. For example, Bastow *et al*. (1982) reported the presence of *B. cereus* in Complan and in 1981 Gill and Gill suggested that raw eggs used in a feed might have been the source of *Salm. enteritidis* infection in a patient.

Mixing and/or diluting the feed ingredients provides another major source of contamination of feeds (Casewell *et al*. 1981, Bastow

et al. 1982) and in the present survey it was found that in 80% of the hospitals additional substances were mixed with the ready-to-use commercial feeds. The most commonly added substance was tap water but in two hospitals distilled water was used. In only two hospitals was the water boiled or sterilised prior to use even though it has been reported that both distilled water and tap water may support the growth of Gram negative bacteria, including Ps aeruginosa and E. coli (Burman and Colbourne 1977, Holmes and Allwood 1979).

Only two hospitals attempted to sterilise the liquidisers and other equipment used to mix the feeds in spite of the fact that there have been numerous reported instances of liquidisers and kitchen equipment being the source of contamination of hospital food (Montgomerie et al. 1970, Casewell 1977, Sharpe et al. 1979, Casewell et al. 1981, Bastow et al. 1982).

In most of the hospitals the feeds were refrigerated between preparation and administration, however, there were three reports of feeds being left at kitchen or ward temperature for between 4 and 12h prior to administration. The results of the present study and those of Bastow et al. (1982) demonstrate that rapid multiplication of contaminants could occur during this time.

Many manufacturers recommend that nutrient containers and giving sets are for single use only. However, in the present survey nutrient containers were often refilled over a 24h period and in two instances

the nutrient containers were used for more than 24h. The results of the present study showed that when the nutrient containers were refilled, the residue of feed in the container contaminated the new feed and resulted in high levels of contamination.

Finally it should be pointed out that over the three years of this survey, there were four definite cases of the feeds being heavily contaminated with micro-organisms

7.2 MICROBIOLOGICAL QUALITY OF ENTERAL FEEDS AND FEED INGREDIENTS

The microbiological criteria for special dietary foods recommended by the International Commission on Microbiological Specifications for Foods (ICMSF; 1974) are summarised in Table 18

TABLE 18 : Recommended microbiological limits for special dietary foods (ICMSF 1974)*

Test	n	c	Limit per g or ml m	Limit per g or ml M
aerobic plate count (APC)	5	1	10^4	10^6
Staph. aureus	10	1	10	10^2
E. coli	5	2	<3	10
B. cereus	10	1	10^2	10^4

* n = number of units in a sample
c = the maximum allowable number of marginal quality units
m = the microbial count that separates good from marginal quality food
M = the microbial count that separates marginal from defective quality food. Values greater than M are considered unacceptable.

None of the units tested in this study exceeded these m or M values for APC, Staph. aureus, E. coli or B. cereus. However, in 1977 the Food and Agriculture Organisation of the United Nations (FAO) established the following microbiological criteria for powdered infant formulae : APC n = 5, c = 2, m = 10^3 and M = 10^5. It seems reasonable to suggest that the criteria for enteral feeds should be as strict as those for infant formulae and, using the FAO criteria, all of the units of Complan and Build-Up equalled or exceeded the m value and could be considered to be of marginal quality. Also, although the ICMSF recommendations are specifically for 'special foods for consumer groups with increased susceptibility' and include 'food for hospitals' (ICMSF 1974), the major problem with enteral feeds is the fact that the feed is kept at ward temperature while being administered and may also be left at ward temperature (or even put onto the top of a warm meal trolley) for extended periods during transport from the diet kitchen to the ward (Anon 1982, Gibbs 1983). Therefore, the results of the experiment in which feeds and feed constituents were incubated at 4, 25 or 37°C for up to 24h, may give a more accurate indication of the numbers of bacteria received by the patient. After 8h at 25°C, counts had reached 10^1 - 10^4 and after 24h, 10^7 - 10^8 organisms ml^{-1}. It may also be argued that the organisms identified are aerobic sporeformers and Staph. albus and therefore 'non-pathogenic', however, presumptive B. cereus organisms were isolated from all six of the contaminated products (Build-Up, Casilan, Complan, Enteral 400, Maxipro and Triosorbon) and Bastow et al. (1982) have reported the presence of B. cereus in Complan.

Since 3.6×10^4 B. cereus organisms g^{-1} of food have been reported to cause food poisoning symptoms in healthy adults (Goepfert et al. 1972), the potential hazard to compromised patients is obvious. The isolation of Bacillus spp. including B. cereus from feeds containing milk or whey proteins is not unexpected since B. cereus can survive pasteurisation (Hobbs and Christian 1973) and both B. cereus and E. coli have been found in spray-dried skim milk (Thomson, Harmon and Stine 1978).

In a review of the role of B. cereus and other Bacillus spp. in food poisoning and other clinical infections, Gilbert et al. (1981) not only emphasise the importance of B. cereus but also discuss the increasing accumulation of evidence that B. licheniformis and B. subtilis act as food poisoning agents. Also, the authors report that all three of these organisms, together with species such as B. circulans, B. macerans, B. pumilus and B. sphaericus, have been reported as capable of acting as opportunistic pathogens to clinically compromised hosts.

An organism of the B. sphaericus - B. brevis group was the main organism isolated from Triosorbon. This feed is used for patients with malabsorption syndrome, inflammatory bowel disease, gastro-intestinal fistulae and liver disease (Merck Ltd.), all of which conditions are likely to make the patients particularly susceptible to infection.

Concern about acceptable levels of microbial contamination in non-sterile pharmaceutical products has led to the proposal of microbiological guidelines for these products. For oral liquids it is suggested that the total viable count should not exceed 10^2 cfu ml^{-1} (T. Munton 1984; pers. comm.). Levels of dosage of these liquids range from 5 to 20ml whereas patients receiving naso-gastric tube feeds receive up to fifty times this, viz. 500 - 1000ml. Therefore at least equally strict, or stricter limits would be appropriate for enteral feeds. Ideally, hospital Bacteriology Departments should make quality checks on all batches of dried products intended for inclusion in enteral feeds and only sterile ingredients should be used in feeds to be given to particularly susceptible patients such as those in intensive care units and those whose natural resistance is lowered either by infection or clinical treatment.

The results of this study on enteral feeds and of those by Bastow et al. (1982) and Gibbs (1983) indicate that it should not be assumed that 'all feeds are packed sterile' as stated by Hanes (1983).

7.3 CONTAMINATION OF ENTERAL FEEDS DURING ROUTINE HOSPITAL USE

The levels of contamination recorded in this study which ranged from 4.5×10^2 to 2.2×10^3 cfu ml^{-1} of residual feed are quite low when compared with the results from studies in other hospitals. For example, in a four week survey of an Intensive Care Unit (ICU) (Casewell and Phillips 1978a), 32 (68%) out of 47 naso-gastric feeds

were found to be contaminated with up to 10^4 klebsiellae ml^{-1}. In a further three month survey samples of 13 milk shakes and dietary milk allowances, 8 ice-creams and 23 naso-gastric feeds were examined and klebsiellae were isolated from 77% of these items (Casewell and Phillips 1978b). Subsequently, Casewell et al. (1981) detected 10^5 - 10^6 mixed coliforms ml^{-1} in naso-gastric feeds in an ICU and Pottecher et al. (1979) reported counts of 10^6 - 10^9 organisms ml^{-1} in feeds given to patients in another ICU. The lower levels of contamination recorded at the Glasgow Western Infirmary may be explained by the fact that only the sterile commercially prepared feeds Ensure, Nutrauxil and Isocal are used in the Intensive Therapy Unit (ITU) of the Glasgow Western Infirmary. Also, over the period of this study the only ingredient added to the feeds was sterile water, and because of the care taken in the preparation of these feeds any contamination occurring during mixing was very low when compared with the results reported by Casewell et al. (1981) and Bastow et al. (1982).

It was also standard practice in the ITU of the Glasgow Western Infirmary to use a new sterile nutrient container and giving set each time a new supply of feed was given to a patient and the results presented in Ch. 5.2.3 indicate that this procedure helps to minimize the growth of contaminants.

On one occasion, strains of Cand. albicans with the same antibiotic resistance patterns were isolated from the gastric aspirate

of a patient (Patient 1) and subsequently from the residual feed in both the nutrient container and giving set of a discarded feeding set. Casewell and Phillips (1978a,b) demonstrated that food strains correspond to those isolated from infected patients when in a study of an ICU with hyperendemic klebsiella infection there was a correlation between food, faecal and patient clinical isolates over a four-week period. In another hospital, Shooter et al. (1969) found Ps aeruginosa in 29 out of 378 items of food destined for patients in wards in which the faecal carriage rate of Ps aeruginosa was high and where patients tended to carry the same strain. Subsequently E. coli was found (Cooke et al. 1970) contaminating 63 out of 873 items of hospital food, and food strains appeared in the stools of five patients following ingestion. Cooke et al. (1969) suggested that the food strains might account for the changing E. coli flora reported in patients in the same ward. More recently Casewell et al. (1981) described a patient who acquired septicaemia with Ent. cloacae from a contaminated enteral feed. Unfortunately it was not possible to carry out further studies on the feeds given to Patient 1 as the patient left the ITU shortly after the feed contaminated with Cand. albicans was examined.

The isolation of a number of Gram negative bacteria including Acinetob. calcoaceticus var. anitratus, K. aerogenes, K. oxytoca, Ent. sakazakii and Alcaligenes spp. from the residual feeds was a cause for concern since it indicated a potential hazard to the patient. In a unit where less care is taken in the handling and replacement of the

feeding sets, rapid multiplication of these organisms could occur (Bastow et al. 1982). Although the numbers of organisms detected in this study were $\leqslant 10^3$ ml^{-1} it is pertinent to add that in a study into the role of salads as a source of potential pathogens Remington and Schimpff (1981) stated that 'for the patient with reduced resistance to colonization due to the systemic administration of antimicrobials, 1g of salad with 10^3 Gram-negative rods is probably sufficient to lead to persistent colonization of the intestinal tract'.

It may also be argued that the main organisms isolated from the remaining feeds were Bacillus spp. and are therefore non-pathogenic. However, once again it must be emphasised that a number of authors have reported that organisms of the genus Bacillus can cause serious infections in clinically compromised patients (Farrar 1963, Pearson 1970, Idhe and Armstrong 1973, Von Gravenitz 1977, Tuazon et al. 1979, Gilbert et al. 1981).

The results of this study showed that contamination of the feeds occurred even when the sterile feed was transferred directly from the can or bottle to the sterile nutrient container, whereas Bastow et al. (1982) reported that there was no contamination of the feed during the procedure of filling the nutrient containers. Possible reasons for these apparently contradictory results may include the fact that although the nutrient containers sampled by Bastow et al. were filled on the ward, they were part of a special study in which it seems

likely that particular care would have been taken with this procedure as compared to the routine handling of these feeds on a busy ITU in the Glasgow Western Infirmary. It should also be noted that no contamination was detected in the nutrient containers filled with sterile feed during the studies of the growth of micro-organisms in enteral feeds under simulated ward conditions described in Ch. 5.2.3. Thus, under carefully controlled experimental conditions it is possible to avoid contamination of the feeds whereas this is not so easy for nursing staff on a busy ward. This view is confirmed by the findings of Schroeder et al. (1983) who, in an evaluation of the procedures for care and preparation of feeding solutions, observed much higher contamination rates under conditions of actual use than in a careful simulation.

7.4 EXPERIMENTAL CONTAMINATION OF ENTERAL FEEDS

In the study of the growth of bacteria in Clinifeed ISO, Triosorbon, Vivonex Standard and Vivonex HN under laboratory conditions, multiplication of all the test organisms to levels in excess of 10^4 organisms ml^{-1} of feed within 8h was observed both at 25 and 37°C in Clinifeed ISO and Triosorbon. Similar results have been reported by Furtado et al. (1980) and Simmons (1981) in studies on the multiplication of bacteria in Osmolite and a Complan-based home-made feed. Also in studies by Fagerman et al. (1984) and Paauw et al. (1984), similar rates of growth were recorded for Ent. cloacae organisms inoculated into samples of Ensure, Travasorb and Vital enteral feeds held at 22.4°C.

The counts for all the test strains in all the feeds remained fairly constant at 10^2 - 10^3 ml^{-1} over 24h at 4°C, and comparison of these figures with those recorded at 25°C demonstrate the importance of storing prepared feeds in a refrigerator.

Half-strength Vivonex also provided an excellent growth medium for the test strains, with the exception of Staph. aureus, counts after 8 and 24h at 25 and 37°C being only slightly less than those in Clinifeed ISO, possibly because of the lower pH (5.5) of Vivonex. The noticeable inhibition of Staph. aureus might be explained by the low pH, the high concentration of glucose solids and the presence of both sorbate and acetate in the formulation. Schmidt, Gould and Weiser (1969) found that 1mg sorbate g^{-1} was effective in preventing the growth of Staph. aureus in artificial cream pies acidified to pH 4.5 - 5.0. Vivonex has a pH of 5.5 and at half-strength contains 0.25 mg potassium sorbate ml^{-1}.

Also in a study of peritoneal dialysis solutions, Zacherle and Charache (1970) found that the factors associated with the inhibition of Staph. aureus were low pH, acetate and dextrose, maximum inhibition being caused by a combination of all three factors.

In studies to examine the effects of time, temperature and preservative on bacterial growth in enteral nutrient solutions, Fagerman et al. (1984) and Paauw et al. (1984) showed that growth was inhibited by potassium sorbate. They added 0.036 or 2.0% by weight of

potassium sorbate to Travasorb, inoculated the solutions with 5 x 10^3 Ent. cloacae organisms ml^{-1} and incubated them at 22.4°C for 12h. After this time they found that the bacterial growth rate was reduced by 70 and 90% respectively as compared with a control sample of Travasorb containing no preservative. They suggested that although potassium sorbate is normally used as a fungistatic agent, it also appears to have a broad-spectrum bacteriostatic action. This has also been reported by Robach (1980) and Sinskey (1980). However, it should be emphasised that in the present study all of the test organisms except Staph. aureus multiplied rapidly in half-strength Vivonex which contained 0.25mg potassium sorbate ml^{-1}.

The lack of growth of all the test organisms at 25°C in full strength Vivonex over the first 8h of the study was probably due to a combination of low pH, high osmolarity, the inhibitory effect of potassium sorbate and an incubation temperature below the optimum of these organisms. Fagerman et al. (1984) and Paauw et al. (1984) also reported that the growth of Ent. cloacae at 22.4°C was inhibited in Vivonex. The increase in numbers of Ps aeruginosa from 10^3 to 10^6 cfu ml^{-1} between 8 and 24h reflects this organism's tolerance of unfavourable growth conditions. At 37°C Ps aeruginosa, K. aerogenes and Ent. cloacae all grew well in full strength Vivonex in spite of the low pH, high osmolarity and the presence of potassium sorbate in the product, whereas Staph. aureus and E. coli showed little growth. The bacteriostatic effect of potassium sorbate claimed by Fagerman et al. (1984) and Paauw et al. (1984) should therefore be treated with

caution, since these authors only report having tested the effect of potassium sorbate on one organism (Ent. cloacae) and at one temperature (22.4°C).

The lack of growth of all organisms in full strength Vivonex HN can be attributed to the high osmolarity of this feed (800 m osm l^{-1}). Similar results were reported by Furtado et al. (1980) and Hoestetler et al. (1982) and Stanek et al. (1983) showed that the growth of a variety of bacterial strains was inhibited in an elemental diet with an osmolarity of 580 m osm l^{-1}.

The presence of aerobic sporeforming bacilli as contaminants in Triosorbon and their rapid multiplication at both 25 and 37°C is of concern since, as previously stated, Gilbert et al. (1981) suggested that a number of Bacillus spp. can cause opportunistic infections in compromised hosts and that some may also be capable of causing food poisoning.

The results of this part of the study emphasize the importance of strict microbiological quality control of ingredients and stringent hygienic precautions during the preparation and handling of all enteral feeds, since bacteria have been shown to survive and multiply in feeds with low pH and high osmolarity as well as in neutral, isotonic feeds.

Various estimates have been made of the number of bacteria that need to be ingested for bowel colonisation to occur, but a number of authors have reported that in healthy volunteers the ingestion of 10^4 - 10^6 *E. coli*, *Klebsiella* spp. or *Pseudomonas* spp. organisms produced detectable faecal counts (Buck and Cooke 1969, Montgomerie *et al.* 1970, Shooter *et al.* 1971). Subsequently, Pottecher *et al.* (1979) suggested that 10^4 organisms ml^{-1} of feed are sufficient to colonise the digestive tract of hospital patients. In the preliminary study of the growth of *E. coli* and *Staph. aureus* in enteral feeds under simulated ward conditions it was found that a level of 10^4 organisms ml^{-1} was reached in 8h in both Clinifeed ISO and Nutrient Broth No.2 in the nutrient containers and in less than 4h in the recipient flasks when the initial inocula were 10^2 cfu ml^{-1}. This initial level of contamination might appear to be rather high until the final count (after 8h) of 10^4 - 10^5 cfu ml^{-1} in the residual feed in the nutrient container is compared with previously reported numbers of organisms in residual feeds in nutrient containers e.g. 10^5 - 10^6 ml^{-1} (Fason 1967), 10^6 ml^{-1} (Casewell 1979), 10^6 - 10^9 ml^{-1} (Pottecher *et al.* 1979) and 10^8 - 10^{10} ml^{-1} (Bastow *et al.* 1982).

Enterotoxin production by bacteria multiplying in the feed could also cause food poisoning. Bergdoll (1973) and Reiser *et al.* (1974) considered that the consumption of less than 1 μg of staphylococcal enterotoxin might be sufficient to produce food poisoning symptoms and Hobbs (1962) reported that the number of organisms needed is 5×10^5 - 1×10^6 g^{-1} of food. The feeding of volunteers with serotypes of *E.*

coli associated with infant diarrhoea produced food poisoning symptoms in healthy adults at levels of 10^6 - 10^8 organisms (Ferguson and June 1952, June, Ferguson and Waifel 1953, Wentworth et al. 1956). The severity of the intoxication is known to be influenced by the type and number of E. coli organisms and the age and physical condition of the person, newborn babies being particularly susceptible (Ayres, Mundt and Sandine 1980).

In the present study a total of 10^6 cfu ml^{-1} was present in the recipient flask within 6h for both Staph. aureus and E. coli. These counts reflect the effect of body temperature upon the multiplication of bacteria in the patient, although other factors, e.g. stomach pH and enzymic activity may adversely affect the rate of multiplication.

However, many patients on nasogastric feeds are treated with antacids or cimetidine which raises the pH of the stomach from 2.0 to approximately 5.5 (du Moulin et al. 1982). This favours the growth of bacteria and inhibits the action of pepsin on staphylococcal enterotoxins (Bergdoll 1970).

Thus the preliminary part of this investigation demonstrated that rapid multiplication of bacteria occurred in both Clinifeed ISO and Nutrient Broth No.2 under simulated ward conditions indicating that Clinifeed ISO acts as a good bacterial culture medium. An

initial inoculum of approximately 10^2 cfu ml^{-1} resulted in the critical level of 10^6 cfu ml^{-1} of Staph. aureus or E. coli being reached within 6h in the recipient flasks. It can be predicted that even with lower levels of contamination and allowing for the controlling influence of stomach pH and other antibacterial conditions, the critical number of 10^6 cfu ml^{-1} could be reached within the 8-12h period over which such feeds are commonly administered to patients.

Similar results were obtained over the first 8h of experiments designed to study the effect of various handling procedures on the rate of bacterial multiplication during the period of administration of enteral feeds. In all experiments it was found that initial contamination of the feed with 10^2 - 10^3 organisms ml^{-1} yielded counts of up to 10^6 organisms ml^{-1} in the feed in the recipient flasks within 6h. Also, it was found that refilling the nutrient containers (Clinifeeding System 3 reservoirs) with sterile feed over a period of 24h gave bacterial multiplication to levels of 10^8 organisms ml^{-1} in the feed in the nutrient containers after 24h, counts of 10^6 - 10^7 organisms ml^{-1} being obtained in the feed in the nutrient containers after only 16h. Counts of 10^3 - 10^4 organisms ml^{-1} recorded on samples of feed taken from the nutrient containers immediately after they had been refilled at 8h demonstrate that the residue of contaminated feed acted as an inoculum for the sterile feed used to refill the containers. In a similar study into the effect of refilling

nutrient containers over 24h carried out by Bastow et al. (1982) viable counts were also reported to have risen from 10^2 to 10^8 organisms ml^{-1} after 24h.

In the present study it was found that the numbers of organisms in the feed in the nutrient containers after 24h were reduced to 10^2 - 10^3 ml^{-1} when they were replaced with new sterile containers containing sterile feed instead of the original nutrient containers being left in situ and refilled with sterile feed. However, the fact that samples of feed taken from the 8h replacement nutrient containers immediately after they had been connected to the system yielded counts of up to 10^1 organisms ml^{-1} showed conclusively that the contents of the replacement containers were still becoming contaminated. The organisms were introduced into each replacement nutrient container on the surface of the connector spike and the internal surface of the air inlet tube of the giving set attached to the new container. Changing the giving sets as well as the nutrient containers, a procedure recommended by many manufacturers of enteral feeding systems, resulted in no organisms being detected in the feed in the nutrient containers after the first 8h of the experiment. However, the actual number of organisms entering the patient's stomach or intestine is shown not by counts on the feed in the nutrient container, but by those made on the feed collected from the end of the enteral feeding tube. Even when the giving set and nutrient container were replaced at 8 and 16h viable organisms continued to be detected in the feed flowing from the end of the enteral feeding tube.

Schroeder et al. (1983) have also reported a similar phenomenon in a simulated study of microbial contamination of enteral feeding solutions. They filled four naso-gastric tubes with nutrient broth containing 10^6 Enterobacter organisms ml^{-1}, clamped them, left them for 2h and then unclamped them and attached them to giving sets. Sterile enteral feeding solution was then run continuously through the tubes at a rate of 60 ml h^{-1} for 24h and Enterobacter organisms were detected in samples taken from the distal end of all four tubes at 0, 12 and 24h.

The results of the present study and that carried out by Schroeder et al. (1983) suggest that once the enteral feeding tubes become contaminated they remain so, and it is possible that these tubes become colonised during clinical use. Thus it is important to recognise that when a patient's intolerance to an enteral feed is being investigated or if it is suspected that contaminants in the feed have contributed to an infection, then simply monitoring the bacteria present in the feed in the nutrient container will not be adequate. Bacteria may be isolated from the enteral feeding tube when none can be detected in the remainder of the system.

The experiment to examine the effect of stopping the flow of feed through the system showed that the rate of multiplication of the organisms in the enteral feeding tube was greater than that of the organisms in the nutrient container. This difference can be attributed to the organisms in the tube being held at 37°C (patient's

body temperature) as opposed to those in the nutrient container at 25°C (ambient ward temperature). This demonstrates that if the feed administered is contaminated then rapid multiplication of organisms will occur in the enteral feeding tube if the system is stopped for an extended period of time. Parallels can also be drawn between the tubing used in beverage vending machines and enteral feeding tubes. Plasticised PVC tubing is widely used in the construction of vending machines and Burman and Colbourne (1977) stated that drinks from these machines may contain flakes of microbial growth encouraged by the warm environment and the periods of little or no flow. Similarly, enteral feeding tubes are at the patient's body temperature and flow of the feed may stop accidentally, due to blockage of the tube, or on purpose to rest the patient's digestive system. During these periods the warm temperature will encourage the multiplication of any contaminating micro-organisms.

The results of the experiments with varying numbers of organisms in the inoculum indicate that with mean generation times of 1.2h in the nutrient container and 0.5h in the "patient's stomach" (recipient flask) then, theoretically, even one organism in a nutrient container containing 1000 ml of feed could multiply and yield sufficient total numbers to cause a clinical problem within 16h. The detection of such a low number in the feed is impracticable and it may therefore be more appropriate to recommend that the hanging time of the feed is reduced.

Results of the study of the feasibility of adding food colouring to enteral feeds to act as indicators of microbial contamination were inconclusive. In the flasks containing Triosorbon plus the azo dyes amaranth, carmoisine, ponceau 4R, sunset yellow or tartrazine, there was a noticeable reduction in colour after 72h at 25°C. The predominant micro-organism isolated at this time was a member of the B. sphaericus - B. brevis group and the viable counts in all flasks were 10^7 - 10^8 organisms ml^{-1}. A similar result was obtained by Fagerman and Dean (1983). They reconstituted an un-named elemental diet with sterile water in an autoclaved blender, added an unspecified quantity of an un-named red colouring material, refrigerated the feed for 24h and then held it at room temperature (unspecified) for 48h. After this time they recorded that a colour change occurred and the viable count was 8.0×10^7 organisms ml^{-1}, the predominant organism being a species of Bacillus.

In the present study it was observed that when the flasks of Triosorbon were incubated at 37°C the colour change occurred more rapidly. A slight colour change was first noted after 18h and after 48h the contents of the flasks containing Triosorbon plus carmoisine, sunset yellow or tartrazine were completely decolourised. The rate of loss of colour in the flasks of Triosorbon which had been experimentally contaminated with E. coli was more rapid than in those containing Triosorbon alone. Fagerman and Dean (1983) also reported that the colour change occurred more rapidly in elemental foods reconstituted with tap water and mixed with an unwashed blender and in

which the predominant contaminant isolated after 24h was E. coli. They suggest that changes in colour of colour-coded enteral feeds should suggest bacterial multiplication which may be hazardous to compromised patients. However, it is significant that in the present study no colour changes were observed over 72h at 4, 25 or 37°C in any of the flasks containing Clinifeed ISO or half-strength Vivonex plus E. coli and colouring materials, even though viable counts of 10^7 -10^8 and 10^8 -10^9 E. coli ml^{-1} were recorded after 72h at 25 and 37°C respectively.

Thus, the results of the present study show that although the change in colour of colour-coded feeds can be linked with the presence of high numbers of bacteria in feeds, the converse is not necessarily true and high numbers of bacteria may be present in feeds in which no colour change has occurred.

Therefore any change in colour in colour-coded feeds should be investigated immediately as a possible sign of heavy bacterial contamination but the addition of colour to feeds should not be relied upon as the sole method for monitoring contamination.

7.5 BACTERIAL ADHESION STUDIES ON EQUIPMENT USED FOR THE ADMINISTRATION OF ENTERAL FEEDS

The adhesion of micro-organisms to surfaces is well documented (Berkeley et al. 1980, Fletcher 1980). Results of the present study demonstrate that residual feed and/or micro-organisms adhering to the

inner surfaces of used nutrient containers could contaminate fresh sterile feed used to refill the nutrient container even if it is carefully rinsed with sterile water prior to being refilled.

Adhesion of micro-organisms to the inner surfaces of enteral feeding tubes could result in patients receiving a clinically significant dose of infecting micro-organisms and/or their toxins over an extended period of time. Consequent hazards to the patient include food poisoning due to bacterial enterotoxins and/or colonisation and infection by opportunistic pathogens. Also, microbial colonisation of the outer surfaces of these tubes may occur in the intestine or stomach and it can be hypothesised that subsequent growth may spread up the outside of the tube and present an infection hazard to the patient, particularly if the tube is left in situ for a long time.

This study has shown that a wide range of commercially available tubes have surface irregularities large enough to trap micro-organisms, and the presence of food particles and bacterial cells was demonstrated in the lumen of fine-bore tubes perfused with experimentally infected feeds. The surface irregularities illustrated were similar to those observed in S.E.M. studies of intravenous catheters by Locci et al. (1981a) and of coronary catheters by Bourassa et al. (1976). There was considerable variation in the surface characteristics of the different types of tubes examined. Both the inner and outer surfaces of PVC, radio-opaque PVC and radio-opaque polyurethane tubes had many surface irregularities including

longitudinal ridges, reticulation, fissures, pits and noticeable roughness due to radio-opaque material. The distribution of radio-opaque material varied in different types of tube. In some tubes it was evenly distributed as small particles, in some it occurred in patches and in others angular chips of radio-opaque material protruded from the tube surface. Similar types of surface roughness have been reported by Muller, Blaschke and Steinmaier (1977) and Locci et al. (1981a) in their studies on intravenous catheters.

In the present study it was found that, in general, the outer surfaces of the tubes appeared to be slightly smoother than the inner surfaces, but major defects, such as deep pits, were observed in the outer surfaces of both radio-opaque PVC and polyurethane tubes. By contrast the surfaces of the silicone tubes appeared to be fairly smooth. Studies on intravenous catheters by Locci et al. (1981b) showed that small numbers of organisms initially lodged in irregularities on the surface of the catheters and subsequently colonies built up. Similar results were obtained in the present study on enteral feeding tubes. For instance, after 24h incubation at 37°C in feeds contaminated with E. coli, Staph. aureus or Bacillus spp., bacteria and particles of feed were observed to have lodged in irregularities on both the inner and outer surfaces of all the tubes tested except for the silicone tubes. Very few adherent bacteria were observed on the surfaces of the silicone tubes after 24h incubation, however, Bayston (1984) reported that when silicone-rubber catheters

were perfused with suspensions of five strains of staphylococci, followed by intermittent perfusion with sterile brain-heart-infusion broth, all the test organisms produced visible micro-colonies in the test catheters within 24-72h.

In the experiments where radio-opaque PVC tubes were perfused with 0.1% (w/v) peptone water contaminated with either Staph. aureus or E. coli the inner surfaces of the tubes had patches of amorphous material deposited on them. Some of the radio-opaque PVC tubes immersed in Triosorbon for 24h were also observed to have patches of the inner surfaces covered with a thin film of transparent material in which rod-shaped bacteria seemed to be embedded. S.E.M. studies of infected intravenous catheters have also shown that bacteria accumulate within amorphous material (Bayston and Penny 1972, Peters et al. 1981, Christensen et al. 1982b, Cheesbrough, Elliot and Finch 1985) and Peters et al. (1982) suggested that production of this material appeared to promote colonisation of the catheters.

Experiments in which radio-opaque PVC tubes were perfused with feed experimentally contaminated with Staph. aureus over 24h showed a steady build-up of material coating the lumen of the tube. However, when tubes were irrigated with sterile water most of the deposited material was removed. It therefore appeared that this procedure, recommended by many of the manufacturers of enteral feeding tubes, was beneficial in limiting the build-up of feed material and bacteria on the inner surfaces of the tubes.

Botta, Costa and Pugliese (1984) recently stated, with reference to fibre-optic endoscopes, that further research was needed to find plastics that are less susceptible to adherence by intestinal bacteria. In their studies on coronary catheters Bourassa et al. (1976) suggested that the use of perfectly regular moulds and more adequate extrusion methods would result in the production of higher quality catheter materials with minimal surface defects. Similar production of enteral feeding tubes with smooth and regular surfaces would help to prevent surface irregularities providing sites for microbial attachment. However, the well documented problem of the adsorption and adhesion of micro-organisms to smooth surfaces (Berkeley et al. 1980, Fletcher 1980, Locci et al. 1981b) leading to subsequent colonisation of the tube, would remain, particularly in tubes left in situ for long periods.

A number of authors have suggested that tube feeds may provide sources of infection which are a particular danger to the debilitated or compromised patient (Casewell et al. 1981, Bastow et al. 1982, Gibbs 1983). However, their concern has been mainly focussed upon feeds contaminated during mixing and handling prior to administration. The present study also highlights another aspect of the problem, since it can be hypothesised that not only may the administration of a contaminated feed lead to the colonisation of the tube, but also the practice of aspirating the stomach or intestinal contents to check the position of the tube could lead to it being contaminated with

micro-organisms from the patient's own flora which, in this new environment, could multiply and produce toxic metabolites.

7.6 CONCLUSIONS AND RECOMMENDATIONS

There are an increasing number of reports showing that the use of contaminated enteral feeds can cause serious infections in hospital patients (Pottecher et al. 1979, Casewell et al. 1981, Casewell and Philpott-Howard 1983). The present study has demonstrated that not only are there many potential sources of this contamination, but also that its effects can be amplified by poor handling procedures. Potential sources of contamination reported in the survey conducted from 1982-1984 included the use of ingredients such as raw eggs, dried milk powder, Complan, tap and distilled water, and the use of inadequately cleaned liquidisers to mix the feeds. Poor handling procedures were also recorded, including refilling of nutrient containers, storage of feeds at kitchen or ward temperature for up to 12h prior to administration, and transporting the feeds from the kitchen to the ward on a warm breakfast trolley. A number of authors have argued that there is a strong case for the bacteriological testing of enteral feeds (Bastow et al. 1982, Casewell 1982) but for this to be of any value it is necessary to define numerical limits for micro-organisms in feeds which, if exceeded, will result in the rejection of the feed. To obtain these numerical limits it is first necessary to ascertain the level at which micro-organisms present a

hazard to the patient due to colonization and possible subsequent infection. The number of organisms that must be ingested by a patient in order for colonization of the intestine to occur will vary according to the patient's treatment and disease. Pottecher et al. (1979) suggested that 10^4 organisms ml^{-1} of feed are sufficient to result in persistent colonization of the digestive tract of hospital patients, but more recently, Remington and Schimpff (1981) stated that a total of 10^3 Gram-negative organisms in 1g of food could lead to persistent colonization of the intestinal tract. There would, therefore, seem to be a strong argument in favour of the use of only sterile ingredients for all hospital patients. However, a survey by Tredger et al. (1981) showed that more than 50% of the hospitals in the UK at that time used hospital-prepared non-sterile feeds and although the survey carried out between 1982 and 1984 indicated a reduction in the use of hospital prepared feeds it also revealed that 80% of the hospitals added substances to the ready-to-use commercial feeds. Also, for non-sterile pharmaceuticals it has been stated that a compromise must be reached between the estimation of the risk of infection from contaminated feeds and the cost of assuring product cleanliness with the available raw materials and procedures (T. Munton 1984; pers. comm.) and a similar case can be made for enteral feeds. The following recommendations are, therefore, put forward for microbiological limits which are applicable to the enteral feed ingredients and the finished products (in the nutrient container) (Tables 19 and 20).

TABLE 19 : Recommended microbiological limits - ingredients for non-sterile feeds

Raw Material	Aerobic plate count cfu ml^{-1} or g^{-1}			Organisms not permitted at any level
	Accept	Marginal	Reject	
Water	$<10^1$	-	$\geqslant 10^1$	*E. coli* *Salmonella* spp. *Pseudomonas* spp.
Other liquids, e.g. milk, emulsions	$<10^1$	-	$\geqslant 10^1$	*E. coli* *Salmonella* spp. *Clostridium* spp. *Staph. aureus* *B. cereus* *Klebsiella* spp. *Pseudomonas* spp.
Solids*, e.g. protein or carbohydrate supplements	$<10^1$	10^1-10^2	$\geqslant 10^2$	*E. coli* *Salmonella* spp. *Clostridium* spp. *Staph. aureus* *B. cereus* *Klebsiella* spp. *Pseudomonas* spp.

* Particular care should be taken with dried products containing milk or whey proteins since these are frequently contaminated with *Bacillus* spp. and *Staphylococcus* spp.

TABLE 20 : Recommended microbiological limits - finished products (in nutrient container prior to administration)

Product	Aerobic plate count cfu ml^{-1}			Organisms not permitted at any level (2)	Maximum recommended hanging time for feed
	Accept	Marginal(1)	Reject		
Sterile feeds	0	-	>0	-	24h
Non-sterile feeds	$<10^1$	10^1-10^2	$\geqslant 10^2$	*E. coli* *Salmonella* spp. *Clostridium* spp. *Staph. aureus* *B. cereus* *Klebsiella* spp. *Pseudomonas* spp.	4h

(1) The lower limit should serve as an 'early warning' level which should be acted upon; the upper level should not be exceeded.

(2) The presence of any Gram-negative organisms is undesirable and in indicative of poor hygiene during preparation.

Enteral feed ingredients

Hospital Bacteriology departments should make quality checks on all batches of dried products intended for use in enteral feeds and reject those with an aerobic plate count (APC) $\geqslant 10^2$ organisms g^{-1}. Checks should also be made to ensure that all ingredients are free from Salmonella spp., E. coli, Klebsiella spp., Pseudomonas spp., Clostridium spp., Staph. aureus and B. cereus.

Particular care should be taken with dried products containing milk or whey proteins since an examination of the microbiological quality of the ingredients presently used in the preparation of enteral feeds has demonstrated that these products are frequently contaminated with Bacillus spp., including B. cereus, and Staphylococcus spp. Liquid ingredients should be sterile where possible and the APC of non-sterile liquids should not exceed 10^1 organisms ml^{-1}. The list of organisms not permitted at any level is the same as for dry ingredients with the particular need to ensure that any water used is free from Pseudomonas spp.

Finished products (in nutrient container)

It is strongly recommended that, where possible, only sterile feed should be used for those patients considered to be a special risk (Appendix F, Page 180). The proposed microbial limits at the start of administration and the recommended hanging times for sterile and non-sterile feeds take into account the fact that in feeds hanging at

ward temperature there will be rapid multiplication of any contaminants present. For instance, it was found that when solutions of milk-based feeds were experimentally contaminated with strains of Staph. aureus, Ps aeruginosa, K. aerogenes, Ent. cloacae and E. coli at levels of 10^2 - 10^3 cfu ml^{-1}, all the test organisms multiplied rapidly. After 24h at 25 and 37°C respectively, viable counts of 10^7 - 10^9 and 10^8 - 10^{10} cfu ml^{-1} were recorded. Since, by definition non-sterile feeds are contaminated at the start of administration, the hanging time for these feeds is limited to 4h in order to ensure that microbial numbers in these nutrient containers will not exceed 10^3 ml^{-1} at the end of administration. This hanging time was arrived at through studies of the rate of growth of E. coli and Staph. aureus in a milk-based feed under simulated ward conditions. In these studies it was found that the counts in the nutrient containers increased over 8h from 10^2 -10^3 to 10^4 - 10^5 cfu ml^{-1} for both organisms. Varying the initial inoculum resulted in parallel growth curves, their being an average increase in numbers of 2 log cycles over 8h (mean generation time = 1.2h). Therefore, it was possible to predict that provided the initial level of contamination was less than 10^2 cfu ml^{-1} and the ward temperature did not exceed 25°C then after 4h hanging time the final number of organisms in the container would not exceed 10^3 ml^{-1}. The recommendation for a maximum hanging time of 4h for non-sterile feeds was also prompted by the fact that the food service industry in the U.S.A. limits the cumulative room temperature storage time of hazardous food products (defined as all food products shown to

be at risk for bacterial contamination and growth) to 4h (Food and Drug Administration 1976).

Initially it is recommended that for a limited period of time (e.g. one month) the Bacteriology departments of hospitals take samples of each batch of raw materials and finished feeds prior to their release for use. It is, however, appreciated that due to the extremely perishable nature of the finished products, it will be necessary to release these for use prior to the microbiological results being available. Nevertheless, by monitoring all the products for a period of time it should be possible to identify the raw materials, kitchen equipment and handling procedures which present a particular risk in each hospital and to act to rectify this. Subsequently, therefore, it may be possible to confine the routine microbiological testing of raw materials to batches of new materials. If this approach is adopted it is also recommended that the departments should continue to make regular routine checks on the microbiological quality of both sterile and non-sterile feeds, prior to, during the course of and at the end of administration to ensure that contaminants are not being introduced from contaminated raw materials or equipment or due to incorrect handling procedures.

The practicality of these recommendations was confirmed during a six-month survey of the contamination of enteral feeds in the ITU of a local hospital. Here the levels of contamination in the residual feed

in the nutrient container ranged from 10^2 to 10^3 cfu ml^{-1}. The only ingredients used were the sterile feeds Nutrauxil, Ensure and Isocal and sterile water. Mixing of the ingredients was done in the sterile nutrient container. Therefore, it could be inferred that the contaminants entered the system from the surface of the cans of feed, from the air or from the skin of personnel handling the systems. Also, reports by Jones (1975) and Gibbs (1983) of nurses testing the temperature of enteral feeds with their fingers highlights the fact that not all personnel are aware of the potential sources and subsequent hazards of microbial contamination of enteral feeds and the need for strict hygiene in their preparation and handling.

Handling Procedures

It is recommended that equipment used for continuous feeding should be sterile as described in the British Standard 6314 (British Standards Institution 1983) and kept in sealed packaging until immediately before use. The importance of this is emphasised by the experiments in which a contaminated nutrient container was refilled with sterile feed over a period of 24h and viable counts rose from 10^2 - 10^3 cfu ml^{-1} at 0h to 10^8 at 24h. Similar results were also reported by Bastow et al. (1982). Also in the present studies to examine the adhesion of bacteria to the nutrient container plastic it was shown that rinsing with sterile water was not sufficient to remove all of the adhering organisms. Therefore the commonly used practice of rinsing the nutrient container with tap water each time it is

refilled is not advisable, and in fact the tap water itself may be a source of contamination (Burman and Colbourne 1977).

In order to minimise the risk of skin contaminants gaining access to the feed it is essential that at no stage should any internal part of the nutrient container or giving set be allowed to come into contact with the skin. Of particular relevance are spikes and air lines inserted into feeding solutions, interiors of empty bags, caps and adaptors.

The possibility of enteral feeding tubes acting as sources of contamination should be considered. S.E.M. studies suggested that it was possible that once these tubes become colonised during clinical use they would remain contaminated due to feed components and bacteria adhering to the tubes and through the entrapment of feed and bacteria at the ends of side-vented tubes.

This was confirmed by studies carried out under simulated ward conditions in which fine-bore enteral feeding tubes were perfused for 8h with a milk-based feed experimentally contaminated with 10^2 - 10^3 Staph. aureus or E. coli ml^{-1}. After 8 and 16h the whole system, apart from the tube, was replaced with new sterile components and sterile feed was perfused through the system. In spite of these procedures viable Staph. aureus or E. coli organisms were still detected in the feed collected from the end of the fine-bore tube

after 24h. These results are similar to those reported by Schroeder et al. (1983) working with Ent. cloacae. Further S.E.M. studies showed that irrigating the tubes with sterile water removed most but not all of the deposited material and may therefore be beneficial in limiting the build-up of feed and bacteria.

Colonisation of the enteral feeding tubes could results in patients receiving a clinically significant dose of infecting micro-organisms and/or their toxins over an extended period of time. Feed issuing from the fine-bore tube may contain bacteria independent of the rest of the system. It is probably insufficient to monitor the bacteria present in the nutrient container. Also, if the feed is accidentally allowed to reflux back to the nutrient container, the whole system would become contaminated.

It is therefore recommended that the nutrient container should not be allowed to hang below the height of the patient's stomach and that the enteral feeding tube should be flushed out with water at least once every 24h and replaced every two weeks.

Guidance for hospital practice

Further details of these recommendations are included in the guidance document prepared by Anderton, Howard and Scott on behalf of the Committee of the Parenteral and Enteral Nutrition Group of the British Dietetic Association (enclosed at back of thesis).

APPENDICES

APPENDIX A

QUESTIONNAIRE ON NASO-GASTRIC FEEDS

PART I - GENERAL

Please underline appropriate response(s) below :

1. Type of hospital

   a) Cottage
   b) Large teaching
   c) General
   d) Maternity
   e) Other (please state)

2. Number of beds (approx.) (please state)

3. Average number of patients in the hospital receiving naso-gastric tube feeds on any given day (please state)

4. What is the average length of time (in days or weeks) for which a patient will be tube fed? (please state)

5. Type of feed use :

   a) Home-made milk feeds
   b) Liquidised ward food
   c) Commercial feeds

6. Of the foods used, what percentage are Commercial Feeds?

   a) 100%
   b) 0%
   c) Other (please state)

PART 2 - COMMERCIAL FEEDS

Please fill in this questionnaire if the hospital uses Commercial Ready-made Sterile Naso-gastric feeds.

Answer all questions that are relevant.

Please underline appropriate response(s) below :

1. Which brands and types of Commercially Made Sterile Feeds are Used? (e.g. Clinifeed 500 Vanilla; Isocal) (please state)

2. Do you add anything to Commercial Feed? YES/NO
   If yes, please state what (including water) -
   how is it added and mixed (please state)

3. What type of water is used?

   a) Tap water from mains
   b) Tap water stored in a tank
   c) Distilled water stored in a container
   d) Other (please state)

4. If additions are made, what type of liquidiser is used?

a) Glass goblet
b) Metal goblet
c) Plastic goblet

5. In addition to tube feeds, what is the liquidiser used for?

a) All types of food
b) Milk food alone
c) Nothing else
d) Other (please state)

6. What method is used for cleaning liquidiser?

a) Very hot water
b) Hot water
c) Warm water
d) Other (please state)

7. How long in advance are feeds prepared or decanted from can?

a) 4h
b) 6h
c) 12h
d) 18h
e) Other (please state)

8. How are they stored after being prepared or decanted from can, but before reaching wards?

   a) In cool area in kitchen
   b) In general kitchen area
   c) In cool room
   d) In refrigerator

9. How are they stored, if in reservoir or bag, on ward?

   a) In kitchen area
   b) On drinks trolley
   c) In ward beside patient
   d) In ward refrigerator

10. If stored in reservoir or bag in a refrigerator, how long before administration are they removed from the fridge (i.e. how much time is allowed for them to warm up so that they are not administered cold?)

   a) 1h
   b) 2h
   c) 4h
   d) Other (please state)

11. How are they administered to patient?

   a) Drip method from glass bottle
   b) Drip method from bag
   c) Drip method from Clinifeed system
   d) Bolus method

12. How are bottles cleaned?

a) Washed thoroughly (machine)
b) Washed thoroughly (by hand)
c) Sterilised

13. If bags or Clinifeed System 3 units are used, are they :

a) Refilled over 24h and then discarded
b) Used often and washed between use
c) Used often and sterilised between use
d) Used once then discarded
e) Other (please state)

14. What is the average length of time per day for feed to pass into patient?

a) 6h
b) 12h
c) 24h
d) Any other length of time - please specify.

15. What is average quantity of feed per patient given per day?

Please state in ml.

16. How much feed does the reservoir, bag or bottle used for administration hold?

a) 300 ml
b) 500 ml
c) 1000 ml
d) 1500 ml
e) Other (please state)

17. How long does reservoir, bag or bottle used take to empty?

a) 3h
b) 8h
c) 12h
d) Other - please give details.

18. If an enteral pump is used, what flow rate is it normally set at?

a) Please state (ml/h).

19. Each time more feed is required by the patient, is the container used :

a) Refilled at bedside (washed daily)
b) Refilled in kitchen (washed daily)
c) Washed in kitchen, refilled and replaced
d) Washed on ward, refilled and replaced
e) New container used each time
f) Other (please state)

20. What type of feeding tube is used?

a) Clinifeed System I
b Ryles tube
c) Other (please state)

21. How often are the patients' feeding tubes changed? (please give details)

PART 3 - HOME-MADE FEEDS

Please fill in this questionnaire if the hospital uses Home-Made Naso-gastric Feeds.

Answer all questions that are relevant.

Please underline appropriate response(s) below :

1. What type of feed is used ?

   a) Liquidised ward food
   b) Own recipe
   c) Elemental

2. If liquidised ward food is used, where is it liquidised?

   a) Main kitchen
   b) Diet bay
   c) On ward

3. What type of water is used in feeds?

   a) Tap water from mains
   b) Tap water stored in a tank
   c) Distilled water stored in a container
   d) Other (please state)

4. If own recipe, what type of milk is used?

   a) Pasteurised
   b) Dried whole milk
   c) Dried skim milk
   d) UHT milk

5. Do you use eggs in own recipe? YES/NO
If yes, what type?

a) Whole raw egg
b) Commercial liquid egg
c) Dried egg

6. What type of liquidiser is used?

a) Glass goblet
b) Metal goblet
c) Plastic goblet

7. What is the liquidiser used for?

a) All types of food
b) Milk food alone
c) Tube feeds only
d) Other (please state)

8. What method is used for cleaning liquidiser?

a) Very hot water
b) Hot water
c) Warm water
d) Other (please state)

9. How long in advance are feeds prepared?

a) 4h
b) 6h
c) 12h
d) 18h
e) Other (please state)

10. How are they stored before reaching wards?

a) In cool area in kitchen
b) In general kitchen area
c) In cool room
d) In refrigerator

11. How are they stored on Ward?

a) In kitchen area
b) On drinks trolley
c) In ward beside patient
d) In ward refrigerator

12. If stored in a refrigerator, how long before administration are they removed from the fridge (i.e. how much time is allowed for them to warm up so that they are not administered cold?)

a) 1h
b) 2h
c) 4h
d) Other (please state)

13. How are they administered to patient?

a) Drip method from glass bottle
b) Drip method from bag
c) Drip method from Clinifeed system
d) Bolus method

14. How are bottles cleaned?

a) Washed thoroughly (machine)
b) Washed thoroughly (by hand)
c) Sterilised

15. If bags or Clinifeed System 3 units, are they :

a) Used often and washed between use
b) Used often and sterilised between use
c) Used once and then discarded
d) Other (please state)

16. What is average length of time per day for feed to pass into patient?

a) 6h
b) 12h
c) 24h
d) Any other length of time - please specify.

17. What is average quantity of feed per patient given per day?

Please state in ml.

18. How much feed does the reservoir, bag or bottle used for administration hold?

a) 300 ml
b) 500 ml
c) 1000 ml
d) 1500 ml
e) Other - please state.

19. How long does the reservoir, bag or bottle used take to empty?

a) 3h
b) 8h
c) 12h
d) Other - please give details.

20. If an enteral pump is used, what flow rate is it normally set at?

Please state (ml/h)

21. Each time more feed is required by the patient, is the container used :

a) Refilled at bedside (washed daily)
b) Refilled in kitchen (washed daily)
c) Washed in kitchen, refilled and replaced
d) Washed on ward, refilled and replaced
e) New container used each time
f) Other (please state)

22. What type of feeding tube is used?

a) Clinifeed System I
b) Ryles tube
c) Other (please state)

23. How often are the patients' feeding tubes changed? (please give details)

Thank you for your co-operation

APPENDIX B

ADDRESSES OF MANUFACTURERS AND SUPPLIERS

i) Manufacturers and Suppliers of enteral feeds and feed ingredients

1.	Abbott Laboratories Ltd., Queensborough 1, Kent, ME11 5EL	Enrich Ensure Ensure Plus
2.	Bristol-Myers Co. Ltd., Station Road, Langley, Slough, SL3 6EB.	Flexical Isocal
3.	Carnation Ltd., Danesfield House, Medmenham, Marlow, Bucks, SL7 2ES.	Build-Up
4.	Cow & Gate Ltd., Clinical Products Division, Trowbridge, Wiltshire, BA14 8XX.	Fortison
5.	Duncan Flockhart & Co. Ltd., 891-995 Greenford Road, Greenford, Middlesex, UB6 OHE.	Prosparol
6.	Express Food Ingredients, 430 Victoria Road, South Ruislip, Middlesex, HA4 OHF.	Express Standard Express High Energy
7.	Farley Health Products Ltd., Tor Lane, Plymouth, Devon, PL3 5UA.	Casilan Complan

8.	KabiVitrum Ltd., KabiVitrum House, Riverside Way, Uxbridge, Middlesex, UB8 2YF.	Nutrauxil
9.	Merck, E. Ltd., Four Marks, Alton, Hants., GU34 5HG.	Peptisorbon Triosorbon
10.	Norwich-Eaton Ltd., Regent House, Woking, Surrey, GU21 5AP.	Vivonex High Nitrogen Vivonex Standard
11.	Roussel Laboratories Ltd., Wembley, Middlesex, HA9 ONF.	Clinifeed Favour Clinifeed 400 Clinifeed 500 Clinifeed ISO Clinifeed Protein Rich Caloreen Nutranel
12.	Scientific Hospital Supplies Ltd., 37 Queensland Street, Liverpool, L7 3JG.	Calogen Enteral 400 Liquigen Maxijul Maxipro HBV

ii) Manufacturers and suppliers of enteral feeding equipment

Aldington Laboratories Ltd.,
Mersham,
Ashford,
Kent.

Argyle,
Division of Sherwood Medical,
London Road,
Crawley,
West Sussex, RH10 2TL.

Franklin Medical,
Turnpike Road,
Cressex Industrial Estate,
High Wycombe,
Bucks, HP12 3NB.

Roussel Laboratories Ltd.,
Wembley,
Middlesex, HA9 ONF.

Viomedex Ltd.,
Gordon Road,
Uckfield,
East Sussex, RN22 4LH.

Vygon (UK) Ltd.,
Bridge Road,
Cirencester,
Gloucestershire, GL7 1PT.

iii) Manufacturers and suppliers of food colouring materials

BDH Chemicals Ltd., Broom Road, Parkstone, Poole, BH12 4NN.	ponceau 4R sunset yellow FCF tartrazine
Pointing Ltd., Prudhoe, Northumberland, England, NE42 6NS.	carmoisine erythrosine BS
Sigma Chemical Co. Ltd., Fancy Road, Poole, Dorset, BH17 7NH.	amaranth

APPENDIX C

DETAILS OF NCTC CULTURES

NCTC 8155	*Enterobacter cloacae*	isolate from tin of dried milk
NCTC 10005	" "	suggested neotype
NCTC 8007	*Escherichia coli*	isolate implicated in infantile gastro-enteritis
NCTC 8603	" "	isolated implicated in infantile diarrhoea
NCTC 8172	*Klebsiella aerogenes*	proposed neotype
NCTC 9997	" "	isolate from ropy milk
NCTC 6750	*Pseudomonas aeruginosa*	suggested working type
NCTC 10332	" "	recommended neotype
NCTC 10652	*Staphylococcus aureus*	prototype strain for production of enterotoxin A
NCTC 10657	" "	produces enterotoxins A and B

APPENDIX D

DEFINITIONS USED IN ENTERAL FEEDING

Disinfection	The destruction of micro-organisms but not usually of bacterial spores; it does not necessarily kill all micro-organisms but reduces the numbers to a level which is not generally harmful to health.
Feeding tubes	Includes nasogastric tubes, naso-jejeunal tubes, orogastric tubes, jejeunostomy, oesophagostomy and gastrostomy tubes.
Giving set	The system linking the nutrient container to the feeding tube including the drip chamber, burette, pump insert, adaptor and 3-way tap.
Handling	Relates to the frequency of feed transfers and the manipulation of equipment (changing bottles etc.).

Hanging time	The total time during which the feed is held in the nutrient container at ward temperature while being administered. This includes periods of time when administration of the feed is interrupted temporarily.
Holding time	The time between the preparation of non-sterile feeds and their final administration to the patient.
Non-sterile feeds	Feeds which may contain live bacteria, e.g. reconstituted powdered complete feeds, commercial pre-packed feeds in liquid or powder form supplemented with nutrients/additives at kitchen, pharmacy or ward level (e.g. water, glucose polymer, fat emulsion, protein concentrate, vitamins, electrolytes, methyl cellulose); home-made feeds prepared in either kitchen, ward or pharmacy from basic materials.

Nutrient container	The vessel from which the feed is directly administered to the patient, e.g. rigid plastic reservoir, glass bottle, plastic bag. In the case of bolus feeding, the nutrient container is the syringe/funnel.
Sterile feeds	Industrially produced pre-packed liquid feeds, which are 'commercially sterile', i.e. no viable organisms can normally be detected by the usual cultural methods employed.
Sterilisation	A process intended to destroy or remove all living organisms.

APPENDIX E

EFFECT OF HANDLING PROCEDURES ON THE GROWTH OF E. COLI AND STAPH. AUREUS IN ENTERAL FEEDING SYSTEMS UNDER SIMULATED WARD CONDITIONS

These figures are the results of replicates of experiments presented in the main text, as follows :

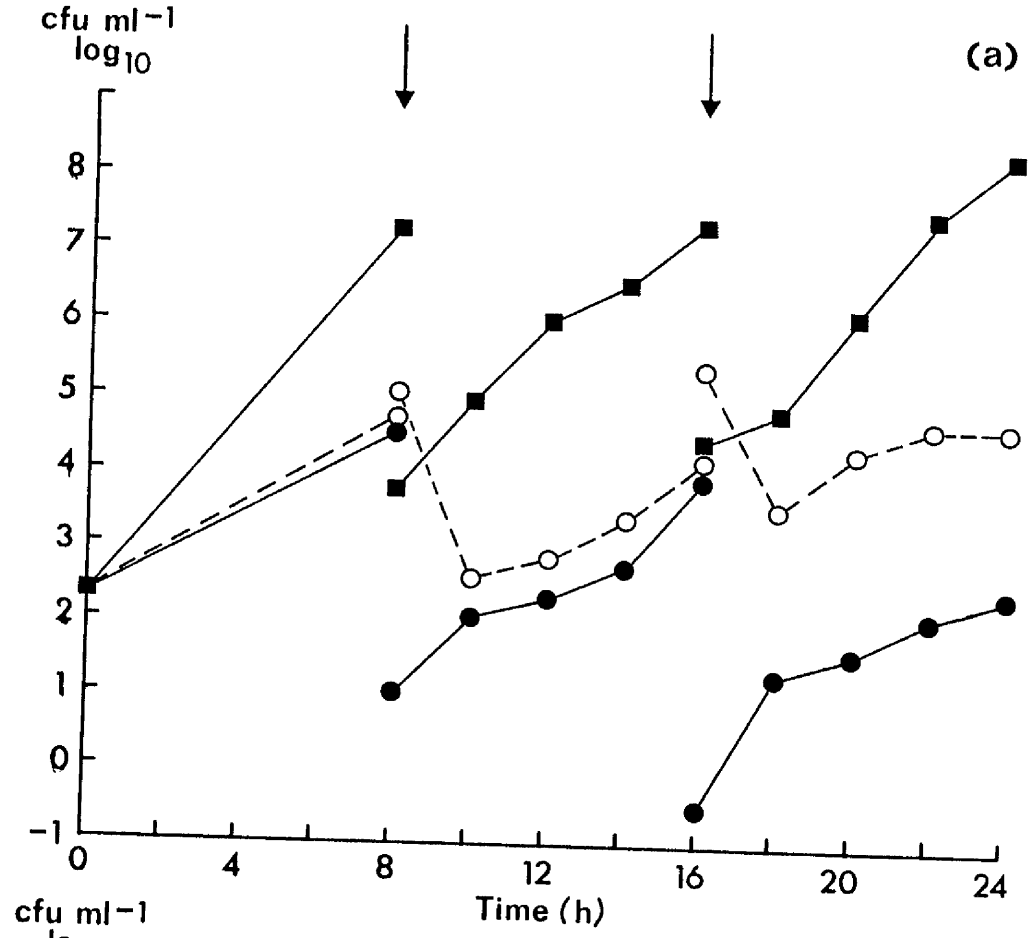
Appendix E figure	Corresponding figure (and page number in main text)
E1	15 (p.86a)
E2	16 (p.86b)
E3	17 (p.87a)
E4	18 (p.87b)
E5	19 (p.88a,b)
E6	20 (p.88c,d)

FIGURE E1 The effect of replacing the contaminated Clinifeeding System 3 reservoir (nutrient container) with a new reservoir containing sterile feed (Clinifeed ISO) on the growth of E. coli in the system.

The reservoir was changed at 8 and 16h (↓).

Samples were taken from the recipient flask (■———■), the reservoir (●———●) and the enteral feeding tube (O- - - O).

((a) and (b) are the results from replicate experiments.)


cfu ml⁻¹
log10
(a)
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-1
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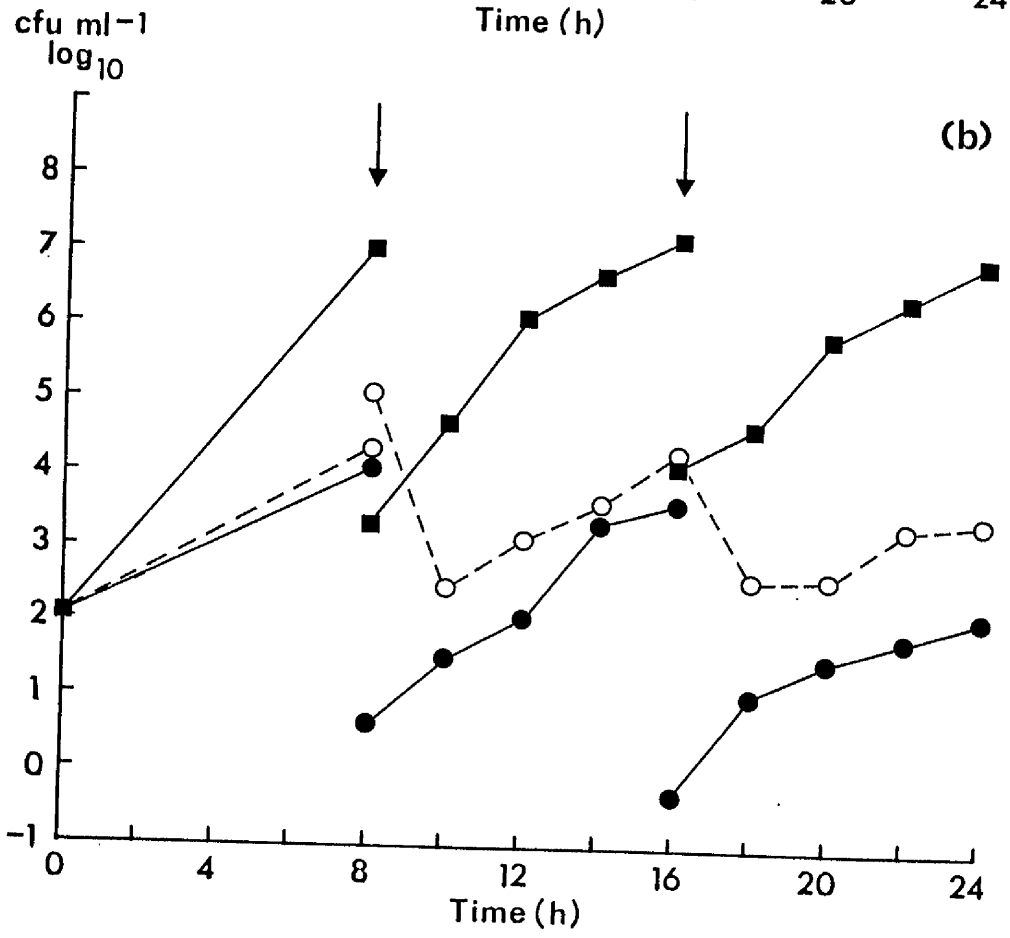
16
20
24
Time (h)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log10
(b)
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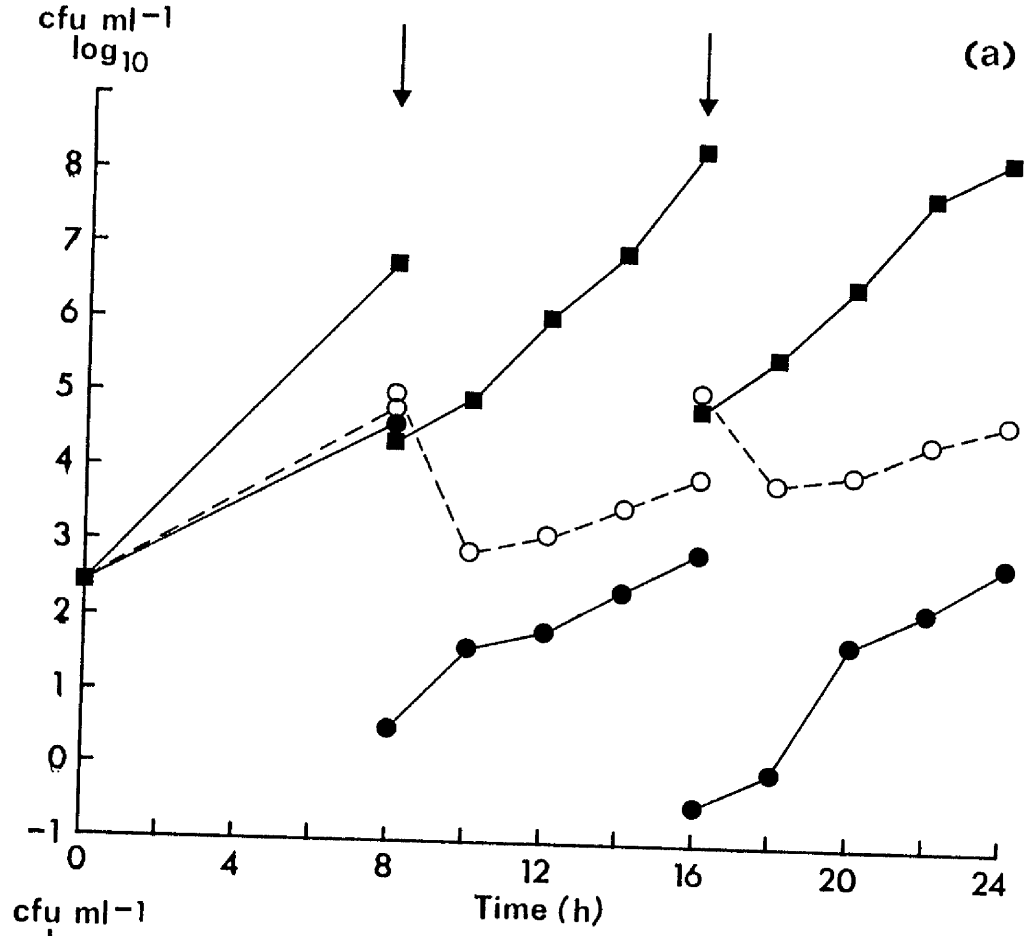
4
3
2
1
0
-1
0
4
8
12
16
20
24
Time (h)

FIGURE E2 The effect of replacing the contaminated Clinifeeding System 3 reservoir (nutrient container) with a new reservoir containing sterile feed (Clinifeed ISO) on the growth of Staph. aureus in the system.

For Key to symbols see Fig. E1 (page 170).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log10
(a)
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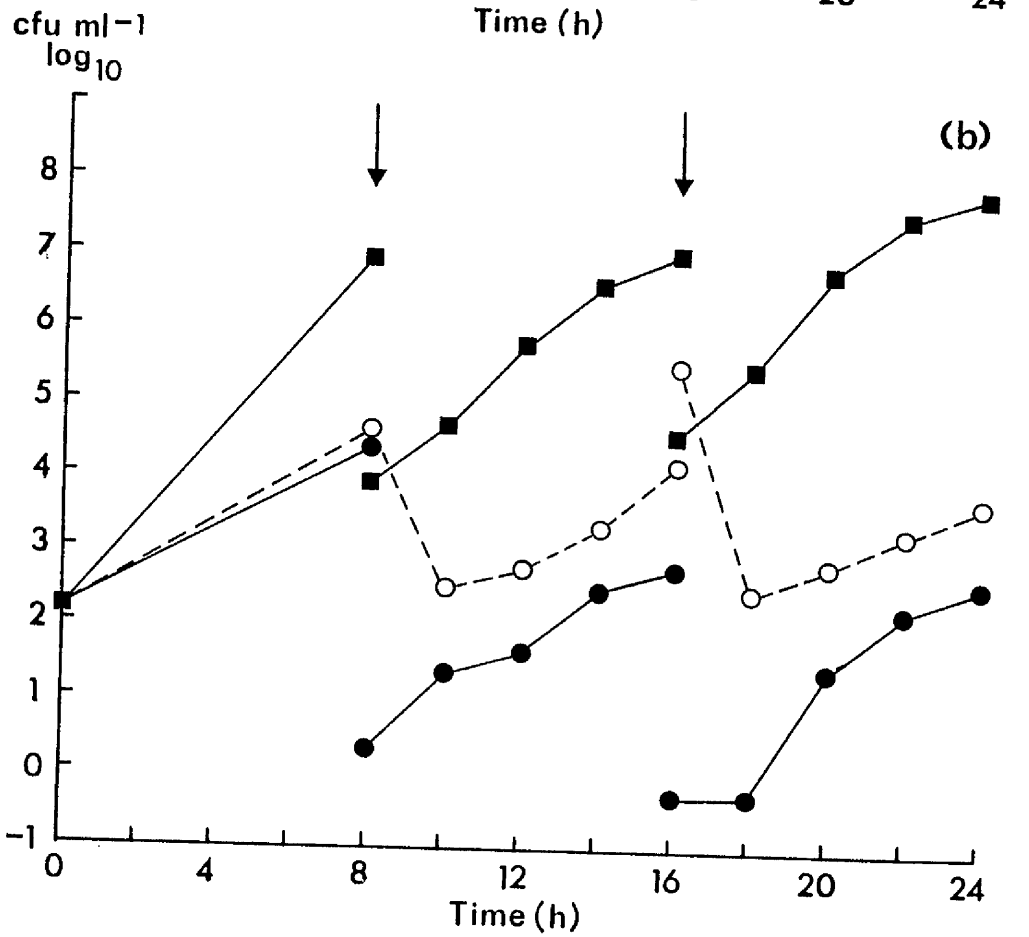
16
20
24
Time (h)


cfu ml⁻¹
log10
(b)
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2
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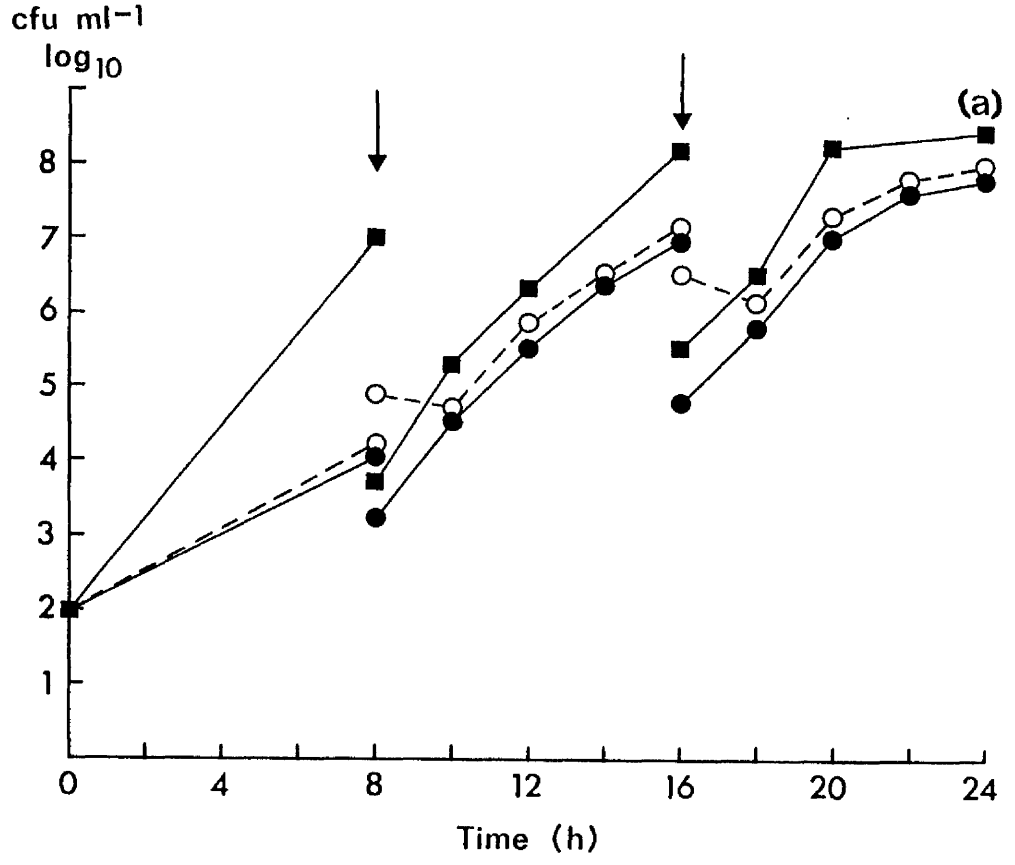
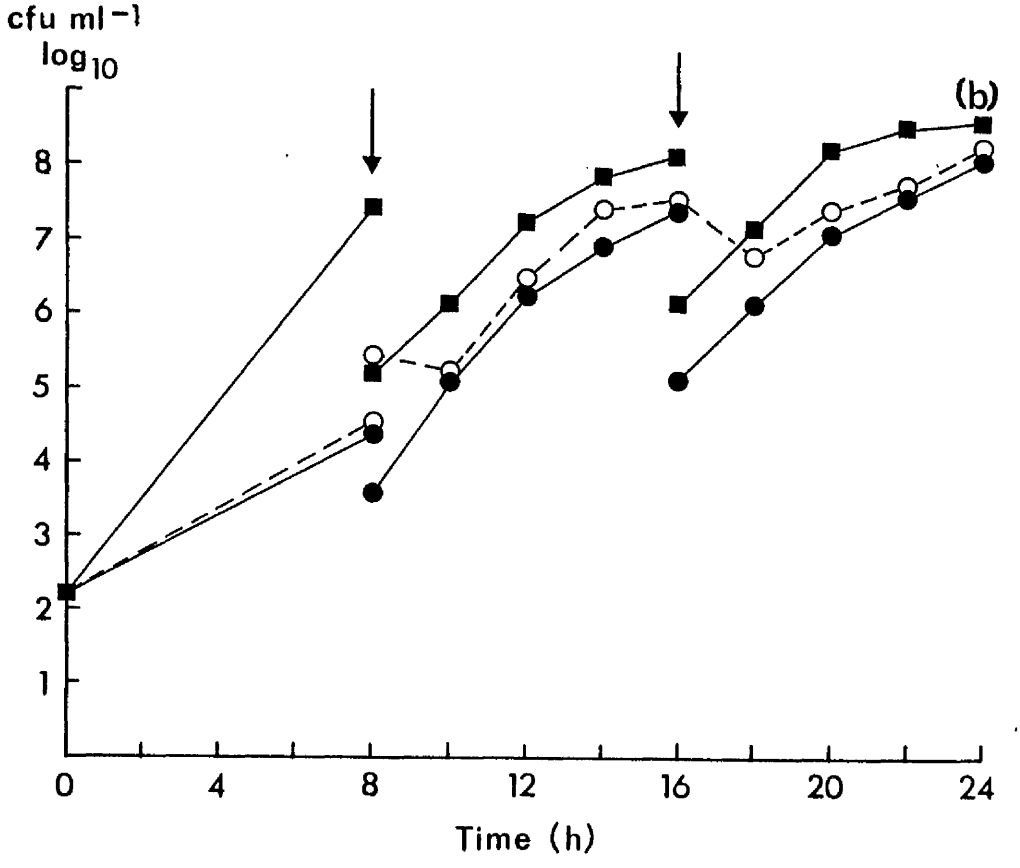
-1
0
4
8
12
16
20
24
Time (h)

FIGURE E3 The effect of refilling the contaminated Clinifeeding System 3 reservoir (nutrient container) with sterile feed (Clinifeed ISO) on the growth of E. coli in the system.

For Key to symbols see Fig. E1 (page 170).


cfu ml⁻¹
log10
(a)
8
7
6
5
4
3
2
1
0
4
8
12
16
20
24
Time (h)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log10
(b)
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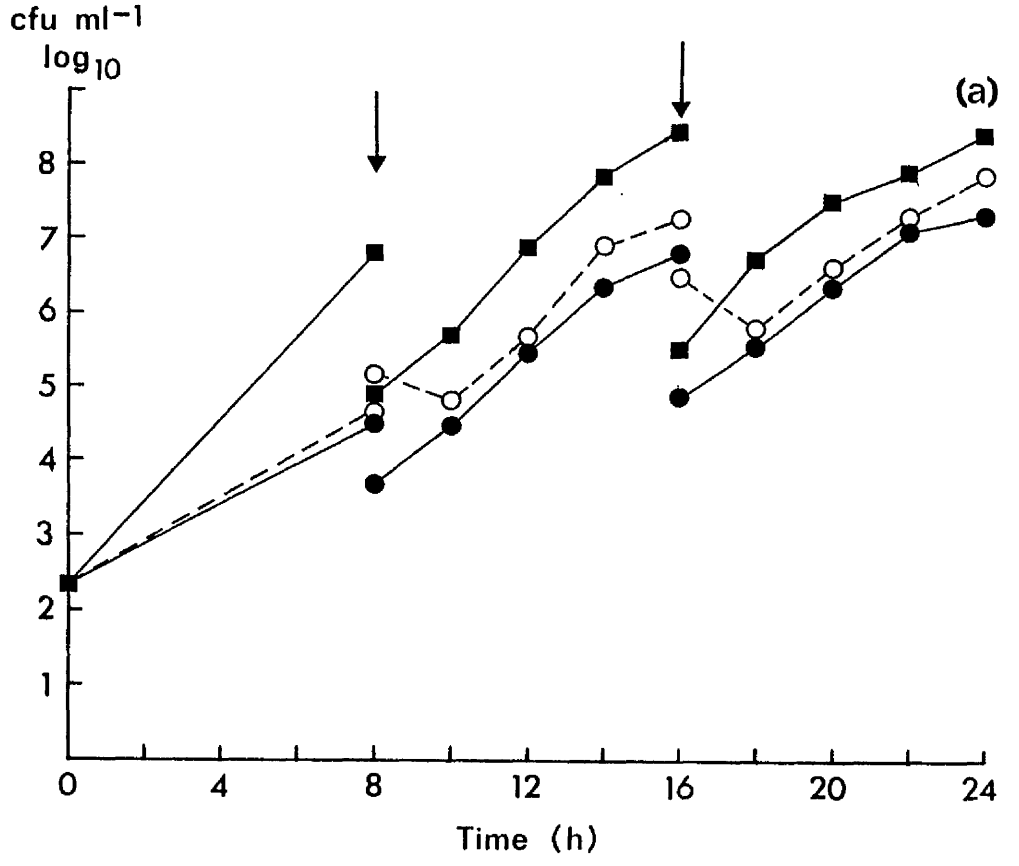
7
6
5
4
3
2
1
0
4
8
12
16
20
24
Time (h)

FIGURE E4 The effect of refilling the contaminated Clinifeeding System 3 reservoir (nutrient container) with sterile feed (Clinifeed ISO) on the growth of Staph. aureus in the system.

For Key to symbols see Fig. E1 (page 170).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log10
(a)
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7
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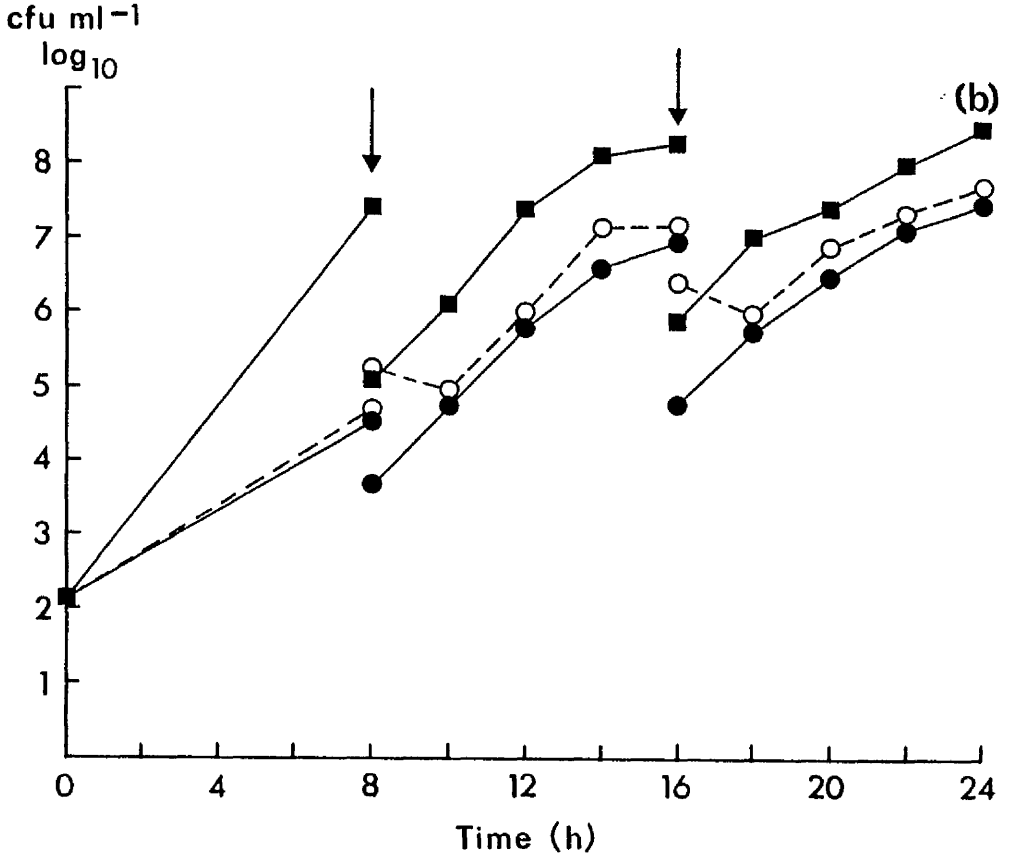
2
1
0
4
8
12
16
20
24
Time (h)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log10
(b)
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2
1
0
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8
12
16
20
24
Time (h)

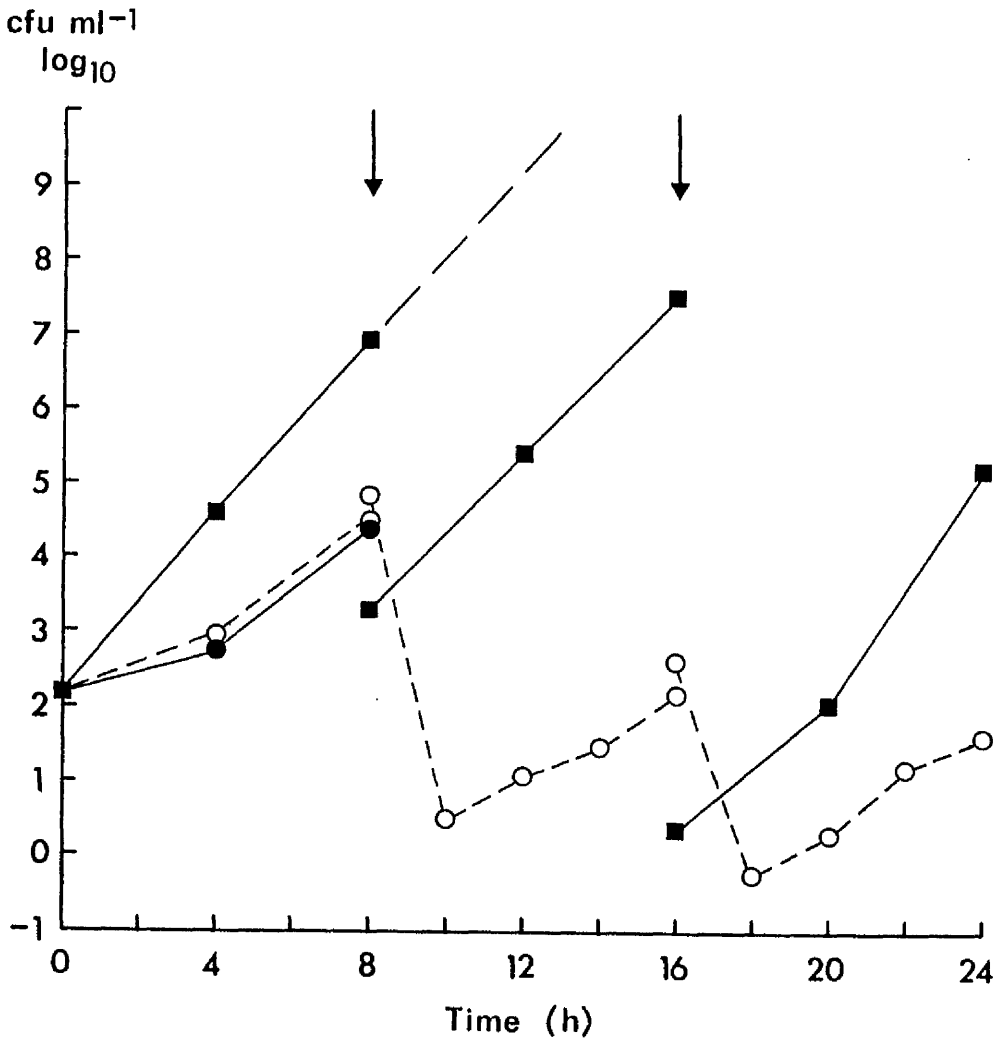

FIGURE E5 The effect of replacing the Clinifeeding System 3 reservoir (nutrient container), the feed and the giving set on the growth of E. coli in the system.

For Key to symbols see Fig. E1 (page 170).

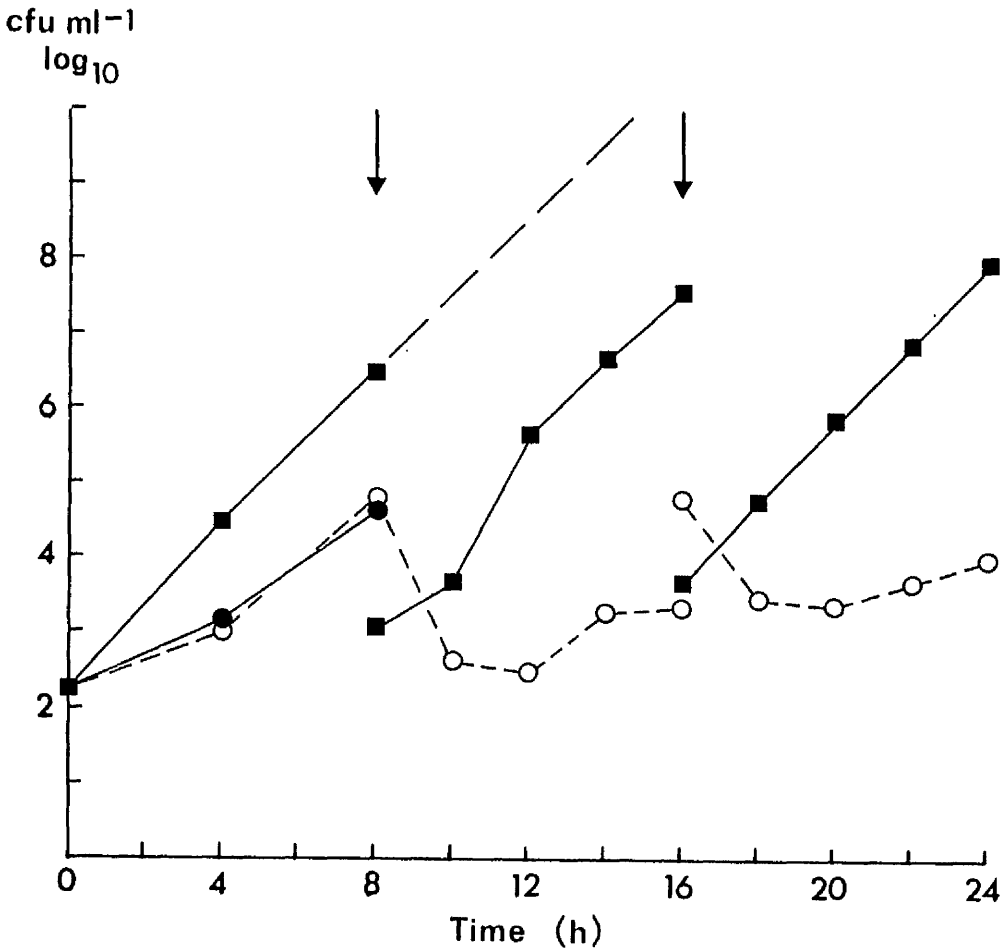

FIGURE E6 The effect of replacing the Clinifeeding System 3 reservoir (nutrient container), the feed and the giving set on the growth of Staph. aureus in the system.

For Key to symbols see Fig. E1 (page 170).

APPENDIX F

PATIENTS AT SPECIAL RISK FROM INFECTION

Some patients are more susceptible to infection and it is in these specific cases that the requirement for sterile commercially prepared feeds and aseptic procedures should be carefully considered. These patients include :

a) Cases of acute infection, sepsis etc., receiving antibiotic treatment;

b) Oncology patients - particularly those on chemotherapy and those with leukaemia;

c) Patients receiving immuno-suppressive treatment for any reason or patients whose immune system is otherwise compromised, e.g. patients with organ transplants, leukaemia, pneumonias, AIDS, some patients with renal failure etc.;

d) Patients with reduced gastric acid secretion, e.g. achlorydria, pernicious anaemia, post gastrectomy or receiving gastric acid inhibitors, e.g. Cimetidine, Ranitidine.

e) Patients being fed by a route which by-passes the stomach;

f) Patients with burns;

g) Neonates;

h) Any patients receiving long term feeding whose injury is particularly associated with recurrent infections, e.g. head injuries.

REFERENCES

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