BACTERIA ASSOCIATED WITH INFLAMMATORY ENTERIC LESIONS IN PIGS

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by

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DEDICATED

TO

MY FATHER

DAVID OKERE OLUBUNMI

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PREFACE

The work described in this thesis was carried out in the Department of Veterinary Pathology, Glasgow University, from Martinmas term 1979 to Martinmas term 1982 under the able supervision of Dr. D.J. Taylor, M.A., PhD, Vet.MB, M.R.C.V.S.

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SUMMARY

A study of bacteria associated with inflammatory enteric lesions in pigs was carried out on 129 pigs from 8 different farms examined post-mortem.

A number of bacterial species were isolated from lesions in the intestinal mucosa and identified. Some of these bacteria were associated with specific pathological changes.

C. coli was isolated from 22 piglets less than 3 weeks of age and from 25 weaned pigs aged 3 weeks and above. Lesions at sites from which it was isolated included thickening of the terminal ileum and enlargement of the mesenteric lymph nodes. The ileal mucosa was often congested and covered with excess mucus, the villi were reduced and cellular infiltration of the lamina propria, the presence of inflammatory cells in the crypts and hyperplasia of the submucosal lymphoid tissue were also common. It was also isolated from the large intestinal mucosa in cases of colitis.

Clostridium perfringens Type A was isolated from the inflamed mucosa of 12 suckling piglets and 11 weaned pigs and adults. Lesions at sites from which it was isolated included congestion of the mucosa and areas of focal haemorrhage and necrosis. The gut contents were in most cases fluid, creamy in consistency with occasional flecks of blood. Histological changes were most pronounced in the small intestine, particularly in the jejunum and ileum. The mucosa was congested and there was villous

atrophy with loss of the epithelial cells, and the presence of red blood cells, shed epithelial cells and inflammatory cells on the mucosal surface.

Treponema hyodysenteriae was isolated from the large intestinal mucosa of 20 weaned pigs with lesions of swine dysentery.

Campylobacter sputorum subspecies mucosalis was isolated from 2 weaned pigs with lesions of proliferative intestinal adenopathy.

B-haemolytic E. coli was isolated from 14 cases of neonatal diarrhoea and from 11 cases of post weaning diarrhoea.

Weakly haemolytic intestinal spirochaetes, differing from T. hyodysenteriae were isolated from one case of spirochaetal diarrhoea.

Fusobacterium necrophorum was isolated from the inflamed intestinal mucosa of 2 sucking and 8 weaned and adult pigs.

The remainder of the bacteria could not be clearly linked with any specific changes, but were in most cases isolated with other bacteria from lesions. Pasteurella multocida (4 cases), P. haemolytica (1) and Corynebacterium pyogenes (8) were only isolated from lesions in animals with respiratory disease.

The other bacteria found included non-haemolytic E. coli (80), Streptococcus faecalis (70), S. faecium (17),

S. suis (4), Unidentified streptococci (3), Staphylococcus hyicus (1), Staphylococcus epidermidis (13), S. aureus (1) Bacteroides fragilis (4), B. vulgatus (9), B. melaninogenicus (1), Fusobacterium fusiformis (1), Pseudomonas aeruginosa (6), Erysipelothrix rhusiopathiae (1), Proteus mirabilis (2), Peptostreptococcus intermedius (3), Unidentified peptostreptococci (6), Bacillus cereus (1), B. licheniformis (10), B. mycoides (3), Unidentified Bacillus spp. (4), Lactobacillus fermentum (2), L. catenaforme (1), Clostridium sporogenes (3), 2 isolates of unidentified clostridium.

C. coli isolated from a 7 day old piglet was used to inoculate hysterectomy derived, colostrum deprived (HDCD) piglets, conventional sucking piglets, conventional weaned pigs and HDCD weaned pigs in 4 experiments.

In HDCD piglets a rise in rectal temperature to 41.1°C occurred within 4 days of infection and a mucoid yellowish diarrhoea containing occasional flecks of blood developed. C. coli was isolated from day 2 from their faeces and from most levels of their intestines at post mortem examination 12 days post inoculation. The infected piglets were in poor condition with thickening, flaccidity and pallor of the ileum and enlargement of the mesenteric lymph nodes. The ileal contents were mucoid, and the mucosa was hyperaemic. Mild villous atrophy and inflammatory changes were seen in the small intestine of the inoculated pigs. Hyperplasia of the submucosal lymphoid tissue was prominent in the ileum. Mild colitis was present in both inoculated and controls. Agglutinating

antibody to the inocular strain of C. coli was present in sera from inoculated pigs at titres of up to 1:640.

Similar but less obvious changes were seen in conventional sucking piglets, weaned pigs and HDCD weaned pigs. The lesions developed within 4 days in the conventional piglets. C. coli was not recovered from sites other than the mesenteric lymph nodes and gut except in HDCD weaned pigs in which it was isolated from the liver. C. coli was not seen in the epithelial cells in histological studies, and did not attach to brush borders. The mild lesions in conventional pigs may have been associated with the existence of widespread C. coli infection and some immunity in the herd of origin.

C. perfringens Type A isolated from a 3 day old piglet was used to inoculate HDCD piglets and conventional weaned pigs in 4 experiments.

Inoculation of HDCD pigs with C. perfringens Type A led to a transient rise in rectal temperature to 40°C and a profuse, creamy diarrhoea with flecks of blood. C. perfringens Type A was isolated in profuse culture from their faeces. Two inoculated animals died or were killed within 72 hours of inoculation and were dehydrated and in poor bodily condition. The serosal surface of the small intestine was congested and it was flaccid with fluid and pasty contents. Flecks of blood and necrotic debris were seen on the congested mucosa on which small haemorrhages, areas of necrosis and villous atrophy was seen. The large intestinal contents were creamy, pasty and contained flecks

of blood. Colitis was present. C. perfringens was isolated only from the jejunum, ileum, caecum and colon.

In weaned pigs, faecal changes were slight and transient and pathological changes restricted to the small intestine which was flaccid with mildly congested mucosa with some necrosis and cell shedding and mucoid frothy contents. C. perfringens Type A did not appear to invade the mucosa.

The relationship of the bacteria isolated during the survey to the lesions in which they were found was discussed and it was concluded that C. coli and C. perfringens Type A could both initiate clinical and pathological changes, particularly in piglets. The mechanisms by which they did so was discussed with reference to related diseases in other species.

CHAPTER 1

REVIEW OF THE LITERATURE CONCERNING ENTERIC DISEASES OF PIGS

Enteric disease in pigs remains a problem for the farmer and veterinarian. It is a major cause of economic loss. This loss results from a combination of death, reduction in the rate of daily liveweight gain and reduced feed conversion efficiency or a combination of all the three factors. It may be present as a clinical problem, as an incidental finding at post mortem examination or as a subclinical problem in which poor growth is the presenting sign. The most common presenting sign of clinical enteric disease is diarrhoea which results from disturbances in various regions of the gastrointestinal tract. It is more common in piglets than in the older pigs and is more often a cause of death in the younger animal.

Many agents are involved, but in recent years a great deal has been discovered regarding the mode of action of the main bacteria, viruses and other agents involved. Both infectious and non-infectious agents may act singly together or in sequence to cause enteric disease and it is often difficult to determine the precise aetiology of any individual enteric syndrome.

The literature describing these infectious and other causes of diarrhoea and enteritis is reviewed below according to the type of agent, beginning with the bacteria.

BACTERIAL CAUSES OF ENTERITIS

Escherichia coli infections

E. coli is commonly associated with enteric disease in pigs especially with neonatal diarrhoea, post-weaning diarrhoea and bowel oedema. At other times it may be associated with enteric disease and at all times it may be accompanied by other enteric pathogens. Enteric diseases of pigs associated with E. coli have recently been reviewed in detail by Wilson (1981).

E. coli is commonly found in the digestive tract. Those E. coli strains that are found in the distal 2/3rd of the alimentary canal in healthy animals do not normally cause disease when introduced to susceptible newborn animals (Wilson, 1981). Some strains are, however, pathogenic to piglets, cause diarrhoea in the field and are capable of producing diarrhoea and septicaemia in very young pigs in experimental conditions.

Many pathogenic strains of E. coli produce haemolysis on sheep blood agar, but the ability to produce haemolysis on blood agar is not a guide to enteropathogenicity, as many haemolytic strains of E. coli are known to be non-pathogenic (Wilson, 1981).

E. coli strains are classified according to their O, K and H antigenic structure (Kauffmann, 1943). The O antigens are often used in diagnostic and epidemiological studies, but the K antigens are of greater significance in pathogenicity and immunity. K88, K99 and 987p antigens

are currently the most frequently encountered K antigens in enteropathogenic strains of E. coli in pigs. The K88 antigen is frequently present in strains enteropathogenic for newborn piglets. Recent studies of the pathogenic strains of E. coli indicate that adhesion and enterotoxin production are essential for disease, although there is evidence for invasion of the intestinal mucosa as a cause of disease (Blood et al., 1979).

The bowel oedema type of E. coli, even though found in the gut, is not associated with specific enteric changes.

Pathogenesis

Extensive studies have been carried out on the pathogenesis of enteric E. coli infections. There are two well recognised properties of enteropathogenic E. coli, namely the ability to adhere to the epithelial cells and the ability to produce toxins, (mainly enterotoxins).

It has been shown that colonisation plays a key role in the pathogenesis of the disease caused by enteropathogenic E. coli, (Moon et al., 1977; Moon et al., 1980). E. coli populations cause disease and reach high numbers in the pig intestine when defence mechanisms such as gastric acidity, other bacterial populations and antibody protection are deficient in some way or when stress or virus infection reduces their protective effect.

Adhesion of E. coli to the intestinal epithelium enables the organisms to overcome the cleansing action of peristaltic motility of the gut and to multiply to large

numbers in the small intestine where they produce and deliver effective levels of enterotoxins in close proximity to the target enterocytes (Smith and Linggood, 1972). The four antigenic types of pili responsible for adhesion are K88ac (Stirm et al., 1967), K88ab (Hohmann and Wilson, 1975), K99 (Smith and Linggood, 1972) and 987p (Nagy et al., 1977).

K88ab and K88ac confer on the bacterium the ability to adhere to the epithelial cells throughout the small intestine, while K99 and 987p antigens only adhere in large numbers to the distal half of the small intestine. The pili of non-enteropathogenic E. coli usually do not carry the adhesive antigens.

K88-positive E. coli adhere to brush borders prepared from small intestinal cells from the majority of pigs (Sellwood et al., 1975). Susceptibility to diarrhoea caused by both natural and experimental infections with K88-positive E. coli is limited to piglets with the adherent phenotype (Rutter et al., 1975; Sellwood, 1979). The inheritance of E. coli K88 adhesion in pigs and identification of non-adhesive phenotypes in a commercial herd was investigated by Snodgrass et al., (1981).

Two main types of enterotoxins are secreted by enteropathogenic E. coli (Smith and Gyles, 1970); namely heat-stable toxin (ST) and heat-labile toxin (LT). All the enterotoxigenic E. coli found in animals secrete ST while some secrete LT with the ST. LT toxin is antigenic but ST toxin is only weakly antigenic (Wilson, 1981). The mechanism of action of the enterotoxins has been well

documented. It is known that they are absorbed into the epithelial cells and change the normal cell metabolism. The intestines of premature pigs are known to be more sensitive to enterotoxins than those of the normal newborn piglets. As pigs grow older, their epithelial cells become less sensitive to enterotoxins. Specific receptors on the intestinal epithelial cells receive molecules of LT toxin. It has been shown by Schafer et al. (1970) that LT toxin stimulates adenyl cyclase, which results in an increase in the cyclic adenosine monophosphate (AMP) concentration in the cells. The result of this activity is an increased transfer of bicarbonate and sodium, and with this, water from the cells to the intestinal lumen. The toxic mechanism of ST toxin on the other hand is not well understood.

It has also been established that, following the multiplication phase in the small intestine, some E. coli strains can invade and destroy the epithelial cells of the mucosa to cause enteritis and subsequent diarrhoea. Systemic spread may occur in this form (Blood et al., 1979).

Immunity to enteric E. coli infections develops both at the systemic and local levels. Specific IgA antibodies directed against E. coli strains encountered by the sow are present in the milk and are adsorbed to the mucosa of the piglet's intestine where they prevent the adhesion or penetration of pathogenic E. coli and thus prevent diarrhoea. From 1-2 weeks of age the piglet intestine is capable of producing IgM antibodies to E. coli and from 2 weeks of age IgA antibodies are produced in

gradually increasing quantities until 6 weeks of age, when the full IgA response to intestinal antigens is operating. This response is protective against E. coli strains present in the normal intestine. Some of these antibodies may also be found in the serum.

Clinical signs

The clinical signs are those of diarrhoea and dehydration.

Neonatal diarrhoea: The piglets are usually born healthy and illness sets in abruptly at about 12 hours after birth. One or two members of the litter may be found dead, others may be moribund even before the onset of diarrhoea. The remaining piglets of the litter show varying degrees of diarrhoea, loss of condition and listlessness. Affected piglets have been reported to vomit in severe cases (Wilson, 1981). The diarrhoeic faeces may be extremely difficult to see on casual inspection as it is often pale or clear in colour and the fluid faecal material just dribbles from the anus down the perineum. The colour of the faeces passed by affected piglets is usually described as yellowish or brownish. Dried crusts of diarrhoeic faeces may be seen on the thighs or perineum and there may also be scalding about the anus. Affected piglets may enter a coma and die or recover with little subsequent loss of condition.

Post-weaning diarrhoea occurs within 10 days of weaning, often within 4-5 days of change of diet. Affected pigs pass greyish or brownish diarrhoeic faeces with no

traces of blood (Blood et al., 1979). The diarrhoea may be transient and resolves within 3-5 days, but it may persist. Death of affected pigs is usually due to dehydration. Chronic post-weaning enteritis may cause permanent stunting in recovered pigs.

The morbidity rate in neonatal diarrhoea may reach 70% of all piglets born and the mortality rate may approach 70% of affected piglets in the first few days of life. It then decreases rapidly so that less than 10% mortality occurs in affected pigs over 2 weeks of age. On the other hand, in post-weaning enteritis, 20 to 50% of all weaned pigs may be affected, but mortality from the uncomplicated disease is often less than 10% of those affected.

Pathological changes

Gross or microscopic tissue changes in the pigs which have died of E. coli enteritis are restricted to the enteric tract. Distension of the small intestine, loss of tone of the intestinal wall, dilation of the stomach, which is often filled with undigested milk curd are the only gross changes (Wilson, 1981) consistently seen at post mortem examination. The anterior small intestine may be mildly inflamed. This is more often seen in post-weaning diarrhoea than in neonatal diarrhoea. Histologically, there may be congestion of the blood vessels in the lamina propria and in very severe cases this may lead to haemorrhage into the intestinal lumen. Villous atrophy is either absent or present in very localised areas of the tract. With the appropriate staining, E. coli organisms are detectable

adhering to the mucosal cells of the intestine. The adherent bacteria may cover the entire villus or may be limited to the crypts of Leiberkuhn.

Bacteriological findings

Pure cultures of β-haemolytic or K88-positive *E. coli* can usually be isolated from the anterior small intestine or K99-positive *E. coli* from the posterior small intestine in *E. coli* enteritis. Isolates may subsequently be identified as known pathogenic serotypes. A simple slide agglutination test has been suggested by Wilson (1981) as being sufficient in most cases.

Diagnosis

E. coli enteritis can be suspected from the clinical signs and pathological findings described above. A presumptive diagnosis suggested by Wilson (1981) is to determine the pH of the faeces, as secretory fluid has an alkaline pH, whereas that from diarrhoeas associated with malabsorption are acid. The diagnosis can only be confirmed by the isolation of pure or profuse cultures of enteropathogenic *E. coli* from the small intestine at sites appropriate to the serotype. *E. coli* can be isolated on blood agar and colonies may be subcultured for identification as *E. coli* by biochemical means and after growth on Minka medium for antigenic analysis. The presence of K antigens such as K88, K99 and 987p are usually considered to indicate pathogenicity. Individual serological strains such as Abbotstown (O149 K88) may also

be identified using appropriate sera. Adhesiveness may be tested using the isolated brush border technique of Sellwood et al (1975). Enterotoxin production is rarely demonstrated and would be carried out using gut loop techniques (Smith and Halls, 1967).or tissue culture techniques. Animal inoculation could be used but is cumbersome (Wilson, 1981).

As viral, other bacterial and protozoal infections may contribute to or cause disease resembling both major types of E. coli enteritis, the presence of these agents e.g. rotavirus, Clostridium perfringens and coccidia should be ruled out before a diagnosis of uncomplicated E. coli enteritis is made.

Salmonella infections

Salmonella infections in pigs are worldwide in distribution but vary markedly in their prevalence from place to place. 558 cases of swine salmonellosis were reported in England and Wales between the year 1968 and 1974 (Sojka et al., 1977). The number of cases appears to be declining in Britain. Less than 200 outbreaks of salmonellosis are now recorded annually in pigs in Britain.

S. choleraesuis used to be the causal organism of 50% of outbreaks of salmonellosis in Britain and is primarily a pig pathogen capable of infecting pig herds for long periods. Early studies by Lawson and Dow (1966) and Harrington et al. (1971) placed the figure as 97% and 62.5% of isolates respectively. Its incidence is decreasing in importance compared with cases involving the isolation of salmonellae

such as S. typhimurium (from rodent-contaminated feed) and exotic salmonellae such as S. saintpaul or S. heidelberg. Infection with the latter organisms occur more often by oral infection rather than by the activation of a carrier state. S. typhimurium has been implicated as an endemic cause of diarrhoeic disease in some pig farms (Heard and Linton, 1966; Jubb and Kennedy, 1970). It has also been reported as the probable causative agent of rectal strictures (Wilcock, 1974).

All salmonella species have similar cultural characters and are divided by their antigenic characters into species. The antigens used are similar to those of E. coli, the O or somatic antigen, the H or flagellar antigens which may occur in two phases and, occasionally, the Vi antigen. Over 1500 serologically distinct species have been named, usually after their place of isolation. Many of these have been recorded from pigs.

Pathogenesis

The sequence of events following ingestion of S. typhimurium has been described in guinea pigs by Takeuchi (1967) and Takeuchi and Sprinz (1967). Following a brief period of intraluminal replication, salmonellae invaded the intestinal mucosa, particularly in the ileum. Fatal bacteraemia occurred 72 hours after inoculation. Mucosal invasion is probably necessary for disease (Giannella et al., 1973). Intraluminal replication is probably not necessary for disease production with large inocula but may be important with small inocula from contaminated feed or water.

Similar studies carried out by Lawson and Dow (1965) in pigs with S. choleraesuis showed that the organism invaded the intestinal wall 6 hours after infection and by 24 hours could be recovered from the mesenteric lymph nodes and draining lymph nodes of the pharynx. Quantitative differences in invasion beyond the mucosa are serotype related but not necessarily absolute. Thus S. choleraesuis in pigs (Smith and Jones, 1967; Wilcock, 1979) is highly invasive and enteric signs may be absent or delayed, while S. anatum has a low invasive potential and causes mild localised enterocolitis. S. typhimurium is of intermediate invasiveness (Wilcock, 1979). The pathogenesis of salmonellosis must therefore be considered as at least two processes, namely local enteric replication with necrosis and diarrhoea and systemic dissemination. Fluid secretion (diarrhoea) is known to occur independently of mucosal inflammation and necrosis as necrosis did not result in increased fluid loss (Kinsey et al., 1976) and fluid loss with resultant diarrhoea occurred without necrosis (Giannella et al., 1973).

Wilcock (1981) has suggested that diarrhoea is the result of decreased sodium resorption and increased chloride secretion, which occur in response to increased mucosal levels of cyclic adenosine monophosphate (AMP). It has been observed in pigs that the severity of diarrhoea corresponds poorly to the severity of gross intestinal lesions. Mucosal inflammation and necrosis occurs in concert with diarrhoea but perhaps independent of it. The fever and toxaemia of septicaemic salmonellosis are both direct effects of endotoxin.

Clinical signs

Salmonellosis in pigs may appear as an acute septicaemia or as an acute to chronic enterocolitis. The septicaemic form is seen most frequently in weaned pigs up to approximately 4 months of age. The disease is less common in older pigs and is usually seen as a diarrhoeic disease of variable duration and severity (Lawson and Dow, 1966; Morehouse, 1972).

Enteric form: Pigs of all ages can be affected although outbreaks are commonest in pigs aged between 3 and 4 months. The rarity of clinical salmonellosis in sucking pigs presumably results from maternal immunity, since neonatal pigs are susceptible to oral challenge with salmonellae and develop a disease comparable to that in weaned pigs (Wilcock, 1978). Outbreaks of salmonellosis characterised primarily or exclusively by diarrhoea may be acute or chronic.

The acute enteric form occurs in younger pigs. Affected animals pass a thin watery yellowish diarrhoea and may be dull and fevered. The faeces have a putrid smell containing mucus, fibrinous casts and in less severe cases, casts of intestinal mucosa (Blood et al., 1979). The disease spreads rapidly to involve most pigs in a pen within a few days. The initial diarrhoea in an individual pig usually lasts 3-7 days, but it is typical of enteric salmonellosis that the diarrhoea often recurs (Wilcock, 1981). There is often reduction of feed intake in affected animals and they are often dehydrated. Other signs that may accompany this stage of the disease are pneumonia, weakness

and paralysis and tremor. In severely affected cases, skin discolouration is present. In the chronic enteric form affected pigs appear severely emaciated and may have intermittent fever. There is persistent diarrhoea which may contain greyish shreds of necrotic epithelium. The presence of spots of blood in the faeces at intervals has been recorded by Wilcock (1981). This form is often preceded by an attack of the acute enteric form. Rectal stricture may be a sequel to enteric salmonellosis caused by S. typhimurium (Wilcock and Olander, 1977a and b).

Septicaemic form: Septicaemia occurs mainly in weaned pigs less than 4 months old, but is rare in conventionally reared sucking pigs. Animals may be found dead or show depression, dullness, weakness or even nervous signs. Affected pigs may be febrile with temperature of 40.5 - 41.6°C. Death may occur within 24 to 48 hours of the onset of disease.

A dark red to purple skin discolouration has been reported in pigs affected by S. choleraesuis (Blood et al., 1979).

Morbidity may reach 50 per cent of susceptible pigs. Mortality from the septicaemic form is almost 100 per cent but is less common in the enteric form.

Pathological findings

The pathological lesions associated with the various forms of salmonellosis have been described by Lawson and Dow (1965), Wilcock et al.(1976)and Blood et al.(1979). There may be no gross lesions in animals which have died peracutely

but there is usually evidence of septicaemia in the form of extensive submucosal and subserosal petechial haemorrhages. Classic signs of a systemic septicaemic disease are present in animals with the septicaemic form. In pigs surviving the first few days of the disease, colitis may occur (Wilcock, 1981).

Focal or diffuse necrotic colitis and typhilitis are seen as the major lesions in pigs which have died of enteric salmonellosis. The spiral colon and caecum are oedematous with fluid contents. The mucosa is reddened, roughened and may be covered with adherent yellow or grey debris. The mesenteric lymph nodes are greatly enlarged, moist and haemorrhagic. Button ulcers are occasionally seen at the ileo-caeco-colic junction. Ileal involvement is often seen with reddening and slight roughing of the mucosal surface. The typical microscopic lesion is focal or diffuse necrosis of the cells of both villous and crypt epithelium. Villous atrophy may be present. The lamina propria and submucosa contain numerous macrophages and moderate numbers of lymphocytes but few neutrophils. The necrosis frequently extends to involve the muscularis mucosae, submucosa and lymphoid follicles resulting in button ulcers (Jubb and Kennedy, 1970).

Diagnosis

Salmonellosis may be suspected on clinical grounds or from the pathological findings described above. The septicaemic form must be distinguished from other causes of sudden death and high fever. The enteric form may be

distinguished from other causes of enteric disease in weaned pigs by the frequent occurrence of high fever absent in diseases such as E. coli diarrhoea and swine dysentery. Signs of systemic involvement such as haemorrhagic lymph nodes may suggest the involvement of Salmonella spp. at post mortem examination.

The presence of the organism may be confirmed by isolation and its isolation may be significant in the absence of other pathogens. The techniques for the isolation of salmonellae vary widely with the nature of the infected material and sometimes with the serotypes sought (Wilcock, 1981). Most laboratories use a variation of tetrathionate or brilliant green enrichment followed by plating on brilliant green or another similar selective inhibitory medium. Incubation of the enrichment medium at 43°C for 24-48 hours has been recommended by Edwards and Ewing (1972) in order to eliminate contaminating bacteria such as Proteus. A pool of ileal wall and adjacent lymph nodes is the specimen of choice for isolation (Wilcock, 1981). The colon is a poor isolation site despite the obvious lesions. Spleen, liver and lung of affected pigs should also be submitted for bacteriological diagnosis. Colonies considered to be those of Salmonella spp. can be confirmed as such by their biochemical characters and by the use of 'H' and 'O' grouping antisera. Serotype identification is based on identification of specific somatic and flagellar antigens by agglutinating serology.

Serological responses are of little value in the diagnosis of salmonella infections unless the serotype

concerned is known when agglutinating antibodies may be detected in the sera of recovered pigs.

Clostridial enteric disease

The clostridial species most commonly associated with enteric disease in the pig is Clostridium perfringens. The majority of papers and reviews describe a haemorrhagic enteritis of piglets associated with C. perfringens Type C, but the presence of other types of C. perfringens, especially Type A, has been described in some enteric disease outbreaks.

C. perfringens is a gram-positive, encapsulated, non-motile anaerobic bacillus. The strains consist predominantly of single cells which are stout, relatively short and have truncated ends. Spores are difficult to demonstrate in smears from ordinary media, but when present, in sporulating medium, they are ovoid and central to eccentric. All C. perfringens strains produce exotoxins. Each type is unique in the number, potency, and biological effects of the toxins it produces but all are biochemically similar. Haemolysis patterns on horseblood agar may differ from type to type.

Clostridium perfringens Type A

C. perfringens Type A strains produce a single major lethal toxin, alpha toxin (lecithinase) and have been associated with enteritis in many animal species. In man it has been reported to cause food poisoning in association with the production of enterotoxin (Hobbs, 1965). There are also reports of both field outbreaks and experimentally

induced enteritis in animals with C. perfringens Type A. These include necrotic enteritis in chickens (Al-Sheikhly and Truscott, 1977a) and calves (Niilo and Dorward, 1971). The production of diarrhoea in rabbits by experimental feeding of C. perfringens Type A has been reported by Duncan and Strong (1969). In the pig, C. perfringens Type A does not receive the same amount of attention as an enteric pathogen. In the past, it was regarded as a part of the normal gut flora (Smith and Jones, 1963). Recently, however, there have been reports of isolation of C. perfringens Type A from enteric lesions in pigs from which no other major pathigen could be demonstrated (Amtsberg et al., 1976).

Moon and Dillman (1972) speculated that it might be an important agent in diarrhoeal diseases of piglets in which no other known aetiological agents could be demonstrated. However, up to the present time, there are no accounts anywhere in literature of the reproduction of enteric disease in pigs using C. perfringens Type A.

In species in which C. perfringens Type A has been investigated, both the vegetative cells and extracts containing toxins have successfully reproduced the enteric lesions. Characteristic findings in the experimental disease are transient diarrhoea in intact animals and the accumulation of clear straw-coloured or blood tinged fluid in ligated intestinal loops (Niilo, 1971; Hauschild et al., 1971). In the experimental disease in lambs, bacterial invasion of the intestinal mucosa does not occur (Hauschild et al., 1967) and the clinical response can be reproduced

by cell free extracts of the causative agent (Hauschild et al., 1970). Necrotic enteritis has also been reproduced by feeding both the vegetative organism and cell free extracts to chickens (Long and Truscott, 1976; Al-Sheikhly and Truscott, 1977a,b). Death can result from enteric infections of animals with C. perfringens Type A (Long and Truscott, 1976) and the organism is frequently isolated from intestinal lesions in dead animals.

Clostridium perfringens Type C

Within the last three decades, C. perfringens Type C has been recognised as the cause of a highly fatal necrotic enteritis, of pigs less than 1 week old, in various parts of the world. Even though young piglets less than 1 week of age are usually affected (Bergeland, 1981), sucking pigs up to a month old are occasionally affected and the disease has also been reported in slightly older pigs as part of the post weaning enteritis complex (Høgh, 1974; Meszaros and Pesti, 1965; Matthias et al., 1968).

In pigs, C. perfringens Type C infection was first described in England (Field and Gibson, 1955) and Hungary (Szent-Ivanyi and Szabo, 1955). Since then it has been reported from various parts of the world (Bergeland, 1981). The disease is relatively uncommon in Britain but is much more important in Hungary and the United States of America.

Pathogenesis

The pathogenesis of C. perfringens Type C infections have been reviewed by Bergeland (1981). The organism is

ingested within a few minutes or hours of birth, and infection is soon established in the jejunum. In the absence of protective colostral immunity, the organism adheres to the intestinal epithelium at the bases of the villi and elaborates toxin to produce the characteristic effects.

The bacteria have been shown to attach to and invade the epithelium of the villi, and they appear to proliferate along the basement membrane. Attachment first occurs at the apices of jejunal villi (Arbuckle, 1972). This is accompanied by desquamation of the epithelium and complete necrosis of the lamina propria of the villi. In peracute cases, massive haemorrhage accompanies the necrosis. The majority of the bacteria remain attached to the necrotic villi and the villi together with the adherent bacteria are sloughed into the intestinal lumen. The precise role of bacterial toxins in the pathogenetic mechanism is not fully understood. The lethal, necrotising beta toxin is the most potent toxin elaborated by Type C strains and is probably the major factor contributing to the development of intestinal necrosis.

Reproduction of the disease by oral administration of bacteria-free culture filtrates has been reported; however, viable Type C organisms were isolated from the intestines of these pigs (Field and Goodwin, 1959; Bergeland, 1965). The characteristic intestinal necrosis appears to result from active invasion of the mucosa by Type C organisms rather than the simple presence of the toxin in the intestinal lumen. Intestinal necrosis has not been

observed in ligated intestinal loops injected with bacteria-free toxin, whereas focal areas of bacterial invasion and necrosis have been seen in loops injected with whole-broth cultures of C. perfringens Type C (Bergeland, 1972). Death may be caused by one or more of the consequences of intestinal necrosis. In some cases there is a secondary bacteraemia. Hypoglycaemia is an important factor in some cases (Field and Goodwin, 1959; Høgh, 1967a). Toxaemia may be a contributing factor in occasional peracute cases in which pig collapse and die suddenly (Bergeland, 1981).

Clinical signs

Considerable variation in the duration of the clinical course of the disease has been described by different research workers. This variation may be evident not only among different herds but also among different litters in the same herd and among littermates. The mortality rate of pigs with clinical signs is consistently very high, and complete recovery is rare. Morbidity appears to vary among different herds (Bergeland et al., 1966; Høgh, 1967b). In the peracute form, sudden death occurs in piglets with little or no evidence of diarrhoea. Others may have haemorrhagic diarrhoea and there may be perineal staining with bloody fluid. Death usually occurs on the first or second day after birth. In the acute syndrome, affected animals may survive for two days after the onset of clinical signs, and commonly die within three days. Throughout the course of the disease they have reddish brown liquid faeces that contain shreds of grey necrotic debris (Blood et al. 1979). They became

progressively gaunt and weak and make only feeble attempts to suck during the last day. In the subacute form, the affected animals have a persistent, nonhaemorrhagic diarrhoea and usually die when 5-7 days old. They remain active and alert and continue to eat, but become progressively more emaciated and dehydrated before death. The faeces tend to be soft and yellow at first and then change to a clear liquid containing flecks of grey necrotic debris.

Chronic cases may have an intermittent or persistent diarrhoea for one or more weeks. Faeces are yellowish-grey and mucoid, and the perineum and the tail may be coated with dried faeces. Affected pigs may remain alert and vigorous for 10 or more days, but their rate of growth is greatly retarded. These pigs may eventually die after several weeks. Survivors failed to gain weight.

Pathological findings

The post mortem lesions associated with enteric infection with C. perfringens Type C have been adequately documented both in Europe and in the United States of America (Barnes and Moon, 1964; Field and Gibson, 1955; Høgh, 1969; Szent-Ivanyi and Szabo, 1955; Bergeland, 1981). The most consistent pathological feature is varying degrees of necrosis and desquamation of the epithelial layer of the mucous membrane of the jejunum, and sometimes of the ileum. In peracute cases, the jejunum is dark red and the lumen is filled with blood stained fluid. The rest of the small and large intestine also contains bloody fluid. The peritoneal cavity may contain 5-10ml of red fluid, and the mesenteric

lymph nodes are bright red. Microscopically, the villi in the jejunum are necrotic and are covered by large bacilli. The epithelium of the crypts may be necrotic. Profuse haemorrhages are present in the mucosal and submucosal layers. Acute cases characteristically have more conspicuous necrosis than peracute cases, but haemorrhage is less evident. The jejunum may be emphysematous and there may be fibrinous peritonitis. The mucosa is yellow or grey, and the lumen contains necrotic debris. Most of the villi have sloughed, leaving a necrotic membrane overlying the submucosa. Submucosal vessels are necrotic and may contain thrombi. The affected portion of the jejunum and ileum in piglets with subacute disease is thickened with a tightly adherent necrotic membrane in place of the mucosa. The intestine of pigs with chronic disease may appear normal from the serosal surface. Close inspection of the mucosal surface reveals one or more areas lined by firmly adherent necrotic material. Microscopically, there is replacement of the mucosa by a necrotic membrane, with numerous bacteria on its underside. The submucosa and tunica muscularis are infiltrated by chronic inflammatory cells.

Diagnosis

A presumptive diagnosis can usually be made on the basis of clinical and gross findings in peracute and acute cases. The observation of haemorrhagic diarrhoea together with a finding necrohaemorrhagic jejunitis at necropsy, strongly suggests a diagnosis of *C. perfringens* Type C enteritis. The presence of an emphysematous segment of jejunum is especially significant. However, the chronic

form of the disease may be confused with necrotic enteritis caused by other agents, such as Isospora suis, rotaviruses and T.G.E. (Bergeland, 1977). Laboratory confirmation of the diagnosis should include the identification of C. perfringens beta toxin in the intestine or isolation of C. perfringens Type C from the intestine. Mouse protection tests have been reported as another useful tool in the confirmation of the presence of C. perfringens Type C toxin. The presence of Gram positive bacteria around the villous outline has been reported as an aid to histological diagnosis (Smith and Jones, 1963; Høgh, 1969). Experimental reproduction of disease with organisms isolated from field outbreaks can also be used to confirm diagnosis (Field and Goodwin, 1959; Bergeland, 1965).

Campylobacters in enteric infections

Campylobacters (formerly Vibrios) have long been associated with enteric diseases in both man and animals. Enteric disease associated with Campylobacters was first reported in pigs by Whiting et al. in 1921. Whiting (1928) proposed that a Vibrio was the cause of swine dysentery since it was seen in large numbers in the lesions and more consistently than any other microorganism. Doyle (1944), James and Doyle (1947) and Roberts (1956b) isolated a Vibrio from field cases and claimed to reproduce swine dysentery experimentally with their isolates.

Extensive changes in the classification and nomenclature of the genus Campylobacter (Vibrio) have occurred since these early reports and they are no longer

considered to cause swine dysentery. More recently, Campylobacter sputorum subsp. mucosalis has been isolated from and associated with proliferative intestinal adenopathy (Lawson and Rowland, 1974; Lawson et al., 1975), proliferative haemorrhagic enteropathy (Rowland and Lawson, 1975, and Love et al., 1977) and necrotic enteritis (Rowland and Lawson, 1974, 1975). It has not appeared in the most recent list of approved bacterial names. Two other Campylobacters have been reported in association with porcine disease. They are C. jejuni which has been isolated from 2 out of 208 faecal samples in normal pigs (Prescott and Bruin-Mosch, 1981) and from faeces, washed intestines and from healthy slaughtered pigs (Sticht Groh, 1982) and an aerotolerant Campylobacter isolated from aborted pig foetuses (Ellis et al., 1977, 1978). Because of the variation in names given to the Campylobacters in the recent past, the important characters of these organisms and the species to which they refer are reviewed briefly below.

The microaerophilic vibrios have been removed from the genus Vibrio, and reclassified in the genus Campylobacter (Sebald and Veron, 1963). A number of species of Campylobacters are found in man and animals. The classification of these Campylobacters has been reviewed by Smibert (1974 and 1978). All Campylobacter species are curved Gram-negative rods. All are oxidase-positive and some produce catalase, a property that serves to divide the genus into two groups. The majority of catalase-positive Campylobacters have been classified as shown below (Table 1).

TABLE 1.

CLASSIFICATION OF CATALASE POSITIVE CAMPYLOBACTERS

	Veron and Chatelain (1973)	Smibert (1974)	Skerman et al. (1980) (approved names)
V.f.ss.venerealis (Florent 1959)	c.f.ss.venerealis		c.f.ss.venerealis
V.f.ss.intestinalis	c.f.ss.fetus	c.f.ss.intestinalis	
V.f.subtype 1 (Bryner et al.1962)	c.f.ss.venerealis Subtype intermedius		
V.fetus (Smith and Taylor 1919);Sebald and Veron, 1963)	C.fetus		C.fetus
V.coli (Doyle, 1948)	C.coli	c.f.ss.jejuni	C.coli
V.jejuni (Jones et al.1931)	C.jejuni	c.f.ss.jejuni	C.jejuni
Related vibrios (King, 1957)		c.f.ss.jejuni	
V.fecalis (Firehammer, 1965)	C.fecalis	C.fecalis	

In addition to these species names, an unnamed aerotolerant campylobacter has been isolated from pig aborted foetuses (Ellis et al., 1978; and Neill et al., 1979)

The cultural and biochemical characters used to separate these species have been listed and reviewed by Florent, 1959; Veron and Chatelaine, 1973 and Smibert, 1974. C. jejuni and C. coli have recently been distinguished by the hippurate test (Harvey, 1980) as described by Skirrow and Benjamin (1980) who showed that C. jejuni produced glycine from hippurate by hydrolysis while C. coli did not do so. This test has enabled the two species to be separated more readily than by the combination of characters previously used. These included the ability of C. coli to grow on medium containing 1:100,000 brilliant green, on which C. jejuni does not grow, and the ability of C. coli to grow at a temperature of 30.5°C at which C. jejuni does not grow.

Catalase-negative Campylobacters These are represented by a single species, C. sputorum (Gibbons, 1973; Prevot, 1940), within which two subspecies are recognised by Bergey, namely C. sputorum subsp. sputorum and C. sputorum subsp. bubulus. C.s. subsp. sputorum has been found in the human oral cavity (Loesche et al., 1965) and in about 3 per cent of faecal samples from normal people (Skirrow, 1979a); while C.s. subsp. bubulus has been recovered from the preputial sac of normal bulls (Florent, 1953), and from intestines of calves (Lederle, 1963). C.s. subsp. mucosalis has been associated with proliferative intestinal enteropathy (Lawson and Rowland, 1974; Lawson et al., 1975); proliferative haemorrhagic enteropathy (Rowland and Lawson, 1975; Love et al., 1977) and necrotic enteritis (Rowland and Lawson, 1974, 1975) in pigs.

Campylobacter coli

C. coli (formerly Vibrio coli) was first isolated by Doyle (1944), but it was not until 1948 that it was given the name Vibrio coli (Doyle, 1948). Since then the organism has been recorded from pig intestines and faeces in a number of different countries. Schmid (1949) in Switzerland; Truszczyenkik (1957) in Poland; Van Ulsen (1953) in Holland; Gorrie (1952) and Roberts (1956b) in Australia. In Britain, the first report of its presence from enteric disease of the pig was by Birrell (1957) who was able to demonstrate vibrios in smears from affected mucosa. Deas (1960) described the first isolation of the organism from enteric lesions of pigs in the south of Scotland. The original isolate of C. coli (Vibrio coli) has been lost and until its recent distinction from C. jejuni (Benjamin and Skirrow, 1980) few of the reports of its isolation were accompanied by any biochemical evidence of its identity with the original description. In addition, for 25 years (1944-1969), it was considered to be the initiating agent of swine dysentery. For this reason, the literature relating to C. coli is complicated and an attempt is given below to review its occurrence in lesions and to interpret the experimental studies which have been reported, often as attempts to reproduce swine dysentery.

C. coli has been recorded in the porcine small intestine by Deas (1960) who isolated the organism from inflamed terminal ileum of weaned pigs with diarrhoea. He produced transient diarrhoea in weaned pigs by feeding

pure cultures of his isolate in experimental studies. Lawson and Rowland (1974) recorded the presence of C. coli in the small intestines of pigs with intestinal adenomatosis but considered that the organism played no part in that syndrome. Birrel (1957) described a syndrome in which yellowish diarrhoea occurred in piglets of three days of age onwards. Mild catarrhal enteritis was noted in the small intestine but vibrios (C. coli) were only isolated from the large intestine.

The association of C. coli with large intestinal disease is less clear. The observations by authors such as Doyle (1944) that C. coli (V. coli) could cause large intestinal disease when fed in pure culture to experimental pigs have been disregarded by workers on enteric disease in pigs in recent years. This lack of attention was partly due to the failure of workers such as Andress and Barnum (1968) to reproduce swine dysentery, the condition under study, by feeding pure cultures of C. coli to weaned pigs and partly due to the demonstration that a spirochaete initiated swine dysentery (Taylor and Alexander, 1971). The accounts by Doyle (1948), James and Doyle (1947) and Roberts (1956a and b), describe the production of diarrhoea 5-27 days after inoculation.

Blood and mucus were seen in varying quantities in the faeces and C. coli (V. coli) was demonstrated in the faeces 2 days following infection. C. coli was also isolated on blood agar plates from the mucosal surface of the terminal portion of the small intestine and the large intestine. However, in the studies described by Warner

(1965), little faecal change other than mild mucoid or transient diarrhoea 12 days after infection was reported. The campylobacter was demonstrated by direct smears and cultures in both the small and large intestines. Similar results were obtained by Andress et al. (1968) and Andress and Barnum (1968) who failed to note the production of clinical signs and reported only mild inflammatory changes and the reisolation of the campylobacter (Vibrios) from the intestinal contents and caecal and colonic mucosal surfaces, thus proving that the organism readily established in most of the pigs inoculated in spite of lack of clinical disease.

It appears therefore that C. coli can be found in lesions and that there is some evidence for its involvement in enteric disease but the results of experimental infections suggest that any syndrome produced is not as dramatic as swine dysentery.

The clinical signs and post mortem findings associated with experimental infections with C. coli (or V. coli) may be summarised as follows: clinical signs have included mild or transient diarrhoea which has occurred within 2-3 days of inoculation or, as long as 12 days afterwards (Warner, 1965) or 27 days (Doyle, 1948). The faeces contained varying amounts of mucus and, in some reports, blood. Inflammation of the terminal ileum was reported by Deas 1960; the small intestine was reported to be empty in infected pigs (Warner, 1965), while necrotic debris containing inflammatory cells was observed in the lumen of the terminal ileum. The large intestine was reported to be distended with fluid or semi-solid mucoid

contents, often with blood specks. Patchy hyperaemia and roughening of mucous membranes were noted in the caecum and colon. The wall of the large intestine was thickened. Inflammatory changes were noted microscopically.

The only recent studies of this type are those reported by Prescott et al. (1982) who infected gnotobiotic piglets with C. jejuni, a related species, and found that mild intestinal changes including increased fluidity of the contents, and moderate inflammation of the colonic mucosa occurred within 5-6 days of inoculation. These studies lend some support to the conclusions drawn above from the literature on C. coli itself.

Campylobacter sputorum subspecies mucosalis

Campylobacter sputorum subspecies mucosalis is often isolated from enteric lesions of pigs. It has never been isolated from any other species. The bacterium was characterised and named by Lawson et al. (1975). C. sputorum subsp. mucosalis produces a typical yellow pigment on blood agar plate. It has been considered as the cause of proliferative intestinal adenopathy (P.I.A.) in pigs because the organism is constantly recovered in large numbers from the lesions and is the only one present in them (Lawson and Rowland, 1974).

Other conditions involving proliferation of porcine intestinal epithelium include porcine intestinal adenomatosis, necrotic enteritis, regional ileitis and proliferative haemorrhagic enteropathy.

The earliest accounts of P.I.A. in the English literature was that of Biester and Schwarte (1931) and Biester et al. (1939). Other contributions to the nature of the disease complex include the studies of Field et al. (1953), Hoorens (1962), Westendrop (1965), Love et al. (1977) and Roberts (1978).

Pathogenesis

It is now known that P.I.A. develops as a progressive proliferation of the immature epithelial cells populated by C. sputorum subsp. mucosalis (Rowland and Lawson, 1981). Once established, the infected cells fail to mature and continuous mitosis follows. The immature cells are not shed and the glands become enormously enlarged and often branched. The changes in the mucosal epithelium may regress or may become necrotic to give the condition named necrotic enteritis. On the other hand mucosal proliferation and erosion may result in changes in the muscular coat of the terminal ileum which becomes hyperplastic to give regional ileitis or "hose pipe" gut.

Clinical signs

Proliferative intestinal adenopathy complex usually occurs as a sporadic disease in a piggery manifesting itself by the appearance of occasional groups or individual animals which fail to make satisfactory weight gains. Clinical cases are observed most commonly in the post weaned fattening pigs between 6 and 20 weeks of age. Affected animals vary from clinically normal to markedly dull and

apathetic. Diarrhoea is not always a feature of this form of the disease. Because the signs are not very dramatic, many cases may not be noticed. Roberts et al. (1979) described one such herd in which close inspection of the production figures allowed the identification of a proportion of affected but clinically normal animals. In fattening herds, during the post weaning period, wasting of a well grown animal with anorexia and irregular diarrhoea is frequently seen (Rowland and Lawson, 1981). Death is not uncommon and is frequently associated with perforations of the hypertrophied ileal wall, leading to a generalised terminal peritonitis.

Porcine haemorrhagic enteropathy occurs more commonly in young adults than in younger growing animals and present a clinical picture of acute haemorrhagic anaemia. Black tarry faeces may be passed by chronically affected pigs. Animals may die without showing any faecal changes.

Recovery from uncomplicated P.I.A. occurs between 4 and 6 weeks after the onset of clinical signs, with a return of appetite and growth rate to normal levels. Recovery from proliferative haemorrhagic enteropathy (Love et al., 1977) also occurs. 12 per cent morbidity and 6 per cent mortality rates have been reported from field studies (Rowland and Lawson, 1981).

Pathological changes

The pathological changes associated with porcine intestinal adenomatosis complex have been described by Emsbo (1951), Rowland and Rowntree (1972), Martisson et al. (1974)

and reviewed by Rowland and Lawson (1981). These changes are known to occur most commonly in the terminal 50cm of the small intestine and the upper third of the spiral colon including the caecum. The magnitude of the proliferation varies widely, but in the developed case the intestinal wall is visibly thickened and the overall diameter is increased. Also commonly seen is the oedema of the subserosa and mesentery. The mucosa is thrown into folds. Histologically, the mucosa is composed of large, branching glands, lined by immature cells. Mitotic figures occur in all crypts. Goblet cells are absent. Electron microscopy reveals C. sputorum subsp. mucosalis lying in the apical cytoplasm of affected epithelial cells. Many cases show little evidence of inflammatory reaction (Rowland and Lawson, 1974; Martisson et al., 1974). In necrotic enteritis, a yellow grey cheesy mass adheres lightly to the wall of the intestine. Histologically, coagulatory necrosis is clearly defined.

In regional ileitis, the whole of the ileum is almost rigid and is referred to as 'hose pipe gut'. Ulceration, usually linear, is always seen on the mucosal surface. Granulation tissue may be present. Hypertrophy of the outer muscle coat is much in evidence. Large intestinal lesions are rare in P.H.E.. Blood clots are seen in the ileum. Few gross changes are seen on the mucosa except for adenomatous thickening. Histologically there is an extensive degeneration of adenomatous epithelium, with accumulation of cellular debris in the crypts.

Diagnosis

There are no laboratory tests currently available to confirm the presence of the P.I.A. complex in a living animal. The clinical signs are always too slight to offer any substantial clue to diagnosis. However, a failure to thrive in the absence of other major pig disease may be the most useful sign (Rowland and Lawson, 1981). The presence of blood in the faeces may be suggestive of P.H.E.

At necropsy the use of modified Ziehl-Neelsen stain on mucosal smears to demonstrate the intracellular organism may be helpful, especially where lesions are minimal. Recovery of C. sputorum subsp. mucosalis in large numbers is now a possible aid to diagnosis, using a selective medium described by Lawson and Rowland (1974). It is usual to isolate the organism in any number from the alimentary tract of conventional pigs unless adenopathy is present. Histological lesions are also a useful aid to diagnosis. The presence of intracellular bacteria has been described by Lawson and Rowland (1975) by the use of silver staining techniques. Immunofluorescent techniques have also been recorded. P.I.A. has proved to be very difficult to reproduce with pure cultures of C. sputorum subsp. mucosalis. It has however been shown that pre-treatment of pigs with benzetinide to reduce gastrointestinal peristalsis allowed mucosalis infection to become established under experimental conditions in weaned pigs (Roberts et al., 1980b).

Treponema hyodysenteriae infection

T. hyodysenteriae is the initiating agent of swine dysentery, a severe mucohaemorrhagic diarrhoeal disease, affecting pigs during the growing-finishing period. Although swine dysentery was first described in 1921 by Whiting et al., the aetiology remained unknown for 50 years. In 1971, Taylor and Alexander at Cambridge University reported the successful isolation and propagation of this pathogenic anaerobic spirochaete. Their work was later confirmed at Iowa State University, and the spirochaete was named T. hyodysenteriae (Glock and Harris, 1972; Harris et al., 1972). Other research workers have since then consistently reported isolation of T. hyodysenteriae from field cases of swine dysentery (Hunter and Ross, 1972; Akkermans and Pomper, 1973; Hamdy and Glenn, 1974).

Pathogenesis

The normal route of infection of pigs with T. hyodysenteriae is by the ingestion of infectious material. T. hyodysenteriae and perhaps other synergistic supporting organisms then multiply within the large intestine (Harris and Glock, 1981). Experimental studies in gnotobiotic pigs revealed the fact that other agents or factors appeared to be required for the production of characteristic lesions. Harris et al. (1972) showed that while T. hyodysenteriae could persist in the colon of gnotobiotic pigs it failed to produce lesions or invade the mucosa. Meyer et al. (1975) could only produce lesions of swine dysentery in gnotobiotic pigs with T. hyodysenteriae in

combination with 4 gram-negative anaerobic bacteria. Some of the other agents used along with T. hyodysenteriae to produce typical disease in gnotobiotic pigs include Bacteroides vulgatus, (Harris et al., 1978); B. vulgatus, Fusobacterium necrophorum, Clostridium spp., Listeria nitrificans, Lactobacillus spp., and non-pathogenic E. coli (Whipp et al., 1979, 1980). However, the failure to produce disease in gnotobiotic piglets with T. hyodysenteriae alone has recently been shown to be due to insufficient numbers of the organism in the inoculum and the other agents are not essential (Whipp et al., 1982).

T. hyodysenteriae can be seen within epithelial cells and the lamina propria in typical lesions but there is no evidence that invasion is essential for lesion production (Glock et al., 1974). A number of experiments have been carried out both in vivo and in vitro to determine the mode of action of T. hyodysenteriae on the mucosal surface. Wilcock and Olander (1979a,b), Knoop et al.(1979) and Knight (1979) demonstrated the attachment of T. hyodysenteriae to animal cells in vitro, but they found that cellular damage and invasion did not occur.

The use of sterile filtrates of broth cultures of T. hyodysenteriae caused no fluid accumulation in ligated colonic loops of pigs or suckling mice (Whipp et al., 1978). Inactivated whole cells and sonically disrupted suspensions of the organism do not cause lesions or fluid accumulation in ligated colonic segments of pigs. Available evidence suggests that T. hyodysenteriae does not invade beyond the

lamina propria of the large intestine.

The studies of pathophysiology of the disease by Argenzio (1980) and Argenzio et al. (1980) have shown that fluid loss as diarrhoea results from colonic malabsorption as a consequence of the failure of the epithelial transport mechanisms to transport sodium and chloride actively from the lumen to the blood. Cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP) levels in colonic mucosa of infected pigs were found to be normal, but their response to theophylline stimulation was markedly reduced. It is therefore suggested that an enterotoxin and/or prostaglandin release from the inflamed mucosa are not involved in the production of diarrhoea. Therefore the pathogenesis of swine dysentery is unlike the diarrhoea induced by enterotoxigenic E. coli or Salmonella spp..

Clinical signs

The clinical signs described by various research workers have been reviewed by Harris and Glock (1981). Diarrhoea is the most consistent sign of swine dysentery but the severity may be quite variable. The onset of the disease is gradual and the early signs are often overlooked. The disease usually spreads gradually through an infected herd, with new animals being affected each day. The first evidence of the disease in most animals is the passage of soft faeces (Alexander and Taylor, 1969) or twitching of the tail, abdominal discomfort, hollowing of the flanks and slight inappetence. A transient fever of up to 40.6°C may occur, but disappears at the onset of diarrhoea which

persists throughout the course of the disease. As diarrhoea progresses, watery stools containing blood, mucus, and shreds of white mucofibrinous exudates are seen with concurrent staining of the perineum. Prolonged diarrhoea leads to dehydration with increased thirst and affected animals become gaunt, weak, uncoordinated and emaciated, and may die.

In the surviving pigs, recovery begins 7-14 days after the onset of clinical signs. Chronically affected pigs may remain stunted and unthrifty. The feed conversion ratio and the daily liveweight gain may be permanently depressed (Taylor, 1976). Immunity may affect the severity and duration of the clinical signs (Terpstra et al., 1968; Olson, 1974). Medicated feeds have also been shown to affect the clinical signs of the disease (Olson and Rodabaugh, 1976a,b).

Suckling piglets are not commonly affected by swine dysentery. They only show a catarrhal enteritis without haemorrhage when infected.

Pathological findings

The pathological lesions associated with the infection of T. hyodysenteriae have been adequately described in the literature (Whiting, 1921; Taylor and Alexander, 1971; Glock and Harris, 1972). Dead pigs are emaciated and dehydrated, with soiling of the perineum. A consistent characteristic of the disease is the presence of lesions in the large intestine but not in the small intestine, often with a sharp line of demarcation

at the ileocaecal junction. The large intestine is usually flaccid and may appear dark or congested with a slimey surface due to oedema of the serosa.

Typical changes in the acute stages of the disease include hyperaemia and oedema of the wall and mesentery of the large intestine. The mesocolic lymph nodes are swollen and pale. The contents are fluid, foul smelling and contain varying proportions of mucus and necrotic materials. The mucosal surface is swollen and congested. As the condition progresses, the amount of oedema within the wall of the large intestine may decrease. Mucosal lesions may become more severe with increased fibrin, which forms firm thick mucofibrinous pseudomembranes containing blood. As lesions become more chronic, the mucosal surface is covered by a fibrinous exudate, often giving the appearance of marked necrosis. Necrosis is, however, superficial.

Microscopic lesions are found primarily in the large intestine but are not specific for swine dysentery. Typical acute lesions include obvious thickening of the mucosa and submucosa due to vascular congestion and extravasation of fluids and leucocytes. There is also hyperplasia of goblet cells and the epithelial cells at the base of the crypts may be elongated and hyperchromic. Focal areas of haemorrhage may be seen and blood is trapped in the overlying mucus. Later changes include the accumulation of large amounts of fibrin, mucus and cellular debris in mucosal crypts and the luminal surface of the large intestine. Superficial necrosis is often observed,

but deep ulceration is not typical. Increased numbers of neutrophils are seen throughout the lamina propria. T. hyodysenteriae may be demonstrated in the lumen and within crypts at all stages of the disease but is most numerous in the acute early phase (Taylor and Blakemore, 1971). Chronic microscopic changes are not specific. At ultrastructural examination, T. hyodysenteriae is seen within cells lining the crypts of the colonic mucosa. The spirochaete infected cells and others adjacent to them often appeared domed with swollen mitochondria, dilated endoplasmic reticulum and reduction in size or loss of the microvilli. T. hyodysenteriae may be found in clusters within some epithelial cells, suggesting that intracellular multiplication may occur (Glock, 1971; Taylor and Blakemore, 1971; Glock et al., 1974).

Diagnosis

The diagnosis of swine dysentery can be made from the history of the outbreak, the clinical signs, gross and microscopic lesions, and is confirmed by the isolation and identification of T. hyodysenteriae. The examination of Gram-stained air-dried or wet smears of faeces for the spirochaete may be useful. The organism may be isolated on a selective medium containing 400 mg/ml of spectinomycin incorporated in blood agar, incubated at 42°C under anaerobic conditions (Songer et al., 1976). A direct fluorescent antibody test, using acetone-fixed faeces smears has been widely used in Britain (Hunter and Saunders, 1977). This test may be used to identify isolates of T. hyodysenteriae and biochemical and other serological

tests such as the growth inhibition test can also be used. Finally the isolate may be inoculated orally into experimental pigs.

Infected or recovered pigs may also be identified by the serum agglutination test or the Elisa test which detect serum antibody.

Other Intestinal Spirochaetes

Spirochaetes other than T. hyodysenteriae are known to colonise the colonic mucosa of pigs. These can be distinguished from T. hyodysenteriae by size, ultrastructure, biochemical and cultural characteristics. While T. hyodysenteriae is known to produce complete haemolysis on blood agar plates, these other spirochaetes are less haemolytic. Less haemolytic spirochaetes were considered by Harris to be non-pathogenic as they failed to initiate obvious clinical or pathological changes in experimental pigs. Isolates of this type have been named Treponema innocens by Kinyon and Harris (1979). Such strains isolated in Britain were designated 4/71 by Taylor (1972) and PWS by Hudson et al. (1976).

There is however a recent report of isolation of a weakly haemolytic spirochaete from diarrhoea of pigs, and initiation of clinical disease in experimental pigs with the isolates (Taylor et al., 1980). The spirochaete designated P43/6/78 is weakly β-haemolytic and was found not to fluoresce with a specific antiserum to T. hyodysenteriae. The disease syndrome reproduced

experimentally was less severe than typical swine dysentery. Diarrhoea, containing clear mucus, with occasional blood occurred in 4 out of the 8 inoculated animals. At necropsy, colitis was seen as the main lesion. Excess clear mucus and punctate haemorrhages were seen on the colonic mucosa and spirochaetes resembling isolates P43/6/78 were recovered from the mucosa. Feed conversion efficiency and growth rate of affected pigs were reported to be reduced compared with the controls. There are as yet no other reports to confirm these findings.

Enteric infections with Yersinia spp.

Yersinia spp. have been recorded in enteric disease in pigs and in normal pig faeces (Toma and Deidrick, 1975; De Barcellos and De Castro, 1981). Until recently the species Y. pseudotuberculosis and Y. enterocolitica had not been properly distinguished and literature published prior to the early 1970s may have referred to Y. pseudotuberculosis when in fact the organism concerned was Y. enterocolitica. In much of the literature an element of uncertainty still remains about the identity of the species isolated, and in this review the species names given by the various authors will be used.

Apart from causing enteric disease in pigs, Y. enterocolitica is of significance in pig husbandry because it is pathogenic for humans causing gastroenteritis and other symptoms (Toma and Lafleur, 1974; Marks et al., 1980). Since it is commonly found in the gastrointestinal tract of pigs, it is a potentially significant zoonosis-

inducing organism. Domestic animals including pigs, have been implicated as potential reservoirs of Y. enterocolitica from many countries including U.S.(Wooley et al., 1980); Canada (Toma and Deidrick, 1975); Denmark (Pederson et al., 1979); and Japan (ZenYoji et al., 1974). In Britain, however, the recent studies suggest that there is a low incidence of carriage of Y. enterocolitica in pigs from the south of England (Walker and Coleman, 1979).

Yersinas are aerobic and facultatively anaerobic, Gram-negative, small pleomorphic, non-sporing and non-capsulated coccobacilli motile in broth culture at 22°C but not at 37°C. The ovoid forms show bipolar staining. The two main species recorded in enteric lesions of pigs are Y. pseudotuberculosis and Y. enterocolitica, eventhough the latter is now reported more often than the former. Cook (1952) described a modified Ziehl-Neelsen staining method that showed Y. pseudotuberculosis as weakly acid-fast.

On the basis of the somatic O antigenic structure Y. pseudotuberculosis has been divided into 5 serotypes. Eight serotypes of Y. enterocolitica have been described and some have somatic antigens in common with Y. pseudotuberculosis.

Pathogenesis

Most natural infections of animals with Yersinia spp. follow ingestion of contaminated feed (Paterson and Cook, 1963). Stress factors are necessary to precipitate disease, as most outbreaks of clinical tersinia infections occur in the winter months. Poor feeding, transportation and other

infections may also contribute to the onset and severity of disease (Henriksson, 1963). It has been suggested that a pre-existing gastrointestinal mucosal lesion may be necessary for oral infection to be effective in establishing enteric lesions (Goret et al., 1955).

Eventhough Y. pseudotuberculosis has been reported from diarrhoea in pigs, the organism is not known to produce enterotoxin (De Barcellos and De Castro, 1981). Some strains of serotype III of Y. pseudotuberculosis are known to produce exotoxin (Haagsma, 1970). Y. enterocolitica, on the other hand, is known to produce heat stable enterotoxins. Mosimabale and Gyles (1982) found the pig gut loop test unsuitable for detection of heat stable enterotoxin; but were able to detect enterotoxin produced by some strains in infant mice. Studies of some of the fatal cases of Y. enterocolitica infections in man have demonstrated an invasive pattern of enteritis with gross ulcerative lesions (Bradford et al., 1974). Similar findings have been reported from experimental infections in mice and monkeys (Carter, 1974; Maruyama, 1973). Very little, if any, work has been done in pigs. A pig model of diarrhoea may be required to elucidate the role of Y. enterocolitica enterotoxin.

Clinical signs

The clinical enteric disease produced by Yersinia spp. infection in pigs has not been properly documented. There are however reports of diarrhoea in pigs from which Y. pseudotuberculosis (De Barcellos and De Castro, 1981)

and Y. enterocolitica (Toma and Deidrick, 1975) were isolated. Generally, the clinical signs of Yersinia infection are non-specific. Morita et al. (1968) described the signs noted in some pigs infected with Y. pseudotuberculosis. These include the oedema of the eyelids, mandible and lower abdomen. Other signs recorded in pigs infected with Yersinia spp. include depression, anorexia, diarrhoea, which may be bloody, and rapid emaciation.

Pathological findings

The information available on pathological changes in Yersinia spp. infected pigs is still very scanty. The lesions vary widely between slight enteritis to necrotic colitis with infection with Y. enterocolitica. In fatal cases of enteric infections of man with Y. enterocolitica, the lesions reported are those of enteritis with gross ulcerative lesions and marked inflammatory exudates (Bradford et al., 1974). Similar findings have been reported from experimental infections in mice (Carter, 1974) and monkeys (Maruyama, 1973).

Diagnosis

Excretion of Yersinia spp. in the faeces is given as an evidence of infection (Mollaret, 1961). Isolation of pure cultures and identification of Yersinia spp. from diarrhoeic faeces and from inflamed mucosa is the only reliable means of diagnosis of enteric infection in pigs. The organisms can be isolated on horse blood agar plates,

MacConkey and selenite broth. Very little information about the clinical signs and pathological findings has been documented or is of immediate importance in diagnosis. Serological diagnosis involving agglutination and haemagglutination of sensitized sheep red blood cells has been described. However, strong cross agglutination has been observed between Y. enterocolitica type 9 and Brucella abortus, Br. melitensis and Br. suis.

Other bacteria often associated with enteric lesions of pigs

A number of bacteria have been reported as being present on inflamed intestinal surface mucosa of pigs or isolated from diarrhoeic faeces. Many of these bacteria have never been investigated in any detail. Most of them are often regarded as acting synergistically with other primary agents to cause specific enteric lesions. In addition to the presence of T. hyodysenteriae, Alexander et al. (1976) reported the presence of Fusobacterium sericola, and Spirillum sulphurinigrans on mucosal scrapings in pigs with early lesion of swine dysentery. Meyer et al. (1975) were able to reproduce swine dysentery in germfree pigs by combining Bacteroides melaninogenicus, Bacteroides fragilis, Fusobacterium necrophorum and Fusobacterium nucleatum with T. hyodysenteriae. Whipp et al. (1979, 1980) have shown that typical lesions of swine dysentery could be produced in germ-free pigs by combining Listeria nitrificans, Selenomonas ruminantium, Lactobacillus species, Bacteroides vulgatus and Fusobacterium necrophorum with T. hyodysenteriae. The importance of the presence of these organisms in the development of enteric lesions has

been an accepted fact but little or no information is available about the role of these bacteria in other enteric lesions. Most of these organisms have failed to cause recognisable clinical signs or pathological changes in gnotobiotic pigs when given in the absence of T. hyodysenteriae.

Other bacteria which have been recorded as either present with or enhancing the effect of other primary pathogens are Acetivibrio ethanolgignens (Robinson and Ritchie, 1981); Lactobacillus acidophilus, L. fermentum and Megasphaera elsdenii (Alexander et al., 1976).

The bacterial flora of the intestinal tract of healthy pigs

The bacterial flora of the various regions of the intestinal tract of the healthy pig has been studied in both qualitative and quantitative terms by a number of authors. In most cases the papers concerned described the species and counts of bacteria present in the lumen and contents of the gut and pay little attention to the mucosal flora. The species considered to form part of the normal intestinal flora of the pig and the order of their appearance in the gut are reviewed briefly below.

Studies of the faecal microflora of the pig have been conducted by several groups of workers, such as Larson and Hill (1955). Willingate and Briggs (1955) studied the flora of the lower tract of pigs and concluded that it was essentially the same as that of the faeces, if corrections for differences in dry matter were imposed. Studies on

the bacterial flora of the alimentary tract as a whole were carried out by Horvath et al. (195) in the U.S. In Britain, methods for studying the bacterial flora of the alimentary tract were described by Smith and Crabb (1961) and later applied to investigation of the bacterial flora of pigs by Smith and Jones (1963). Other authors who had investigated the intestinal flora of a healthy pig include Pesti (1962); Kolacz et al. (1970); Hill et al. (1970) and Dickinson and Mocquot (1961).

The intestinal tract of the newborn pig is initially free from bacteria, but within 24 hours of birth, dense populations of bacteria are present. The whole alimentary tract of the newborn piglet becomes colonised with large numbers of E. coli, Clostridium perfringens, Streptococci and Lactobacilli (Smith and Jones, 1963; Wilbur et al., 1960). After ingestion these bacteria multiply in the stomach contents, which contain very little acid in the first 24 hours of life, and also in the intestine. By the second day of life, the pH of the stomach contents is sufficiently low to suppress the multiplication of all organisms except the Lactobacilli, which then establish themselves as the principal bacterial inhabitants of the stomach. Studies to estimate the number of viable bacteria/organisms in the contents of the intestinal tract of pigs showed that the site of greatest microbial activity appeared to be the large intestine; while the site of least activity appeared to be the stomach (Horvath et al., 1958).

The bacteria listed above are present in all the regions of the alimentary tract of the piglet. Quantitative differences exist from one region to the other however. As the pig becomes older, other organisms appear in the gut. These include Bacteroides, Veillonellae, Peptostreptococci, and yeasts, some of which are restricted to the large intestine. Alexander et al. (1976) reported the isolation of Lactobacillus acidophilus, L. fermentum, Megasphaera elsdenii and Selenomonas ruminantium in the colon of healthy weaned pigs. Bacteroides spp. are also reported to be predominant in the colonic mucosa of healthy pigs. Streptococcus faecium and Streptococcus faecalis are the two commonly reported species of streptococci in the gut of pigs.

Apart from the tendency of E. coli counts in the small intestinal contents to increase 3 to 7 days after weaning, no significant changes are noticed in the bacterial content of the gut at weaning (Smith and Jones, 1963). As the pigs grow older, there is a tendency for bacterial numbers to fall and the numbers of organisms voided in the faeces shows a fairly steady decline in aerobic cultures.

Streptococci spp. multiply to a lesser extent in the small intestine and to a much greater extent in the large intestine. In the weaned pig, C. perfringens is absent from the stomach and duodenum. It is usually found in the lower part of small intestine and large intestine. Bacteroides are found only in the large intestine of healthy pigs. Veillonellae are more common in pigs over 14 days

old, being present in the stomach, small intestine and large intestine. Yeasts are much more common in weaned pigs than in unweaned pigs. The intestinal flora of pigs has been shown to be influenced by diet. In pigs fed on an alkaline diet, flooding of the alimentary tract with bacteria was observed (Smith and Jones, 1963). All rectal organisms, with the exception of the total anaerobes and lactobacilli, were found to be lower in numbers when lactose, as compared with starch, was the carbohydrate fed (Wilbur et al., 1960). In all diseased animals the numbers of bacteria in the parts of the alimentary tract other than those directly involved in the pathological process were found to be similar to those in healthy animals.

VIRUSES OF THE PORCINE ENTERIC TRACT

Members of a number of virus groups have been demonstrated in the intestinal contents or in enteric lesions of the pig. Some of these such as the coronaviruses (transmissible gastro-enteritis and epidemic diarrhoea viruses) and the rotaviruses are widespread and responsible for recognisable diarrhoea syndromes. Others such as enteroviruses infect the enteric tract and may cause disease on a wide scale but are rarely associated with specific enteric syndromes. Yet others such as adenoviruses, parvoviruses, reoviruses, astroviruses and caliciviruses (Bridger, 1980) have been demonstrated but are of unknown significance.

Coronaviruses

(a) Transmissible Gastroenteritis (T.G.E.) virus

Transmissible gastroenteritis is a highly contagious viral enteric infection of pigs resulting in a very high mortality in pigs under 2 weeks of age. The mortality rate in pigs over 5 weeks of age is very low.

T.G.E. is known to be caused by a coronavirus which is relatively host-specific for pigs.

Pathogenesis

Ingestion is undoubtedly the commonest portal of entry for the virus; following which it selectively infects the mucosa of the small intestine; multiplies and causes a rapid and extensive loss of functional epithelial cells (Hooper and Haelter, 1966b). The marked loss of absorptive cells, surface area and enzymes results in a generalised decrease in absorptive capacity. Intestinal absorption of fat, glucose, sodium, iron and chlortetracycline has been shown to be impaired in pigs with T.G.E. (Ackerman et al., 1972; McClung et al., 1976). Thus, materials entering the small intestine by ingestion or secretion are poorly absorbed and tend to pass along to the colon. When the amount of this material exceeds the absorptive capacity of the colon diarrhoea results. The reduction of enzymatic activity in the small intestine disrupts the digestion and cellular transport of nutrient and electrolytes. Retention of unhydrolysed lactose in the lumen of the small intestine causes retention of fluid

and even withdrawal of fluid from the tissues of the body contributing to the diarrhoea and body dehydration (Masek and Stěpanek, 1975). It has also been shown that hypersecretion contributes to diarrhoea in T.G.E. (Butler et al., 1974). If affected cells in the tips of the villi, especially in the distal duodenum and jejunum, are lost lowering of the height of the epithelium and shortening of the villi results (Hooper et al., 1966b). The virus is not known to infect the colonic epithelium (Butler et al., 1974). Dehydration and metabolic acidosis coupled with hyperkalaemia are known to be the ultimate cause of death (Cornelius et al., 1968).

Clinical signs

Pigs of all ages are affected by T.G.E.. Signs are most characteristic in non-immune herds. The disease has an incubation period of 24-48 hours (Blood et al., 1979). This is followed by an explosive outbreak of diarrhoea. In Britain T.G.E. typically causes an acute disease which is short-lived in one unit, most incidents resolving themselves within 4 to 6 weeks (Wood, 1979). The diarrhoea is profuse, watery, green or grey in colour, sometimes with a foetid odour and containing curds of undigested milk. Vomiting may occur in piglets under 3 weeks of age and piglets under 1 week of age may show pink flushing of the skin. Affected piglets are dehydrated, lose weight and finally die. The mortality rates range from 100 per cent in animals under 1 week of age to 25 per cent in piglets over 2 weeks of age (Bachmann et al., 1972). Agalactia, vomiting, inappetence and transient diarrhoea have been

reported in sows affected by the disease.

Pathological changes

The pathological findings in pigs that died with T.G.E. have been described by Hooper and Haelterman (1966b); Cartwright (1968); Woode, (1969), and reviewed by Bohl (1981). Dead piglets are dehydrated but in good condition. Lesions are confined to the gastrointestinal tract. The lesions are more prominent in piglets than in adult pigs. The stomach is often distended with curdled milk. Gastric lesions were described by earlier workers (Hooper and Haelterman, 1966b) but have not been reported in recent papers. The small intestine is distended with foaming fluid content. The intestinal wall is always very thin. The mesenteric lymph nodes are often congested, and urate deposits may be seen in the renal pelvis and ureters (Wood, 1979). Villous atrophy is extensive in areas of small intestine distal to the first 10cm of the duodenum (Bohl et al., 1978). Histological changes in the small intestine are difficult to detect and consist mainly of alteration of cell types in the mucosal epithelium (Trapp et al., 1966). This cellular alteration has been confirmed by the use of transmission electron microscopy (Thake, 1968; Wagner et al., 1973).

Serum antibodies occur regularly in recovered pigs but local IgA immunity appears to be important in protection against the disease.

Diagnosis

A presumptive diagnosis of T.G.E. can be made on the basis of epizootiology and clinical signs of diarrhoea throughout the herd, deaths in the young piglets and the pathological findings. Demonstration of the virus or circulating antibodies are necessary for confirmation of diagnosis. T.G.E. should be differentiated from a disease in pigs caused by a coronavirus-like agent, distinct from T.G.E. (Pensaert and De Bouck, 1978; Chasey and Cartwright, 1978). The direct fluorescent antibody test (F.A.T.) described by Konishi and Bankowski (1967) and modified by Lucas and Napthene (1971) is used in Britain as confirmatory test. Serum neutralisation tests are also used. Serum antibodies appear within 10 days of infection (Norman et al., 1973).

(b) Epidemic diarrhoea (virus)

Epidemic diarrhoea of pigs has been described as a highly contagious disease characterised by vomiting diarrhoea and inappetence of pigs of all ages. This diarrhoea syndrome was first described in England by Wood (1977) and in Belgium by Pensaert and De Bouck (1978) and De Bouck and Pensaert (1980). The disease has since been reported in other parts of the world. The syndrome appears to be due to a coronavirus distinct from T.G.E. and H.E.V. and material in which it occurs can reproduce a syndrome resembling the classic disease (Tyrrell et al., 1978).

Pathogenesis

The pathogenesis of epidemic diarrhoea has been reviewed by Pensaert (1981) and is similar to that of T.G.E.. In partially immune piglets only a localised area of the gut may show villous atrophy.

Clinical signs

These resemble those of T.G.E. but differ from them in that mortality is usually restricted to piglets of less than 1 week of age and is not a major feature of the disease in non-immune herds. In Type I of the disease, suckling and young weaned pigs are rarely affected. Mortality is rare in the absence of intercurrent disease and where water is freely available. In Type II disease, pigs of all ages can be affected and some deaths occur in young piglets. Recovered pigs show no after effect of the disease but a few may remain unthrifty.

Pathological findings

Very limited reports of pathological lesions of Type I is available. In Type II disease, lesions are confined to the small intestine. The stomach is usually empty or filled with bile-stained fluid. The intestines are pale, often with fluid content. There is lowering of the height of the villi in the small intestine of the baby pig. Ultrastructural studies reveal the affected epithelial cells to have shortened or depleted microvilli and cytoplasmic changes. Virus particles are present in the cytoplasm within vacuoles of smooth endoplasmic

reticulum from which they have been seen to bud (Chasey and Cartwright, 1978).

Type I disease was first recognised in Yorkshire in 1971. Since then it has been seen in Lancashire, West Midlands, East Anglia and Aberdeen. Type II disease was first described in 1977 by Wood

Diagnosis

It is very difficult to differentiate epidemic diarrhoea from a typical explosive outbreak of T.G.E. on clinical signs alone, except for some minor differences. Mortality rate is lower, death rarely occurs in piglets infected in the second week of life and virus dispersion within the herd is somewhat slower. The simultaneous presence of clinical signs in older animals differentiate the disease from rotavirus. Direct immunofluorescence test of frozen section of piglets has been used to confirm diagnosis by Pensaert (1981). Confirmation of diagnosis in older pigs is more difficult.

Rotaviruses

Rotaviruses have been associated with diarrhoea in suckling pigs and during the immediate post-weaning phase, from 2 to 56 days of age. The disease is most severe in younger animals and at the post-weaning phase, when a significant level of mortality may result (Lecce et al., 1976; Woode et al. 1976a) The loss of growth in the recovered piglets has proved to be the most important effect of the disease. Rotavirus diarrhoea in pigs was first recorded

by Woode (1974) in England.

Pathogenesis

Following oral infection, the virus infects the epithelial cells, particularly of the middle small intestine. Holmes et al. (1976) suggested that the rotaviruses are activated in the intestine to an infectious form by enzymes such as lactase; or trypsin (Theil et al., 1977). The enzymatically mature epithelial cells on the distal half of the villi appear to be especially susceptible to infection (Bohl et al., 1978). Virus replication follows infection of cells. The cells are rapidly sloughed into the lumen and partially replaced by epithelial cells that are much less susceptible to infection, thus resulting in an immediate self limiting infection. Villous atrophy, malabsorption and diarrhoea result. Virus particles are shed in large numbers in the faeces.

Clinical signs

The incubation period of diseases varies from 18-24 hours after which depression, anorexia, and reluctance to move are noticed. Vomiting may be seen. Profuse diarrhoea, which is watery and light brown in colour develops within 36 hours of infection. In milk fed pigs, the diarrhoea is yellow with floccules floating in a whey-like fluid. There is a rapid loss of condition. Dehydration and death are most likely to occur in pigs that are only a few days old. Mortality is variable and usually occurs after 3-7 days of diarrhoea, at which time

the piglets are severely dehydrated. Clinical signs may regress 4-6 days after infection but loose faeces may persist for 7-14 days. Thirty-three per cent of affected pigs may die. A mortality rate of 100 per cent has been recorded in gnotobiotic piglets between 0-5 days of age.

Pathological findings

Dead animals appear dehydrated. The lesions are restricted to the small intestine which is distended with creamy fluid contents. Villous atrophy is common. Gross lesions may be found from the duodenum to the terminal ileum, depending on the severity of infection. There is flattening of the remaining epithelial cells with loss of the brush border and vacuolation of the cytoplasm.

Serum antibody can be detected by serum neutralisation or fluorescent antibody but serum titres are low.

Diagnosis

Rotaviral diarrhoea has been observed mainly in 8-35 day old pigs (Bohl et al., 1978). Rotavirus should be considered as a possible cause of disease in suckling pigs over 7 days of age and in newly weaned pigs of any age (Woode et al., 1976a,b). The clinical signs must be distinguished from those of T.G.E., mostly by mortality in young pigs, and from colibacillosis by vomiting and lack of response to antibiotic. The diagnosis is confirmed by immunofluorescent staining of the mucosal surface (Bohl et al., 1978), direct electron microscopy of faecal

specimens (Woode et al., 1976a), or by the detection of serum antibody in recovered pigs (Lecce et al., 1976, 1978; Woode et al. 1976b). Experimental reproduction of disease with viral isolates or faecal materials have proved a valuable diagnostic technique when other tests fail (Bohl et al., 1978).

Enteroviruses

Enteroviruses have been isolated from and are known to be present in enteric lesions in the pig. They are frequently isolated from the faeces of piglets with diarrhoea. Enteric strains of porcine enteroviruses appear to be ubiquitous. Transmission of infection occurs by the fecal-oral route. Endemic infection with several serogroups of these viruses can be demonstrated in conventional herds and is probably maintained in groups of weaned pigs (Bohl et al.1972). Infection is normally acquired by piglets shortly after weaning when maternal antibody protection is lost, and pigs from several litters are mixed together; and it persists for at least several weeks. Adult pigs rarely excrete enteroviruses, but they are known to have high antibody levels. Pigs of any age are, however, fully susceptible to infection with a sero-group to which they have not previously been exposed.

Natural infection follows an ingestion of the virus and it is well established that initial replication of the virus occurs in the tonsils and intestinal tract (Baba et al., 1966; Long et al., 1966).

In natural field infection, transient diarrhoea has been described as the major enteric clinical sign seen in infection with enteroviruses. Diarrhoea has also been produced experimentally by enteroviruses in piglets believed to be free of other pathogens. The diarrhoea is mild and relatively transient (Derbyshire, 1981).

The ileum and the large intestine are most frequently infected and contain higher titres of virus than the upper small intestine. It has also been clearly demonstrated that when piglets are inoculated parenterally with porcine enteroviruses, the virus rapidly infects the intestine multiplying particularly in the cells of the lamina propria. The virus is known to persist in the large intestine for several weeks.

No specific changes have been associated with intestinal enterovirus infections. They do not appear to cause the villous atrophy characteristic of coronaviruses and rotaviruses infections.

In the investigation of diarrhoea, virus isolation from intestinal tracts may be attempted, but the virological findings should be interpreted cautiously, since enteric infections with enteroviruses are common in healthy pigs. Serological identification of the virus isolate by the use of immunofluorescence and immunoperoxidase straining has been suggested by Watanabe (1971) and Derbyshire (1981).

Adenovirus

The first association of porcine adenovirus with enteric disease of pigs was recorded by Haig et al. (1964) who isolated the virus from a rectal swab from a 12-day old piglet with diarrhoea. Four serotypes of porcine adenovirus are known, and serological surveys indicate that infection is widespread in pig population. Antibodies to the virus are found in 70-80 per cent of slaughtered pigs in Britain. The virus is excreted in the faeces most frequently in the post-weaning period (Derbyshire et al., 1966), while adult pigs rarely excrete the virus, even though they have high serum antibody levels.

Type 4 was isolated from the brain of a pig, with enteritis as one of the clinical signs (Kasza, 1966). Other serotypes have been from piglets with diarrhoea (Genov and Bodon, 1976). Faecal-oral route is the main way of transmission of the virus. Clinical enteric syndromes associated with porcine adenovirus infection are rarely identified in the field in Britain. Diarrhoea has consistently been reproduced in experimental infection of pigs with Type 4 porcine adenovirus (Shadduck et al., 1967); and has also been observed as one of the clinical signs in experimental infection of pigs with other serotypes of the virus (Harkness et al., 1971; Derbyshire et al., 1975).

Following ingestion of the virus, viral replication occurs in the tonsil and lower intestinal tract (Sharpe and Jessett, 1967; Shadduck et al., 1968). Viral excretion in the faeces is known to continue for several weeks after infection. No specific enteric lesions have been described

in adenovirus affected pigs except for the presence of nuclear inclusions in villous epithelium with lymphocytic cellular infiltration of the underlying lamina propria in Type 3 infected piglets (Jericho et al., 1971).

Adenovirus infection is diagnosed by viral isolation and serology.

Reoviruses

Reovirus isolates (Type 1) have been obtained from British pigs with respiratory and enteric problems (McFerran and Connor, 1970). Antibodies to Type 1 and Type 2 were found in 30-40 per cent of pig sera in Britain in 1971 (McFerran et al., 1971), while 57 per cent of herds were found to be infected. Type 1 virus was most common in south west England and west Scotland and Type 2 in the east Midlands and north Scotland.

Pigs are known to be infected through the oral and respiratory routes. The main site of viral replication is the alimentary tract and to a lesser extent the respiratory tract.

Infection of pigs with reovirus is followed by excretion of virus in the faeces within 24 hours. The virus excretion may continue for about 14 days (McFerran et al., 1971). Haemagglutination inhibition antibody develops rapidly and is first detected on the 7th day and the peak is attained on the 11th to 21st day post infection. No description of any enteric lesions has been recorded in reovirus-infected pigs.

In natural outbreaks, viruses are recovered from faeces, nasal washing, lungs, spleen and liver, mediastinal and mesenteric lymph nodes.

Viral isolation on tissue culture is useful in diagnosis. Immunoelectron microscopy ensures that viral particles are seen and positively identified on infected tissues. Reoviruses are seldom, if ever, seen on direct electron microscopic examination of faeces.

PARASITIC AGENTS

Both the metazoa and protozoa are known to be involved in enteric diseases of pigs. In Britain, enteric parasitic infection in pigs is generally subclinical and therefore difficult to diagnose except by laboratory and necropsy procedures. The relative rarity of nematode infection in Britain is probably due to the modern system of husbandry, where the majority of animals are kept on concrete for much of their lives. Where they occur, internal parasites continue to be an economic factor in pork production. Subclinical infections inhibit weight gain and decrease feed conversion; therefore the time required to reach market weight is longer. Lesions of the alimentary tract and other organs are responsible for these setbacks and may result in condemnation of carcases at slaughter. In many cases the lesions associated with nematode infections are poorly described.

Hyostrongylus rubidus

Infection of pigs with Hyostrongylus rubidus has been reported in Britain. The progressive move toward large intensive units in Britain has been accompanied by a fall in the prevalence of the parasite. The parasite is still a major problem in many other countries of the world.

H. rubidus is only found in the pig's stomach. It has not been reported from any other host or any other organ and is usually found only in adults.

The adult worm occurs in the stomach. The adults are 4-9 mm long, slender and reddish, and they are found closely applied to the gastric mucosa. Parasitic moults are known to occur in gastric glands following ingestion of L_3 (Kendall et al., 1969).

The pathogenesis of infection depends upon invasion of the gastric glands with resulting loss of parietal cell function. The studies on the pathogenesis of H. rubidus reported by Kendall and Small (1974), show that little tissue disruption is known to result from larval penetration of the gastric mucosa. There is however, a tissue reaction to the presence of the larger larvae. There is no evidence to suggest that H. rubidus is a serious hazard to pigs unless secondary factors are involved.

The clinical disease is predominantly seen in the lactating sow where marked weight loss is observed despite adequate feeding. The weight loss has been observed in some cases, to continue after the litter is weaned.

Although diarrhoea is not present, the faeces may be dark intermittently. Chronically affected animals are dull, lethargic and have depraved appetites.

The necropsy findings are slight and always confined to the gastric mucosa. The adult worms sometimes may cause a slight hyperaemia of the gastric mucosa or formation of eroded areas and ulcers. When pigs are killed about 4 days following infection, nodules containing the larvae are seen on the mucosal surface.

Diagnosis is always based on clinical signs of weight loss and a history of poor food conversion rates in sows and gilts which have previously grazed on permanent pasture. The adult worms may also be demonstrated in the stomach wall at necropsy. Demonstration of eggs in the faeces is not a definite mode of diagnosis as these eggs might be confused with those of Oesophagostomum spp.

Other stomach worms had been reviewed by Dunn (1978).

Ascaris suum

Ascaris infection is known to be common in all pig rearing areas of the world, including Britain. The ascarid is the largest gastrointestinal parasite of pigs. Adult A. suum resides in the small intestine of infected pigs. Heavy infestations of the intestine with adult ascarid worms is known to cause digestive disturbances and resultant poor growth (Blood et al., 1979). This appears to be the major source of the economic loss associated with infection.

Infection occurs by ingestion of larvated eggs (L_2). Development to adult takes place after migration by the hepatic-tracheal route (Corwin et al., 1981).

Clinical signs are rarely evident even in heavy infections. Signs are only marked in young pigs. In 4 to 5 month old pigs, poor growth and resistance to other diseases are prominent (Blood et al., 1979). Occasionally, diarrhoea, pot belly and unthriftiness are noticed in herds where infections occur (Corwin et al., 1981). Adult worms may be vomited up and occasional cases of obstructive jaundice and intestinal obstruction or rupture occur (Blood et al., 1979).

Few, if any, intestinal lesions specific to Ascaris infection have been described.

Diagnosis of Ascaris suum infection cannot be based on clinical signs, as these are variable and difficult to correlate with the egg counts passed in the faeces. Adult worms are often passed in the faeces. Recognition of worms both in the faeces and at necropsy is an important aid to diagnosis. New methods of diagnosis, based on larval antigenic activity are being used experimentally for diagnosis (Stevenson and Jacobs, 1977; Benkova and Boroskova, 1978).

Other small intestinal worms have been reviewed by Corwin et al. (1981). These include Strongyloides ransomi, Macracanthorhynchus hirudinaceus and Trichinella spiralis.

Oesophagostomum

Oesophagostomum infections have been reported to cause diarrhoea in pigs, but infections are more frequently associated with poor weight gain. Oesophagostomum is the most common helminth in British pigs. Eighty-five per cent of sows and 45 per cent of weaners are known to be infected (Pattison et al., 1980). The parasitic burden in many of the infections does not appear to be heavy enough to cause clinical disease characterised by diarrhoea and weight loss. In heavy infections, the clinical disease is produced.

O. dentatum and O. quadrispinulatum are the two major species found in pigs in Britain (Taffs et al.1969) The adult worms, about 1.5cm long, occur in the colon and caecum. Infection occurs by ingestion of L_3; the latter enter the mucosa and develop to the adult stage through two moults, the first moult occurring within a nodule. Diarrhoea and reduction of weight gain occur in heavy infection. Anorexia and emaciation may also be seen in some cases. Experimental reproduction of diarrhoea and anorexia with heavy doses of larvae of Oesophagostomum has been reported (Poelvoorde and Berghen, 1981).

At necropsy, diffuse enteritis is found as the main lesion. Nodules are produced on the large intestinal mucosa as a host reaction (Blood et al., 1979). The distribution of these nodules have been seen from the caecum to the distal rectum (Taffs, 1966). Petechiation has been described at the site of entry of larvae into the mucosa

(Jacobs, 1969). Encapsulated larvae may be seen deep in the ruptured muscularis mucosa (McCracken and Ross, 1970). The walls of the caecum and colon become oedematous. Localised fibrinonecrotic membranes of neutrophils and necrotic debris have been seen where larvae escape to the lumen. These inflammatory changes may gradually resolve, although a few nodules may remain. The mechanical damage to the mucosa of the large intestine in very heavy infection may be sufficient to provide an environment conducive to the proliferation of other pathogens.

Necropsy is often the only certain method of diagnosis as the clinical signs are non-specific. Recognition of eggs in the faeces may aid diagnosis; but the eggs are similar to those of Hyostrongylus (Taffs, 1966). Larval culture is the best method of differentiation between these two (Honer, 1967).

Trichuris suis

T. suis is known to reside in the caecum and colon, with migration limited to the walls of the gut.

T. suis infection is common in porkers and baconers in Britain, but the numbers present are usually low. In other countries it is sometimes responsible for a severe ulcerative typhilitis (Corwin et al., 1981).

Infection follows the ingestion of infective eggs. All subsequent development occurs in the mucosa of the caecum and colon.

In heavy infection, irritation of the intestinal mucosa may result in diarrhoea, sometimes accompanied by the passage of mucus and blood. Experimental reproduction of the disease with heavy doses of eggs has been reported (Beer and Lean, 1973; Hass and Collins, 1973). Bacterial infection of the lesions has been reported.

The pathology of the affected gut may range from virtually no host tissue reaction (Beer, 1973), to a catarrhal enteritis with oedema and nodule formation (Powers et al., 1960; Batte et al., 1977).

Pigs are most susceptible to clinical diarrhoea or bloody scours due to T. suis in the 2 to 6 months of age. Immunity appears to keep infections low thereafter (Powers et al., 1959). Normal bacterial flora of the gut is known to affect the severity of the disease and infectivity of larvae, as eggs fail to hatch in gnotobiotic pigs (Rutter and Beer, 1975).

Necropsy is the best method of diagnosis of Trichuris infection. Detection of eggs in the faeces may be less helpful in diagnosis as Trichuris sp. do not lay eggs continuously (Powers et al., 1960).

Coccidia

Intestinal coccidiosis in swine is caused by the genera Eimeria and Isospora. Until recently coccidiosis was thought to be very rare in Britain; but Roberts (1980) showed that it is a widespread subclinical condition. The presence of coccidia in the faeces of piglets with diarrhoea

has also been reported by Roberts et al.(1980) and Scanford et al. (1982). Of the nine described species of coccidia, Isospora suis is the commonest species reported in both subclinical and clinical disease in Britain (Roberts and Walker, 1982; O'Neill and Parfitt, 1976).

The life cycles of coccidia in the pig are rather complex and involve both asexual and sexual cycles (Hoefling and Todd, 1981).

The severity of coccidiosis due to I. suis is thought to depend on the number of oocysts ingested. The parasites are transmitted in the faeces as unsporulated oocysts. Coccidiosis usually affects the young piglets with the older pigs acting as carriers. In field cases, diarrhoea occurs most commonly in piglets 5-15 days old with I. suis (Roberts and Walker, 1982), Sanford and Josephson, 1981).. Piglets of 3-21 days old can also be affected.

Pathogenesis

Following the infestation of sporulated oocysts, sporozoites are liberated into the lumen of the intestine. These penetrate the intestinal cells and round up to form trophozoites. The subsequent developmental stages which involve both the asexual and sexual cycles result in the destruction of the host cells. The intrinsic ability to destroy a certain number of the host cells determines the pathogenicity of species of coccidia.

Clinical signs

Diarrhoea is the predominant clinical sign of infection with I. suis. The diarrhoea persists for 4 to 6 days. The faeces are fluid and pasty in consistency and range from yellow to greyish in colour (Sanford and Josephson, 1981). Brown coloured diarrhoea had been recorded in some outbreaks. Blood is rare in the faeces. The diarrhoea may be self limiting in some outbreaks and the main clinical signs are emaciation and stunting of the affected pigs. The poor growth is evident when affected pigs are compared with unaffected piglets (Roberts and Walker, 1982). Morbidity is usually high in herds that are at risk of infection. Mortality is however variable depending on several environmental factors, and the presence of other co-existing enteric disease problems.

Pathological findings are characterised by an acute enteritis that is limited to the jejunum and ileum. A greenish adherent, fibronecrotic membrane is reported to be present throughout most of jejunum and ileum (Sanford and Josephson, 1981). Other reports of pathological ~~lesions~~ changes include a yellow fibrinonecrotic pseudomembrane loosely adherent to the hyperaemic mucosa (Hoefling and Todd, 1981). Moderate to severe villous atrophy in the jejunum and ileum has been reported as the most prominent histological change, but various asexual and sexual stages of coccidia can be seen in the vacuoles of the villous epithelial cells. Multifocal erosions with necrosis of the villous tips and occasionally more diffuse mucosal necrosis with fibrinocellular exudate are seen. Mature oocysts are not,

however, seen in the epithelial cells or necrotic exudates. Pathological changes rarely occur in the large intestine.

Diagnosis

Faecal flotation examination of the faeces is of little value in clinical diagnosis of coccidiosis as oocysts may not be shed during the diarrhoeic phase of the disease. Oocysts are detected in the faeces of the recovered pigs, but normal pigs may shed coccidial oocysts. A definitive diagnosis of coccidial infection is made by the examination of the jejunum and ileum for the endogenous forms of coccidia either by histopathological examination or by the examination of stained impression smears. Experimental reproduction of the enteritis by feeding *I. suis* to neonatal piglets with varying degrees of severity depending on the size of initial dose of sporulated oocysts has been reported by Stuart *et al*. (1980).

Various bacterial and viral infections may co-exist with coccidiosis. It is of value therefore to eliminate these other diseases.

Cryptosporidial infection of piglets

Reports of natural cryptosporidial infection in pigs are still few. These include those of Kennedy *et al*. (1977); Morin *et al*. (1976) from U.S.A.; and Links (1982) from Australia. However, Tzipori *et al*. (1980), Moon and Bemrick (1981) and Tzipori *et al*. (1981a) have succeeded in producing infection and diarrhoea in newborn piglets following oral inoculation with calf ileal contents; faeces

and calf ileal scrapings respectively. Cryptosporidium has been shown to be an important agent in diarrhoea diseases of other species.

Cryptosporidia are generally found in the microvillous brush border of host epithelial cells. They infect the alimentary tract of young animals (Kennedy et al., 1977; Snyder et al., 1978; Hoerr et al., 1978).

The life cycle of Cryptosporidium is similar to that described for other coccidia. Infection follows ingestion of contaminated feeds with the faeces containing oocysts of cryptosporidium.

Clinical signs

In previous reports, no clinical significance could be attributed to the presence of cryptosporidia in naturally infected piglets (Kennedy et al., 1977; Morin et al., 1976). However, diarrhoea has been reported recently in 4 infected piglets in Australia (Links, 1982). In experimentally infected 1-2 day old colostrum-fed piglets, excretion of cryptosporidia was closely related to the presence of diarrhoea (Tzipori et al., 1980; Moon and Bemrick, 1981). Clinical signs of inappetence, vomiting and diarrhoea and shedding of the oocysts in faeces have also been reported in experimentally infected piglets (Tzipori et al., 1981b). In the report, the clinical response varied among 4 litters exposed in first week of life, from moderate illness with anorexia, vomiting and diarrhoea, to subclinical infection only. The variations were much greater between litters than within them, indicating that external factors such as

level of maternal immunity or the nature of the inoculum, could have been responsible.

Pathological findings

The gross pathological changes described in natural infection of piglets with cryptosporidium are non-specific. Diphtheritic inflammation of the mucosa of the ileum, caecum and colon has been recorded in 1 out of 4 infected piglets (Links, 1982). Mild inflammation or absence of changes were recorded in other cases. Enterocolitis has been described in experimentally infected piglets (Tzipori et al., 1980; Tzipori et al., 1981a,b).

Microscopic changes in natural infection of piglets are minimal. Cryptosporidia can be detected in the brush border of villous epithelial cells in the small intestine. They are most common over the tips of the villi and occurred in decreasing numbers down the side of the villi (Links, 1982). The organisms have also been reported to be present in the colon (Kennedy et al., 1977). In experimentally infected piglets, the most consistent histological changes observed in the intestine include congestion, moderate to severe villous atrophy, fusion of villi, replacement of columnar mucosal epithelium by low cuboidal cells and infiltration with neutrophils (Tzipori et al., 1980). Moon and Bemrick (1981) also reported increased numbers of mononuclear cells in the lamina propria and distension of some large intestinal crypts with debris, neutrophils and cryptosporidia. Tzipori et al. (1981a) reported that the parasite was observed throughout the small

and large intestine attached to epithelial cell surfaces and its presence was associated with extensive mucosal damage. Electron micrographs revealed the various forms of the life cycle of cryptosporidium embedded in the microvilli of villous epithelial cells and free in the lumen (Tzipori et al., 1981b).

Diagnosis

Diagnosis of cryptosporidial infection in piglets is most commonly made by histological examination of the intestinal mucosa. The demonstration of oocysts in the faeces as a means of diagnosing cryptosporidium infection in piglets is unsatisfactory. Even in acute cases of diarrhoea, shedding of the oocysts began 1 to 3 days after the onset of illness (Tzipori et al.1981). In less acute cases detection of oocysts in faeces is extremely difficult and often based on observation of very few oocysts (Snodgrass et al., 1980).

Giemsa stained smears of faeces or intestinal mucosa have been used to detect cryptosporidia in pigs (Tzipori et al., 1980).

The prevalence of cryptosporidial infection in pigs is unknown; a serological study conducted recently on a variety of species, including pigs, suggests that cryptosporidium infection may be widespread (Tzipori et al. 1981a).

Balantidium coli

Balantidium coli, a ciliated protozoan, is found in pigs and is known to be a secondary invader of lesions already present on the intestinal mucosa. On its own, it cannot penetrate an intact intestinal mucosa (Corwin et al., 1981). Its ability to invade secondary lesions is said to be due to the production of hyaluronidase, which breaks down the ground substance and enlarges the lesion. The effect of this secondary invasion is seen clinically as anaemia, enteritis and reduced growth in affected young pigs.

Sow's faeces are the principal source of infection for baby pigs (Negru et al., 1964). Balantidium coli reproduces in the intestinal lumen by binary fission. Resistant cysts are found after conjugation has taken place.

The importance of Balantidium coli infection in Britain has never been assessed. Faecal examination for the presence of large numbers of trophozoites or cysts can be an aid to diagnosis of infection. Trophozoites and cysts can also be identified from intestinal contents and mucosa scrapings at necropsy and the organism may be seen in histological sections.

Toxoplasma gondii

Diarrhoea has been described as one of the clinical signs of field infection with Toxoplasma gondii in pigs (Dubey et al., 1979). The importance of this agent in enteric disease in Britain has not been studied. The majority of cases of Toxoplasmosis are subclinical infections.

The main route of infection is said to be through the ingestion of infective oocysts or encysted bradyzoites.

Intestinal ulceration has been reported as part of the pathological findings in clinical cases of swine Toxoplasmosis (Cole et al., 1974).

MYCOTIC ENTERITIS

Mycotoxicosis of pigs is known to occur as a result of fungal growth on damp feed or the inclusion of affected grain in the ration. A number of clearly defined syndromes have been described in Britain involving some moulds, but little information is available about enteric involvement. However, a natural occurrence of mouldy corn toxicosis which apparently caused enterocolitis in pigs has been reported by Blevi's et al. (1969). Aflatoxin B, was detected in feed and Penicillum rubrum, Aspergillus spp. and Rhizopus spp. were isolated from the ration fed to the pigs in the herd.

In the field outbreak, a diarrhoea with a purple-red bloody faeces was noted as the main clinical sign of the disease.

Experimental reproduction of the disease by feeding the isolated fungi resulted in a mild syndrome. The onset of the disease was sudden, occurring within 3 to 7 days following feeding with infected ration.

At necropsy, the haemorrhagic enterocolitis was peculiarly demarcated, beginning abruptly in the lower

jejunum and ending in the lower colon. The microscopic lesions were those of haemorrhagic and necrotic enteritis, and were mainly found in the ileum and large intestine.

In the diagnosis of mycotic enteritis, other conditions such as swine dysentery, proliferative intestinal adenopathy and mercury poisoning should be eliminated.

OTHER FACTORS

Husbandry and management factors may predispose or contribute to piglet diarrhoea. These factors are also very important in the control of enteric diseases. The first hours of a newborn piglet's life are the most dangerous. At birth it is small, weak, and highly vulnerable because of its lack of circulating or surface antibodies. The provision of the essential environmental and other cares is vital if fatal enteric diseases are to be prevented. If piglets are to thrive they must be warm and dry on well-drained floors. It should be ensured that they obtain colostrum and essential nourishment.

It is known that the condition of the sow may also affect the outbreak of diarrhoea in piglets. If the sow is fed too much high energy feed in the week or the week following farrowing, her piglets are said to develop scour (Blood et al., 1979). Certain substances, when present in food, may start piglets scouring.

Deficiency of certain nutrients in sow feed (particularly Vitamin A) or iron deficiency in the piglets will predispose to coliform scours (Whitehair and Miller,

1981; Alexander, 1982). Vitamin E deficiency is also known to increase the incidence of diarrhoea in pigs (Jorgensen and Wegger, 1979).

Vague and poorly documented conditions known as deprivation scours have also been described. They start in the first week of life and may affect 1 or 2 pigs in a litter. Affected pigs become gaunt, rough coated, dirty, and thin with a chronic brown watery diarrhoea. At necropsy, the carcass is pale. The stomach and intestines are empty or nearly so. Brown soft watery contents in the large intestine occur usually with staining around the perineum. Lack of colostrum or milk and/or other adverse environmental factors like wet, cold, draught, dirty, poorly ventilated housing; bad management and husbandry are said to be the cause

CHAPTER 2

GENERAL MATERIALS AND METHODS

Many of the materials and methods described in this chapter were used throughout the study.

Bacteriological materials and methods

(a) Media and reagents used

The following media and materials were used throughout the study for general cultivation and maintenance, were prepared according to the manufacturer's instructions and used in the form given below.

1. Tetrathionate broth (Oxoid) dispensed in 10ml volumes.

2. Salmonella shigella agar plates (SS) (Oxoid).

3. Deoxycholate citrate agar plates (DCA) (Oxoid).

4. MacConkey agar plates (Oxoid).

5. Nutrient agar plates (Oxoid).

6. Cooked meat medium (Oxoid) dispensed in 10ml and 50ml volumes.

7. 7 per cent sheep blood agar plates.

   7ml formolised sheep blood (Gibco-Biocult Ltd.) were added to 100ml Blood agar base No.2 (Oxoid).

8. 7 per cent horse blood agar plates.

   7ml defibrinated horse blood were added to 100ml Blood agar base No.2 (Oxoid).

9. Basal medium for the identification of Campylobacter species.

(a) Semi-solid form.

Brucella broth (Difco) was added to 0.12 per cent Oxoid agar No.3. This semi-solid medium was dispensed in 15ml amounts in 16mm screw cap Universal bottles.

(b) Solid form.

Brucella broth (Difco) was used with 1.2 per cent Oxoid agar No.3 and poured into plates.

10. Selective medium for catalase positive Campylobacters.

Freeze dried antibiotic supplement SR69 (Oxoid) was incorporated in 7 per cent horse blood agar at the rate recommended by the manufacturer.

11. Selective medium for catalase negative Campylobacters (Lawson and Rowland, 1974).

0.5ml of 0.5 per cent Brilliant Green (BDH Ltd) was incorporated into 150ml of Blood agar base No.2 and 11ml of horse blood was added.

12. Reinforced Clostridial Medium (Oxoid) was prepared with the addition of 2.5 per cent Oxoid agar No.2, and 7 per cent horse blood.

13. Nutrient broth.

Nutrient broth No.2 (Oxoid) was prepared and was used when broth media were required.

14. Serum broth.

10 per cent horse serum (Gibco-Biocult) was added to the nutrient broth described above.

15. Chocolate agar.

Chocolate agar was prepared by the methods used for horse blood agar but heated to 75-80°C for 10 minutes.

16. Physiological saline.

Physiological saline was used throughout the study. The sodium chloride concentration was 0.85 per cent in deionised water. It was sterilised before use.

(b) <u>Conditions of cultivation</u>

1. Atmospheric conditions.

Cultures were incubated in aerobic, microaerophilic and anaerobic conditions. Cultures for aerobic incubation were placed directly into the incubator.

Microaerophilic conditions were produced by evacuating loaded anaerobic jars with no catalyst (Gallenkamp Ltd., Whitley Scientific Ltd.) to a pressure of 20 inches of mercury using a vacuum pump. The jars were then flooded with a 5 per cent carbon dioxide and 95 per cent hydrogen mixture (British Oxygen Co.Ltd., Special Gases Division).

Anaerobic conditions were produced by evacuating anaerobic jars, containing the cultures and fitted with cold catalysts to a pressure of 24 inches of mercury using a

vacuum pump. The evacuated jars were then filled with the carbon dioxide:hydrogen mixture. Gassed jars were evacuated once more and gassed again in order to flush out any remaining oxygen. The inoculation of plates for anaerobic culture was performed rapidly and inoculated plates were returned to an aerobic atmosphere as soon as possible in order to reduce the period of time for which cultures were exposed to oxygen.

2. Temperature of incubation.

Cultures were incubated at 37°C unless specified. For some organisms, temperatures of +4°C; 22°C; 30.5°C; 42°C 44°C and 45°C were used in the identification process. They were produced as follows: Incubation at +4°C was carried out using a refrigerator operating at +4°C; at 22°C, cultures were incubated at room temperature. Temperatures of 25°C; 30.5°C; 42°C; 43°C and 45°C were obtained by using accurately-adjusted water baths. Cultures of microaerophilic or anaerobic organisms were placed in anaerobe jars containing the appropriate atmosphere and the jar was placed in the water bath for the appropriate period.

3. Period of incubation.

All primary cultures on routine non-selective media were incubated for 24 hours, examined and then reincubated for 48 and 72 hours. Cultures were not discarded until five or more days after inoculation. Selective media were incubated for 24 hours prior to examination (Reinforced Clostridial Medium) and 48 hours (Campylobacter medium).

They were also reincubated for 48 and 72 hours. Fastidious organisms were incubated for up to 5 days.

(c) Identification of bacterial isolates

1. Colonial morphology.

Many different colony types were noted in the initial cultures and each colony type seen was recorded and subcultured to provide pure sub-cultures. The examination of colonies was made by unaided eye and under the dissecting microscope. The colonies seen were described in terms of their morphological characters such as size, elevation, outline, colour and their effect on the medium and these were recorded. Colonies were presumptively identified by these characters and their identity confirmed by further tests.

2. Methods of identification.

All isolates were identified using the methods outlined by Cowan and Steel (1974), supplemented where necessary by reference to Bergey's Manual (1974) and the Anaerobe Laboratory Manual (1977). Specialist publications were consulted for the identification of Campylobacters. The tests used and the materials used in each test are given here and variations from the methods and criteria for assessment described by Cowan and Steel, Bergey and the Anaerobe Laboratory Manual are given below where appropriate. Biochemical tests were carried out in the atmospheric and temperature conditions appropriate to the organism under test. The tests used in identification

were as follows:

A. Morphology of bacterial cells.

Smears made from lesions and from colonies were prepared on slides, air dried and heat fixed. They were stained by Gram's Method (Soltys, 1963). The stained slides were examined under the oil immersion lens. This method made it possible to determine the cellular morphology of the bacteria and their reaction to Gram stains which were recorded. The modification of Moeller's spore stain described by Cowan and Steel (1974) was also used.

B. Motility test.

The hanging drop technique was used on suspensions prepared from both cultures or from growth on solid media after 24 and 48 hours incubation. Cell motility was also studied using phase contrast microscopy at a magnification of 970x.

Motility of anaerobic bacteria was determined by growth in a semi-solid medium stab and the presence or absence of diffuse growth of the organism was noted and recorded.

C. Growth at temperatures other than 37°C.

The ability of organisms to grow at temperatures other than 37°C was determined by culture of the organism under test on blood agar or in nutrient broth under the appropriate atmospheric conditions. The presence of growth was recorded. The various temperatures were obtained as described above.

D. Biochemical tests.

Oxidase tests were carried out using filter paper dipped in the reagent (1 per cent NNN'N - tetramethyl-P-phenylenadiamine dihydrochloride, B.D.H. Ltd.). Cultures were streaked on to the paper using a platinum wire.

Catalase tests were carried out by emulsifying loopfuls of 24 hour or 48 hour cultures of the organism under test grown on nutrient agar or blood agar with a few drops of 3 per cent hydrogen peroxide (B.D.H. Ltd.) on a microscope slide. Suspensions were examined for bubbles of gas immediately and after five minutes.

Oxidation and fermentation tests (O/F) were carried out to determine the effect of the organism on OF basal medium (Difco) containing 1 per cent added glucose (Analar). Results were assessed after 2, 4 and 8 days.

Utilisation of sugars. This was tested using sugar broth base No.2 prepared using the following ingredients in the basal medium: Lab Lemco beef extract (Oxoid) 5g; Oxoid bacteriological peptone 10g; sodium chloride (May and Baker) 3g and disodium hydrogen phosphate (May and Baker) 2g were dissolved in 1 litre of deionised water and steamed for 30 minutes. When the medium was cool, the pH was adjusted to 7.2 to 7.3 and 12ml of indicator (0.1g Bromothymol blue, May and Baker; 2.5ml 0.1M NaOH Analar and 47.5ml of distilled water) were added per litre. This medium was dispensed in 100ml volumes. One gram of the appropriate sugar was added to 100ml of this medium. When each sugar was dissolved it was dispensed in 4ml volumes

into bijou bottles containing durham tubes. The bottles were then sterilised by tyndallisation. Bijou bottles were inoculated with colonies of bacteria under test. The results were recorded after 24 hours but inoculated cultures were not discarded until 7 days after inoculation.

Indole production was determined by growing the culture in 1 per cent peptone water (Oxoid) and testing with Kovac's reagent.

Gluconate oxidation was determined by growth in medium containing potassium gluconate (B.D.H. Ltd.).

Decarboxylase tests were carried out using Bacto Decarboxylase Medium Base (Difco) with the addition of 0.5 per cent L Lysine, L ornithine, L arginine (Analar) where appropriate.

Methyl red (MR) and Voges-Proskauer (VP) tests were carried out after cultures were grown in M.R.V.P. medium (Oxoid) for 2 days at 37°C. The production of acid was tested for by adding 2 drops methyl red solution. After completion of this test, the V.P. test was carried out by adding 0.6ml of 5 per cent α-naphthol (Analar) in absolute ethanol and 0.2ml 40 per cent potassium hydroxide (Analar) aqueous solution to another aliquot of the culture.

Nitrate reduction was determined on cultures grown in nutrient broth supplemented with 0.1 per cent potassium nitrate (Analar) and incubated for 5 days. They were then tested for the presence of nitrite.

Hydrogen sulphide production was assessed in two ways.

(i) On Triple Sugar Iron agar (TSI) - Oxoid. Slopes of TSI were inoculated and incubated in aerobic, microaerophilic and anaerobic conditions where appropriate for 24 and 48 hours. The production of hydrogen sulphide was indicated by a black colour.

(ii) The sensitive hydrogen sulphide test. This was conducted by suspending filter paper strips saturated with lead acetate (B.D.H. Ltd.) in universal bottles of the basal medium described above containing 0.02 per cent Cysteine-HCL (B.D.H. Ltd.). If any portion of the paper strips became blackened, the isolate was considered hydrogen sulphide positive by the sensitive hydrogen sulphide test.

Hippurate hydrolysis was determined by growing cultures in hippurate broth prepared by the method of Cowan and Steel using sodium hippurate (Hopkins and Williams Ltd.) and incubated with an uninoculated control tube at 37°C for 4 days in the appropriate atmospheric conditions. Another test was used for the identification of campylobacters (see below).

Coagulase tests were carried out by the slide method using citrated rabbit plasma collected in the department.

Citrate utilisation was determined using the modified Koser's citrate described by Cowan and Steel.

Arginine hydrolysis was examined by the inoculation of cultures into Arginine broth prepared by the method described by Cowan and Steel incubated with a control tube at 37°C for 2 days, followed by test with Nessler's reagent (B.D.H. Ltd.).

Phosphatase production was determined using phenolphthalein phosphate agar prepared using phenolphthalein phosphate (B.D.H. Ltd.). This was lightly inoculated to obtain discrete colonies, incubated for 18 hours at 37°C and examined. O.1ml of ammonia solution (sp.gr.O.88O, B.D.H. Ltd.) was used.

Starch hydrolysis was determined on nutrient agar containing 1 per cent starch (B.D.H. Ltd.) sterilised at 115°C for 1O minutes. Lugol's Iodine used in the test was prepared with 5g Iodine, 1Og potassium iodide and 1OOml distilled water.

Haemolysis was determined by streaking cultures on 7 per cent sheep blood and 7 per cent horse blood agar plates. These were incubated at 37°C for 24 or 48 hours and haemolytic activity around discrete colonies was recorded.

Growth on MacConkey agar was recorded after incubation for 18-24 hours at 37°C in the appropriate atmospheric conditions. Some organisms required a prolonged incubation period.

Litmus milk (Oxoid) was used in 10ml quantities and incubated for 7 days at 37°C when the result was recorded.

Gelatin liquefaction was determined using Nutrient Gelatin (Oxoid) after 15 days of incubation at 37°C or at room temperature. Cultures were placed in a refrigerator at 4°C for 1 to 2 hours before being recorded as positive.

Aesculin hydrolysis was determined by the inoculation of 10ml quantities of aesculin broth prepared by the method described by Cowan and Steel.

Urease production was determined by the inoculation of Urea Broth Medium (Oxoid) with added Urea solution 40 per cent (Oxoid). Inoculated broth was incubated and examined after 18-24 hours.

ONPG (O-nitrophenyl-β-D-galactopyranoside) tests were prepared and carried out by the method of Cowan and Steel.

Growth in potassium cyanide medium was carried out using nutrient broth with potassium cyanide (May and Baker).

Malonate utilization was determined using Malonate-phenylalanine Medium (Oxoid).

Appearance on egg yolk agar was determined on medium prepared as follows:

Five per cent Egg Yolk Suspension (Oxoid) was added to sterile molten Nutrient Agar Base No.2 (Oxoid) at

55°C and plates were poured immediately. Cultures were inoculated onto plates and incubated at 37°C in the appropriate atmospheric conditions. The changes in the medium were recorded.

Digestion of cooked meat was determined following the inoculation of synthetic Cooked Meat Medium (Oxoid) and incubation at 37°C for 14 days.

Digestion of inspissated serum was determined by using Loeffler's serum slopes prepared with horse serum.

Casein hydrolysis was assessed on milk agar prepared using 100ml Blood Agar Base No.2 (Oxoid) and 35ml of sterile skimmed milk.

Ammonia production was determined by the addition of Nessler's reagent to peptone water (Oxoid) culture which had been incubated for 5 days at 37°C.

Growth in media with increased sodium chloride concentration was assessed on nutrient agar plates containing the required concentration of sodium chloride (May and Baker).

E. Lancefield's test for streptococcus species.

The test employed was that in use in the Bacteriology Department at Glasgow Veterinary School and was carried out as follows:

(i) Pure cultures were inoculated into Lancefield broth composed of Nutrient broth No.2 (Oxoid) and 1 per cent glucose (Analar) and incubated at 37°C overnight.

(ii) The bacteria were removed by centrifugation at 1500g for 10 minutes.

(iii) The supernatant was discarded and the deposit was suspended in 2ml of N/20 hydrochloric acid in 0.85 per cent sodium chloride solution.

(iv) The suspension was heated in a boiling water bath for 5 minutes, cooked and centrifuged at 1500g for 10 minutes.

(v) The supernatant fluid was removed and several drops of 0.02 per cent phenol red solution were added. Sufficient N sodium hydroxide (May and Baker) was added carefully to turn the indicator red.

(vi) Tests were carried out in tubes (Microtube).

(vii) A drop of each grouping serum (A,B,C,D,E,F,G Wellcome Reagents Ltd.) was placed in each tube and overlayed by a drop of the antigen prepared as above.

(viii) Tubes prepared by the method described above were left on the rack for 10 minutes. A positive reaction consisted of the formation of a white, flocculent

precipitate at the junction of the extract and the homologous serum after 10 to 30 minutes at room temperature.

F. Tests for the identification of Campylobacters

Campylobacters were identified by the methods described by Veron and Chatelain (1973); Smibert (1974, 1978); Skirrow and Benjamin (1980) and Harvey (1980).

Glycine tolerance was assessed by the inoculation of 15ml of the semi-solid brucella medium described above containing 1 per cent glycine (Sigma Ltd.). This was incubated aerobically for 6 days at 37°C, and any growth was considered to indicate glycine tolerance.

Sodium chloride tolerance was assessed on semi-solid brucella medium containing 3.5 per cent sodium chloride (May and Baker) inoculated and incubated aerobically for 6 days at 37°C. Any growth was recorded.

Selenite reduction was determined on the solid brucella medium containing 0.1 per cent sodium selenite (Sigma Ltd.), inoculated and incubated microaerophilically. The reduction of selenite was indicated by a change of colour in the medium to a deep orange colour. The result was recorded after 2 to 3 days incubation.

Glucose tolerance was assessed by the inoculation of semi-solid brucella medium containing 8 per cent glucose (B.D.H. Ltd.). Cultures were incubated aerobically for 6 days at 37°C. Any growth was recorded.

Bile tolerance was determined using the semi-solid brucella medium supplemented with 1 per cent ox bile (Oxoid). Inoculated medium was incubated aerobically for 6 days at 37°C. Growth was recorded.

Nalidixic acid (NA) tolerance was determined on 7 per cent horse blood agar plates containing 40µg of nalidixic acid (Sigma Ltd.) per ml. Plates were inoculated and incubated microaerophilically. Any growth on this medium was recorded after 2 to 3 days.

2,3,5 triphenyltetrazolium chloride (TTC) tolerance was determined by the ability to grow after 2 to 3 days microaerophilic incubation at 37°C on 7 per cent horse blood agar plates containing 1mg of TTC (Sigma Ltd.) per ml.

Nitrate and nitrite reduction were determined by the inoculation of semi-solid medium containing 0.1 per cent potassium nitrate (Analar). Inoculated cultures were incubated aerobically for 6 days at 37°C and were then tested for the presence of nitrite and then nitrate.

Hydrogen sulphide production was determined by the inoculation of semi-solid medium containing 0.02 per cent cysteine hydrochloride (B.D.H. Ltd.). Inoculated cultures were incubated at 37°C with filter paper strips impregnated with lead acetate solution, which were suspended from the screw caps. The filter paper strips were examined daily for blackening.

Brilliant green tolerance was determined on 7 per cent horse blood agar plates containing 1:33,000 and

1:100,000 of brilliant green (B.D.H. Ltd.). Plates were inoculated and included microaerophilically at 37°C for 2 to 3 days. Growth on the plates was recorded.

Rapid hippurate hydrolysis was determined by the method described by Harvey (1980) and Skirrow and Benjamin (1980)

The growth of isolates at 30.5°C and 43°C (Skirrow, 1977 and Skirrow and Benjamin, 1980) was employed in addition to the other growth temperatures used by Veron and Chatelain.

G. Maintenance of cultures

Each pure culture isolated was inoculated on to tryptone soya agar or blood agar slopes in universal bottles as appropriate to the isolate, incubated for 24 hours in appropriate conditions and stored at room temperature. Duplicate cultures were made of each isolate, one being stored at +4°C.

Campylobacter isolates were maintained by subculture onto blood agar at 8-day intervals. All cultures were maintained in the microaerophilic conditions described above at room temperature following initial inoculation at 37°C for 48 hours.

Isolates of anaerobic bacteria were maintained in Robertson's cooked meat medium.

Selected cultures were freeze dried using the method described by Garvie (1967) within the number of passages specified in the appropriate chapter and then stored at +4°C.

Sources of animals for field survey

Animals used in the survey studies were submitted for post mortem examination from pig farms around Glasgow. Details of these farms are given in Chapter 3.

Some of the animals were alive when submitted but many had died within the 24 hours prior to examination.

The ages of the animals ranged from 1 day old to adult. Most were aged between 1 day and 6 weeks. Older pigs between the ages of 10 to 12 weeks were also examined. Animals used for swine dysentery experiments were also examined at slaughter for the presence of other bacterial infections.

Euthanasia

The small pigs were killed by the intravenous or intracardiac administration of Pentabarbitone sodium (Euthatal, May and Baker Ltd.); followed by exsanguination. The older pigs were killed by electric stunning and exsanguination.

Post-mortem techniques

Thorough post-mortem examination was carried out as soon as possible after death. Particular attention was paid to the gastrointestinal tract and the organs of the abdominal and thoracic cavity were examined for the presence of gross lesions. The gross changes noted were recorded. Both the appearance of the mucosal surfaces

and the consistency of the mucosal contents were noted.

. Sample lengths of the intestine were opened and the various segments were carefully examined for the location and distribution of the gross lesions after the mucosa had been rinsed. Samples for bacteriological examination were taken from the liver, and both small and large intestine in the first few cases examined in the survey (Nos. P_1 to P_{15}); and thereafter from the stomach, duodenum, jejunum, ileum, caecum, colon, liver, and mesenteric lymph nodes; and in some cases the gall bladder. Samples of small intestine were opened in sterile physiological saline and viewed by low power microscopy to observe the villous architecture. Its appearance was recorded.

Histological examination by light microscopy

Samples were fixed in 10 per cent formol saline for at least 48 hours before trimming and embedding. The samples were embedded in paraffin wax and sections were cut and mounted on glass slides. Sections of all samples were stained with haematoxylin and eosin while some were stained by Gram's method (Twort) (Drury and Wallington, 1967). Sections of the intestine from some animals were stained by the method of Young (1969) in order to demonstrate campylobacters and spirochaetes. All the stained sections were examined under the light microscope. The results were recorded.

Methods of sampling for bacteriological examination

Bacteriological examination was carried out on all samples in the following ways. Rinsed intestinal mucosa, the liver, mesenteric lymph nodes, the gall bladders with obvious gross lesions; faecal samples in cases of diarrhoea were used for bacteriological examination.

Sampling of specimens

Mucosa was rinsed with physiological saline. The surface was then seared lightly with a hot spatula as was that of organs such as the liver, gall bladder and mesenteric lymph nodes. Material was taken from the seared area and from the faecal samples using a stiff bacteriological wire loop for direct and cultural examination.

(a) Direct examination

Direct smears were made on clean glass slides, air dried and heat fixed then stained by Gram's method. They were examined under the light microscope using oil immersion. Some of the fixed smears were stained by Koster's method where modified acid fast organisms were suspected. The morphology and the reaction of the bacteria to the stains used were noted and recorded.

(b) General cultural examination

Each sample was inoculated onto a 7 per cent blood agar plate and a MacConkey agar plate for aerobic incubation. It was also inoculated onto a horseblood agar

plate and in the survey and campylobacter studies onto selective medium for campylobacters and incubated microaerophilically. One horse blood agar plate was also inoculated and incubated anaerobically. On some occasions chocolate agar plates were also inoculated and incubated aerobically. The bacterial colonies seen were recorded and identified by the methods described above or in the sections on special bacterial examination.

(c) Cultural examination for specific bacteria

(i) Detection of Salmonella species from faecal samples and from the intestinal lumen

A loopful of the faecal material was inoculated into tetrathionate broth to which 0.2ml of iodine solution had been added. The inoculated broth was incubated at 37°C for 24 hours. A loopful of the broth culture was then streaked onto SS agar plates and also DCA agar plates.

The inoculated plates were examined for non-lactose fermenting colonies after 24 hours of incubation at 37°C under aerobic conditions.

Urea broth was inoculated with non-lactose fermenting colonies on SS and DCA plates. The inoculated urea broth was incubated for 18 to 24 hours.

Colonies that failed to hydrolyse urea were further tested by stabbing and streaking onto Triple Sugar Iron agar slant to determine hydrogen sulphide production. Slide agglutination tests were carried out on the suspected salmonella colonies, with specific known salmonella

antisera (Wellcome Reagents Ltd.). The identity of any positive cultures would have been confirmed biochemically.

(ii) Campylobacters

Samples of intestinal mucosa, faeces and other organs were examined for the presence of catalase positive campylobacters using the campylobacter selective medium described above. Catalase negative campylobacters were sought by the inoculation of the selective medium of Lawson and Rowland, 1974, described above. Inoculated plates were incubated under microaerophilic conditions. Suspect colonies were recorded and sample colonies identified further using the methods described above.

(d) Anaerobic enteric spirochaetes

Spectinomycin blood agar plates were inoculated and incubated under anaerobic conditions at 37°C for 48 hours and then examined. Plates were reincubated for longer periods in some cases. Colonies which appeared to be those of anaerobic spirochaetes were examined further by microscopy to confirm their identity.

(e) Clostridium species

Reinforced clostridial medium was inoculated and incubated under anaerobic conditions. Colonies which appeared on this medium were recorded and sample colonies examined further by the methods described above. Clostridial antisera (Wellcome Reagents Ltd.) were used. Colonies considered to be those of Clostridium spp. and

isolated on non selective medium were examined in this way.

Virological examination

These were carried out on faecal samples taken from a small number of pigs with diarrhoea and intestinal contents of killed animals. Each sample was suspended in sterile physiological saline and centrifuged at 750g for 45 minutes. The supernatant was discarded and the sediment resuspended in sterile physiological saline and centrifuged in ultracentrifuge at 11,000g for 15 minutes as a clarification run. The supernatant was taken for further processing, while the sediment was discarded. The supernatant was spun at 30,000g for 45 minutes. The pellets were resuspended in few drops of normal saline and negatively stained. The negatively stained samples were examined by electron microscopy for the presence of viral particles by Dr. H. Laird. The presence or absence of viral particles and their nature was recorded.

Parasitological examination

Faecal samples and intestinal contents from animals from both the survey and the transmission experiments were examined for the presence of nematode eggs, coccidial oocysts and cryptosporidia using the methods described below. Both nematode eggs and coccidial oocysts could be detected by methods (a) and (b), cryptosporidia were demonstrated by method (c) and evidence of both coccidial and cryptosporidial infection was seen in histological sections stained with H and E.

The examinations were carried out by the Department of Veterinary Parasitology and were reported on the basis of the presence or absence of significant numbers of eggs or oocysts and their reports may not reflect the absolute incidence of infection.

(a) Modified McMaster method

Three grams of faeces were mixed thoroughly with 42ml of tap water in a glass beaker. The faecal suspension was blended in a homogenizer (MSE Scientific Instruments). The blended faecal suspension was poured through a '100' mesh sieve and the filtrate was collected in a clean dry bowl. The filtrate was transferred to centrifuge tubes and centrifuged for 2 minutes at 1,500 rpm (700g). The supernatant was discarded. The packed sediment was emulsified and suspended in saturated salt solution (NaCl) until the volume was equal to that of the initial filtrate. The tube was inverted several times until the sediment was evenly suspended. Two chambers of a McMaster slide (Gelman Hawksley Ltd.) were filled by using a clean pasteur pipette.

All the oocysts or nematode eggs within the ruled area of each chamber were counted using 2/3ins objective and x10 eyepiece. The mean number of oocysts found was calculated.

(b) Salt flotation technique

Faecal samples were emulsified with tap water and the coarse particles were removed by centrifugation 700g. The sediment was placed in a tube which was then filled with saturated salt solution and the contents mixed by inverting several times. The tube was then placed in a centrifuge and salt solution was added until the meniscus appeared convex. A 3/4 x 3/4 in. coverglass was placed on the top of the tube which was then centrifuged for 2 minutes at 1,000 rpm (700g). The coverglass was carefully removed with a deliberate upward movement and placed on a clean slide and observed by microscopy. The oocysts and nematode eggs present were noted and counted.

(c) Examination for cryptosporidium

Scrapings of ileal mucosa and faecal samples were examined for cryptosporidium using light microscopy. Giemsa stained smears of faeces or intestinal scrapings were used to detect cryptosporidia by the method described by Pohlenz et al. (1978). The presence of organisms with the morphology of cryptosporidia was recorded.

Transmission experiments

(a) Source of experimental animals

All pigs used in the study were of minimal disease origins (Northern Pig Development High Health Status) and were obtained from the University of Glasgow Animal Husbandry Department. They were of 3 types, conventional

weaned pigs aged 8-10 weeks, conventionally farrowed sows and litters, and hysterectomy-derived piglets and weaned pigs.

Conventional weaners

These were produced by normal farm practice and when obtained had been weaned and maintained in flatdeck accommodation since weaning at 3 weeks of age. All were individually identified using a numbered ear tag. They were housed in unheated pens with concrete floors and straw bedding. They were fed on a barley-based ration containing no non-nutrient additives standard to the Department of Animal Husbandry. Water was freely available. Their farm of origin, Farm 3, is described further in Chapter 3.

Conventionally farrowed litters

One litter was used. It was farrowed in isolated accommodation adapted as a farrowing pen.

The conventional sucking piglets reared in isolation were housed in a loose box with background and creep heating. They were allowed free access to the sow. The isolated piglets were fed solely by the sow with no supplementation. Single Iron Dextran injections of 1ml (Imposil, Fisons Pharmaceuticals Ltd.) were given intramuscularly during the first 2 days of life.

Hysterectomy-derived colostrum deprived

The hysterectomy-derived colostrum deprived piglets were produced and reared by the general methods described

by Betts and Trexler, 1969. Hysterectomy was carried out on the 112th day of pregnancy. After stunning the sow electrically, the whole uterus was removed aseptically into an antiseptic solution in a large bin. The piglets were removed from the uterus on a disinfected operating table. The umbilical cords were clamped and then ligated after respiration was firmly established.

The piglets were housed individually in cages placed in racks of 9 in a temperature controlled room. The room temperature was kept approximately at 35°C for the first week and gradually reduced to 30°C in the second week.

The piglets were fed on evaporated tinned milk (Carnation Foods Ltd.). The feeding started about 2 hours after delivery with 20ml diluted milk. The piglets were fed 4 times per day. Water was available ad libitum. The feeding was supplemented with vitamins (Abidec Multivitamins, Park Davis Ltd.) for the first few days of life. Each piglet was injected intramuscularly with 1ml of Iron Dextran (Imposil, Fisons Pharmaceuticals Ltd.) on the second day of life.

After each feed, the feeding trays were washed in the solution of Chloros (10 per cent alkaline sodium hypochlorite, I.C.I. Ltd.) and then rinsed in hot water. The number of feeds was decreased from 4 times to 3 times daily from the 6th day of life. The milk fed was gradually increased in amount to 50ml at each feed.

When diarrhoea occurred in animals being reared (Experiment 4), it was controlled by oral administration of sulphadiazine and trimethoprim mixture (Tribrissen, Wellcome), at a dosage of 1.1ml per kg body weight. The treatment was combined with the reduction of the amount of the milk fed to such piglets. Some were treated with Ampicillin (Penbritin, Beecham Animal Health) at the dosage of 4-12ml per kg body weight orally for 5 days.

Piglets which were kept for more than 3 weeks were placed on the floor of rearing pen with a water trough and creep feed (Sucklercare pellets, No. 402; B.O.C.M. Silcock Ltd.), available ad libitum. They were fed on small amounts of evaporated milk mixed with the creep feed in feeding trays until they were weaned at 3 weeks of age. After weaning, creep feed was provided ad libitum as the sole source of food.

The diet of the pigs was changed to non-pelleted and non-antimicrobial containing feed at 6 weeks of age.

(b) Experimental procedures

Experimental design

All experiments were carried out with appropriate controls. Animals were divided into control and infected groups and maintained in isolation in separate housing. In conventional and weaned pigs this was done in separate pens outside which stood a disinfectant footbath and for hysterectomy-derived colostrum deprived piglets, animals were placed in separate cage blocks within the same

temperature controlled room.

Inocula

The inoculum used in each experiment was prepared from an isolate which had been cloned twice after isolation and freeze-dried by the method described by Garvie (1967). Freeze-dried cultures were reconstituted for each experiment, passaged once on horse blood agar and used to prepare the inoculum. Thickly inoculated blood agar plates were incubated at 37°C under the appropriate atmosphere for 24 or 48 hours, depending on the organism. The surface growth was harvested in sterile physiological saline and used to inoculate the experimental animals. Viable counts of organisms present in the inoculum were determined using the method described by Miles and Misra (1938), using horse blood agar plates and sterile physiological saline for dilution. The inoculated plates were incubated at 37°C for 24 hours after which the colonial growth on the surface of the plates was examined. The plates were reincubated for another 72 hours and examined daily. The number of colonies on each plate was counted and the number of viable organisms in each dilution was calculated.

Inoculation

All pigs, except the conventional sucking piglets, were inoculated orally after withholding food for the previous 24 hours. Pigs were forcibly fed with various amounts of the inoculum (stated under each experiment) in an attempt to give the required dose. The amount used was recorded and is given under the appropriate experiment.

Clinical observations

The general bodily condition of the experimental pigs was noted daily. Appetite and the consistency of the faeces were noted. Rectal temperatures were taken and recorded daily. Any deviation from this general pattern of clinical examination is discussed under the specific experiments.

Feed consumption and weight gains were measured weekly and recorded in some of the experiments.

Rectal swabs were taken daily from all the experimental animals prior to and after inoculation. Rectal faeces were taken from individual animals when faecal changes were noted. Both faeces samples and rectal swabs were examined for bacteria, and faeces samples were examined for parasites and viral particles as described above. Virological and parasitological examinations were carried out particularly when evidence of diarrhoea was seen. Details are recorded under the appropriate experiments.

Serum samples

Blood samples were collected from the anterior vena cava of weaned pigs before inoculation. Blood was obtained from all pigs at the end of experiments by collection from the stick wound in the thoracic inlet made with a dry knife during exsanguination. Clotted samples were left at room temperature for about 5 hours, and later stored at 4°C in the refrigerator for 24 hours or overnight. The blood was

spun down in a centrifuge at 750g for 20 minutes. The serum was collected, centrifuged again at 750g for 10 minutes and was stored at -20°C in 5ml amounts in bijou bottles until needed.

Post mortem examinations

These were carried out by the methods outlined above as were histological and bacteriological examinations.

Serological examinations

Only a limited number of serological tests were carried out. The test used throughout the study for the demonstration of antibody to the bacteria being examined was the tube agglutination test. The Nagler reaction was, however, used in an attempt to demonstrate the presence of antibody to lecithinase in animal studies of C. perfringens Type A.

The tube agglutination test used for the determination of serum antibody to both C. perfringens Type A and C. coli was that described for C. jejuni by Butzler and Skirrow (1979).

Each organism was heavily streaked on horse blood agar plates and incubated at 37°C in the appropriate atmosphere for 24 or 48 hours. The surface growth harvested from the agar plates with 0.5 per cent formol saline (FBS), which was prepared with 1ml of formaldehyde and 200ml of normal physiological saline.

Formalized whole cell antigens were prepared by suspending the cells in FBS. Concentrated whole cell antigen thus produced was stored in the refrigerator at +4°C overnight or for 24 hours.

Prior to use in the tube agglutination test, the concentrated antigen was processed further in order to obtain a standard concentration of antigen. Stored antigen was diluted in fresh 0.5 per cent formaldehyde-saline solution and centrifuged for 20 minutes at 750g. The supernatant was discarded and the cells were resuspended in 0.5 per cent formol-saline. The process of washing was repeated twice at 750g. The cells were finally resuspended in 0.25 per cent formol-saline solution. The final cell concentration was adjusted to a density of tube No.8 using Wellcome Opacity tubes (Wellcome Reagents Ltd.). The test was conducted using phenol saline solution (1g phenol crystals, M & B, in 200ml of physiological saline) as a diluent to give the final following dilutions of serum 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280. 0.5ml of antigen was added to each tube already containing 0.5ml of the serum dilution. The suspensions were mixed thoroughly and incubated at 37°C in a water bath for 24 hours.

Inoculated tubes were then observed for signs of agglutination. The results were read by tilting the tubes. The titre of the serum was recorded only when no auto agglutination in the antigen control tube was observed. The tubes were stored in the refrigerator at +4°C overnight

and the results were read once more. A further 24 hours' incubation in water bath was necessary in some cases to obtain a complete agglutination.

Nagler reaction

Half of an egg yolk agar plate was treated with C. perfringens antitoxin A (Wellcome Reagents Ltd.), while the other half remained untreated. The plate was sown with a pure culture of C. perfringens Type A, and incubated under anaerobic conditions at 37°C for 24 hours. The plate was examined for the presence or absence of an opalescent zone of precipitated lipid around each colony released from lecithin in the egg yolk by lecithinase.

All results were recorded.

CHAPTER 3

THE SURVEY OF FIELD MATERIAL

INTRODUCTION

The pigs used in this survey came from a small number of farms and were mainly young animals. In spite of the limited nature of the source material a large number of bacterial species were isolated and identified. In many cases, examination for all possible pathogens could not be carried out and the scanty history of the cases and their state of preservation limited the information which could be obtained. The results have been presented below according to the conditions identified, the bacteria isolated and the presence or absence of lesions at the site of isolation. The results which follow are a summary and partial interpretation of the detailed findings, a case by case summary of which is included at the back of this thesis as Appendix 1.

MATERIALS AND METHODS

The majority of the materials and methods used have been described in Chapter 2. The 129 pigs concerned came from 8 farms, details of which are given below with the number of pigs submitted from each.

Farm 1

This was a 270 sow herd run as part of a mixed farming enterprise within 20 miles of Glasgow. Most rations were home mixed and did not contain antimicrobials. The

exceptions were creep rations which always contained non nutrient additives the identity of which changed from time to time during the survey. A number of enteric diseases had been demonstrated on this unit in the past and its productivity had been closely monitored over a period of 10 years. The enteric diseases identified included coccidiosis, neonatal diarrhoea not known to be associated with the presence of rotavirus or B-haemolytic E. coli, post weaning E. coli diarrhoea, spirochaetal diarrhoea and proliferative intestinal adenopathy.

Seventy-four pigs which had died or which were chronically ill were submitted to the Veterinary School for diagnosis on a regular basis.

Farm 2

This was a 120-sow unit supplied with breeding stock by a breeding company (Pig Improvement Co.Ltd.). Few enteric diseases other than post weaning diarrhoea associated with a K88-bearing E. coli were known to occur. Non nutrient additives were used only in creep feed to which penicillin was added towards the end of the survey to control streptococcal meningitis. The post weaning diarrhoea was controlled by a combination of individual dosing with antibiotic, vaccination of the sows and piglets with an oral E. coli vaccine (Intagen, B.O.C.M. Ltd.) and the use of a highly digestible diet. Ten pigs which had died suddenly and which were suspected of having streptococcal meningitis were sent for examination.

Farm 3

Thirty-three pigs from this farm, the University of Glasgow Animal Husbandry Department's farm, were submitted for diagnosis or following experimental studies into the therapy of swine dysentery. The farm is mentioned briefly in Chapter 2 and Chapter 4, Studies 1 and 2, but a full description is given here.

A small herd of 35 sows and two boars of Northern Pig Development High Health Status stock is maintained by the Animal Husbandry Department. Sows are housed throughout and fed on a ration containing no non nutrient additives. Weaning is at 4 weeks of age. Weaned pigs are placed in flat decks from 4-8 weeks of age and then placed in pens in the fattening section of the house. Creep feed containing non nutrient additives is given from 10 days until 6 weeks of age.

Rectal stricture, epidemic diarrhoea, and the spirochaete of spirochaetal diarrhoea had all been identified in the herd.

Farm 4

Four unweaned pigs were submitted by a local practitioner for diagnosis of an enteric problem. No history of the farm was provided.

Farm 5

Three pigs were submitted from a herd in which congenital tremor was present. The pigs concerned were less than one week of age and no history of the enteric

disease situation was available for that farm.

Farm 6 .

Two sucking piglets were submitted from a large conventional breeding herd in Eastern Scotland for the diagnosis of an enteric disease problem. E. coli postweaning diarrhoea was present and an enteric problem of unknown cause was also present in the sucking pigs.

Farm 7

Two pigs thought to have swine dysentery were submitted for confirmation of the diagnosis following failure to respond to tylosin treatment.

Farm 8

One pig with exudative epidermitis was submitted from a farm stocked by the same company as Farm 2 for confirmation of the diagnosis.

Post mortem examination was carried out by the methods described in Chapter 2. All pigs were examined for gross changes but samples for histological examination were taken only from animals which had died recently or which were killed. A general bacteriological examination was carried out on all regions of the gut of all pigs but examination for anaerobic spirochaetes was carried out only on the large intestinal mucosa of weaned pigs. These were principally from Farm 3. C. sputorum ss. mucosalis was sought specifically using the Lawson and Rowland selective medium in pigs from Farms 1, 3, 4, 5 and 7. A total of 72

pigs was examined. Reinforced Clostridial medium was used routinely in the survey on pigs from Farms 1, 2, 3 and 4 and it was used in 90 cases.

RESULTS

A. Conditions diagnosed in the pigs included in this survey

The following diseases were diagnosed in the pigs used in this survey.

E. coli enteritis Fourteen neonatal pigs from Farms 1, 2 and 6 were considered to have E. coli enteritis as were 7 weaned pigs from Farms 1 and 2.

Swine dysentery was diagnosed in 17 weaned pigs from Farms 3 and 7. Those from Farm 3 had been used in experimental studies of the therapy of the disease.

Spirochaetal diarrhoea was diagnosed in one weaned pig from Farm 1.

Proliferative intestinal adenopathy and its associated conditions of proliferative haemorrhagic enteropathy and necrotic enteritis were diagnosed in 2 weaned pigs from Farm 7.

The rectal stricture syndrome was diagnosed in a pig from Farm 3 but was also seen in pigs from Farm 1 which were not included in this series.

'Bloody gut' This syndrome was diagnosed in 3 pigs from Farms 2 and 3.

Rotavirus diarrhoea was confirmed in 3 piglets from Farm 3.

Viral enteritis other than rotavirus was identified in 2 piglets from Farm 5 but the nature of the virus could not be determined.

Local lesions and evidence of enteritis were identified in a number of animals in the survey but no final diagnosis could be assigned to the syndromes seen. These conditions are described below and correlated with the bacteria found in them.

A number of non-enteric conditions were diagnosed and these are listed in detail in Appendix 1 of this thesis. No evidence of salmonellosis, epidemic diarrhoea, transmissible gastroenteritis, nematode infections, cryptosporidial or coccidial infections was found in the survey. Pigs from Farm 3 were found to be infected with coccidia when killed and examined in Study 1, Chapter 4 and with cryptosporidia in Experiment 2, Chapter 4.

B. Bacterial species isolated from intestinal mucosa in the survey and their relationship to lesions

The bacteria isolated are listed below.

Escherichia spp.

Non haemolytic E. coli

B-haemolytic E. coli

Streptococcus spp.

B-haemolytic streptococci, some of which belonged to Lancefields group D and were identified as S. faecium

α-haemolytic streptococci, which belonged to Lancefields Group D, mostly S. faecalis, S. faecium and S. suis

Non-haemolytic streptococci, some of which were Group D and identified as S. faecalis and S. faecium

Unidentified streptococci

Campylobacter spp.

C. coli

C. sputorum subsp. mucosalis

Clostridium spp.

C. perfringens Type A

Non haemolytic C. perfringens

C. sporogenes

Unidentified Clostridium spp.

Treponema hyodysenteriae

Non T. hyodysenteriae spirochaetes

Bacillus spp.

B. licheniformis

B. mycoides

B. cereus

Unidentified bacilli

Proteus mirabilis

Peptostreptococcus spp.

P. intermedius

Unidentified peptostreptococci

Bacteroides spp.

B. fragilis

B. vulgatus

B. melaninogenicus

Fusobacterium spp.

F. necrophorum

F. fusiformis

Lactobacillus spp.

L. fermentum

L. catenaforme

Unidentified lactobacilli

St phylococcus spp.

S. aureus

S. epidermidis

S. hyicus

Pseudomonas aeruginosa

Corynebacterium pyogenes

Pasteurella spp.

P. multocida

P. haemolytica

Erysipelothrix rhusiopathiae

In addition, the yeast Torulopsis glabrata was isolated from one animal from Farm 3.

Escherichia coli

Non-haemolytic E. coli

Non-haemolytic E. coli was isolated from the mucosa of some regions of the gut in 80 pigs. It was completely absent from 40 pigs and was present only in small numbers in the mucosa of the duodenum, jejunum, ileum caecum and colon in those animals in which it was present (Table 2). It was present in both normal and abnormal mucosa and in the absence of serotyping or pathogenicity determination its significance is uncertain. No further attempt to relate its presence to lesions was made.

B-haemolytic E. coli

B-haemolytic E. coli was isolated from 24 unweaned animals of 2 days to 2½ weeks of age, and from 13 weaned animals and adults from Farms 1, 2, 3, 4 and 6. It was identified by the methods described in Chapter 2. It could be identified easily in primary cultures on sheep blood and MacConkey agar by its colonial morphology, and its identity as E. coli was confirmed by biochemical tests.

The criteria for the diagnosis of E. coli enteritis were reviewed in Chapter 1. Using these criteria, B-haemolytic E. coli were present in significant numbers in the duodenum, jejunum and ileum in 14 sucking and 7 weaned pigs and these animals were considered to have E. coli enteritis. The organism was accompanied

TABLE 2. Sites from which non-haemolytic E. coli were isolated in 80 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation							Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	MLN	Liver	
3	+	+	+	-	-	-	-	9,10,62
8	+	+	+	+	-	-	-	2,12,20,39,38, 117,120,123
20	+	+	+	+	+	-	-	1,13,15,16,22, 24,25,26,42,43, 50,58,70,73,90, 95,100,110,121, 126
19	++	++	+	+	+	-	-	3,4,5,19,21,29, 34,36,41,44,47, 48,49,61,63,88, 96,101,116
13	-	+	+	+	+	-	-	6,8,11,27,45,46, 74,76,78,86,98, 102,115
8	-	++	+	+	+	-	-	80,81,83,104,105, 109,111,124
3	-	-	+	+	+	-	-	107,108,109
6	-	+	-	+	-	-	-	7,14,53,68,92, 129

MLN = Mesenteric Lymph Nodes

by a number of other bacteria. In the small intestine these included Streptococcus spp., non-haemolytic Clostridium spp., non-haemolytic E. coli and C. coli. In the large intestine, intestinal spirochaetes, Bacillus spp. and Lactobacillus spp. were also isolated. In one case rotavirus was also present.

In 18 cases B-haemolytic E. coli was isolated in pure culture from the mucosa of the small intestine. In these animals the gross lesions found resembled those described for E. coli enteritis in Chapter 1. One observation not generally reported was that the villi were only slightly reduced in height and that the large intestinal mucosa was normal in appearance regardless of the consistency of its contents. In the three cases in which E. coli was not present in pure culture, the mucosa appeared more hyperaemic than in the uncomplicated infection.

In all 18 cases, fluid faeces were present in the rectum at post-mortem examination. The histological changes seen were minimal in those animals examined. Inflammatory changes were prominent histologically in 2 complicated cases in which gross inflammatory changes were present. Inflammatory cells were present in the lamina propria and the villi were stunted. Small blood vessels in the mucosa and submucosal layers were slightly distended or congested.

The distribution of B-haemolytic E. coli in the intestines and an approximate guide to the numbers of organisms isolated is shown in Table 3. The other bacteria present are given under the individual case numbers in Appendix 1. Gram negative bacteria with the morphology of E. coli were present in smears made from all sites from which the organism was isolated.

Campylobacter coli

Campylobacter coli was isolated from 22 piglets less than 3 weeks of age and from 23 weaned pigs aged 3 weeks and above from Farms 1, 2, 3, 4, 6 and 7. It was isolated both on horseblood agar plates and on the selective medium containing the Oxoid supplement (Chapter 2). In all cases growth was more obvious on the selective medium and all the isolates identified presumptively on both non-selective and selective media were identified as C. coli on the basis of the characters described below.

The colonies were 2-5mm, greyish, flat, translucent with irregular edges, or spreading along the direction of streak and at times tending to swarm and coalesce (Fig. 1). In gram stained smears the morphology of the organisms was typical of campylobacters (Fig. 2). Isolates from each pig tested were found to be catalase positive, oxidase positive, to grow at 30.5, 37, 42, 43, 44 and 45°C but not at 25°C or in aerobic conditions. All isolates grew on media containing 1mg TTC per ml. (Fig. 3), and 3.5% sodium chloride and on medium containing 1:100,000 Brilliant Green

TABLE 3. Sites from which B-haemolytic E. coli were isolated in 32 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation								Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	MLN	Liver	
1	+	+	+	+	+	+	+	+	16
1	+	++	++	+	+	+	-	-	17
1	+	++	++	++	+	+	+	+	109
6	+	+++	+++	++	+	+	-	-	1,3,13,21, 63,104
5	-	+	+	+	-	-	-	-	22,56,53, 96,120
6	-	+	+	+	+	+	-	-	5,7,14,15, 64,122
1	-	+	+++	++	+	+	-	-	18
6	-	++	++	+	+	+	-	-	69,92,106, 111,121, 128
1	-	++	++	++	++	++	-	+	113
9	-	+++	+++	++	+	+	-	-	2,19,29,30, 40,61,105, 115,116

MLN = Mesenteric Lymph Nodes

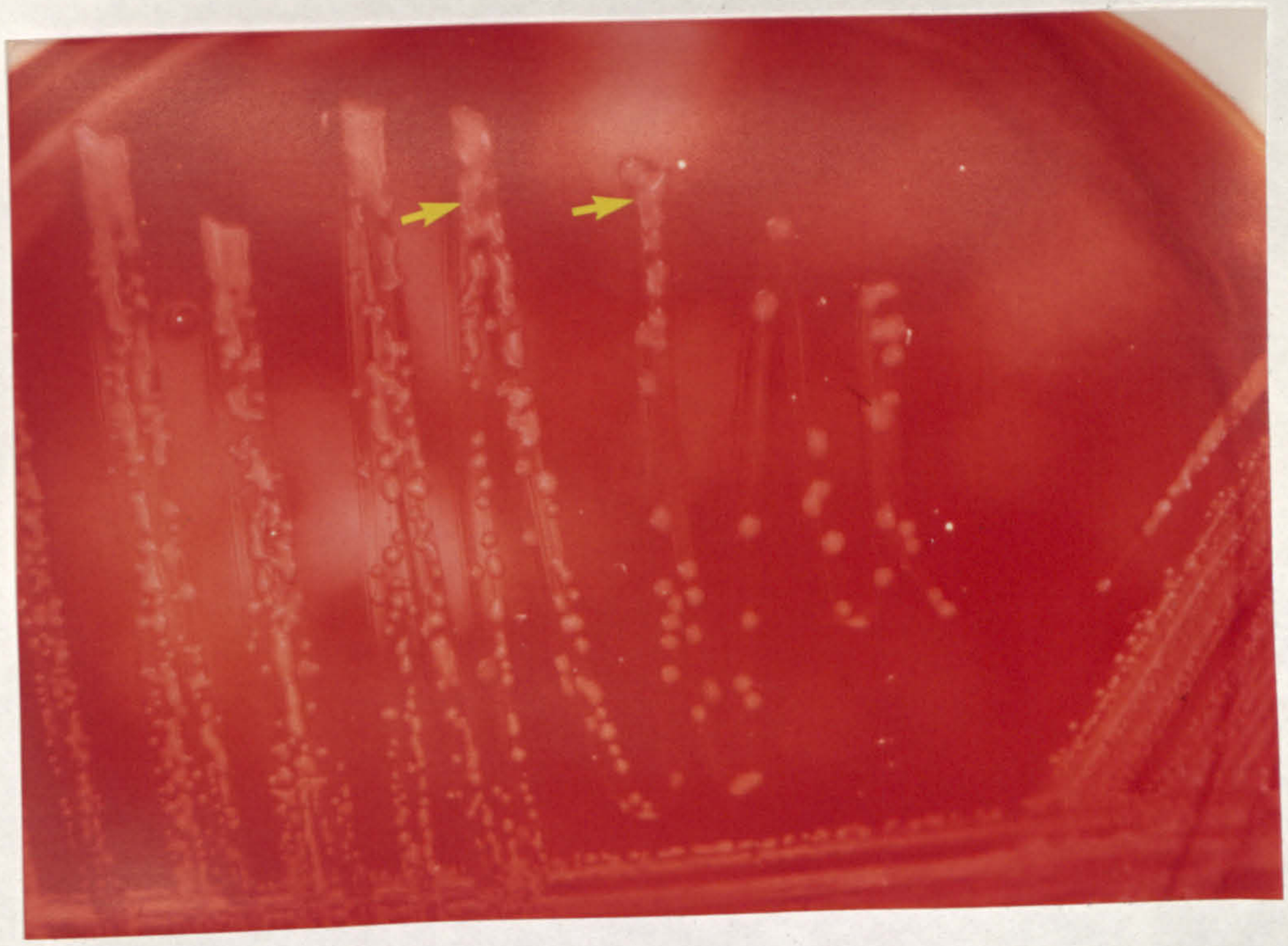


FIG. 1: Colonial morphology of C. coli isolated from Piglet 36, 48 hour culture on horse blood agar at 37°C in microaerophilic conditions.
Note coalescing colonies (arrow).

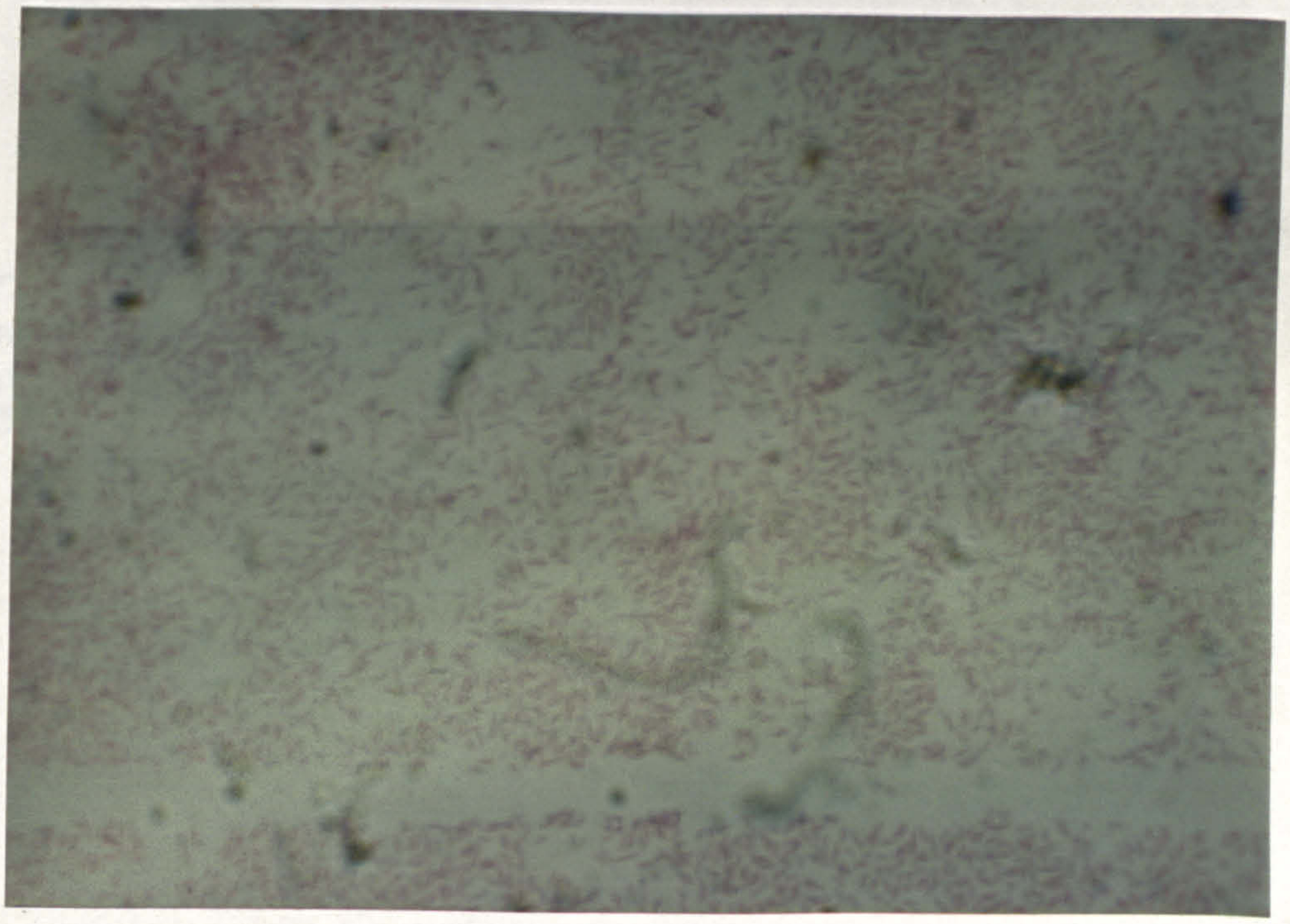


FIG. 2: Smear of a colony of C. coli from the plate shown in Figure 1.
Gram x 1200

FIG. 3: Growth of C. coli on an agar plate containing 1mg T.T.C. per ml after 48 hours incubation at 37°C in microaerophilic conditions. The red colour is associated with growth.

but not on the 1:33,000 dilution or on nalidixic acid medium. All isolates failed to produce hydrogen sulphide from T.S.I.medium but produced some hydrogen sulphide when tested with lead acetate papers after growth in cysteine hydrochloride medium. All isolates reduced nitrates and selenite but none of the 47 isolates examined hydrolysed hippurate (Fig. 4). This latter character, with the ability to grow in 8% glucose and at 30.5°C distinguished these isolates from C. jejuni and confirmed their identity as C. coli.

Large numbers of colonies of C. coli were isolated from the ileum and caecum of some animals. Less commonly, moderate to heavy growths were isolated from the jejunum and colon. In 3 animals it was isolated from the gastric mucosa in small numbers and in 7 animals from the duodenum. In only 3 animals was the organism recovered from sites outside the gastrointestinal tract, in all cases as scanty growths from the mesenteric lymph nodes (Table 4).

In no case was C. coli isolated in pure culture. Other bacteria such as Gram-positive cocci, Gram-negative bacilli, and, on some occasions, Gram-positive rods were seen in smears made from the mucosa from which C. coli was later isolated. Curved or spiral Gram negative rods with the morphology of campylobacter were seen in smears made from the mucosa at all sites from which moderate or profuse cultures of C. coli were recovered (Fig. 5).

Beta-haemolytic E. coli, non-haemolytic E. coli, faecal streptococci, and Clostridium species were also

FIG. 4: The rapid hippurate hydrolysis test with C. coli and C. jejuni. C. coli (left) is negative when compared with the positive reaction of 2 isolates of C. jejuni (centre and right).

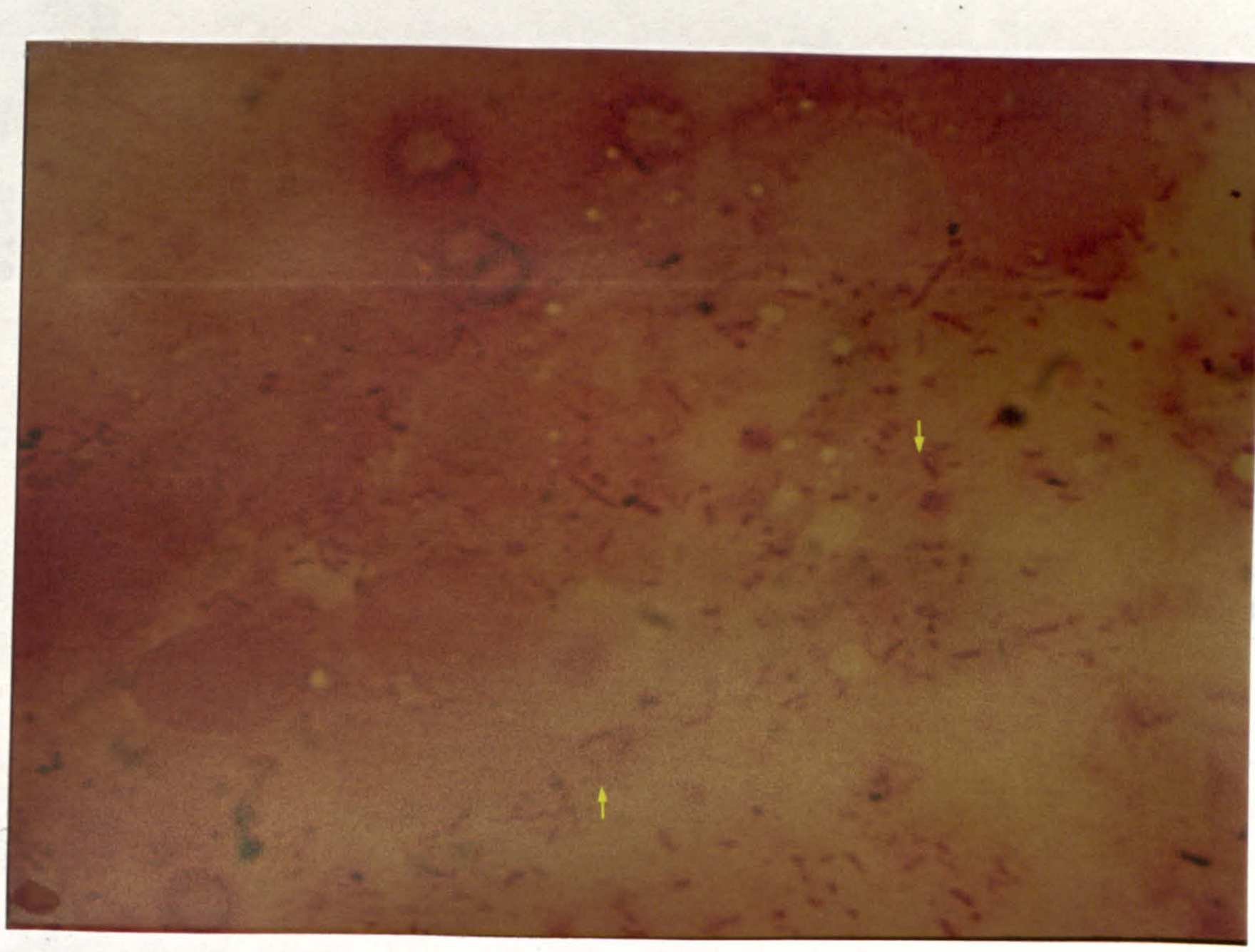


FIG. 5: Smear of the ileal mucosa of Pig 36, at a site from which C. coli was isolated.
Note the curved rods (arrows).
Gram x 1200

TABLE 4. Sites from which C. coli was isolated in 45 cases examined at post mortem and the relative abundance of colonies.

Number of cases	Site of isolation							Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	MLN	
1	+	+	+	+	+	+	-	12081
1	+	+	+	+++	+++	++	+	12181
1	+	+	++	+++	++	+	+	9480
1	-	+	+	+	+	+	-	11581
2	-	+	+	++	++	+	-	10780 11981
1	-	+	++	++	+	+	-	118
5	-	-	+	+	+	+	-	35,37,87, 00,116
11	-	-	+	++	++	+	-	12,32,33,36 40,54,58,59 67,114,124
1	-	-	++	++	++	++	-	125
1	-	-	+	+++	++	+	-	57
8	-	-	-	+	+	+	-	34,47,48,76, 79,98,100,123
3	-	-	-	+	+	-	-	81,84,111
2	-	-	-	++	++	+	-	5,105
1	-	-	-	++	++	+	+	31
1	-	-	-	++	++	++		75
5	-	-	-	-	+	+	-	50,51,77,80,89

MLN = Mesenteric Lymph Nodes

isolated in most cases. In 9 animals ranging in age from 7 days to 6 weeks profuse cultures of C. coli were recovered from the small intestine and no other pathogens could be demonstrated. In a further 13 animals aged 3 days - 8 weeks small numbers of colonies of B-haemolytic E. coli or C. perfringens Type A were isolated along with the profuse cultures of C. coli. The history, gross, microscopic and bacteriological findings in these two groups were similar but those for the 9 animals considered to have fewest complications are described below.

In the remaining 23 animals few colonies of C. coli were isolated (often less than 5 colonies from each site), and in all cases, other bacteria were present.

The 9 animals in the group apparently infected with C. coli as the sole pathogenic bacterium had diarrhoea at the time of death. No consistent features of the diarrhoea were noted but all animals were in fair to poor condition. These findings also applied to the second group of 13 pigs only one of which did not have diarrhoea.

In all 9 of the animals in which C. coli was considered important, gross changes were noted in the small intestine or its contents. The serosal surface of the small intestine appeared pale and fleshy, particularly in the distal portion. The contents of the small intestine were fluid in consistency and the mucosa was inflamed or congested particularly in the lower jejunum and ileum (Figs.6 and 7). In these animals, the villi were stunted in both the jejunum and the ileum and the wall of the ileum

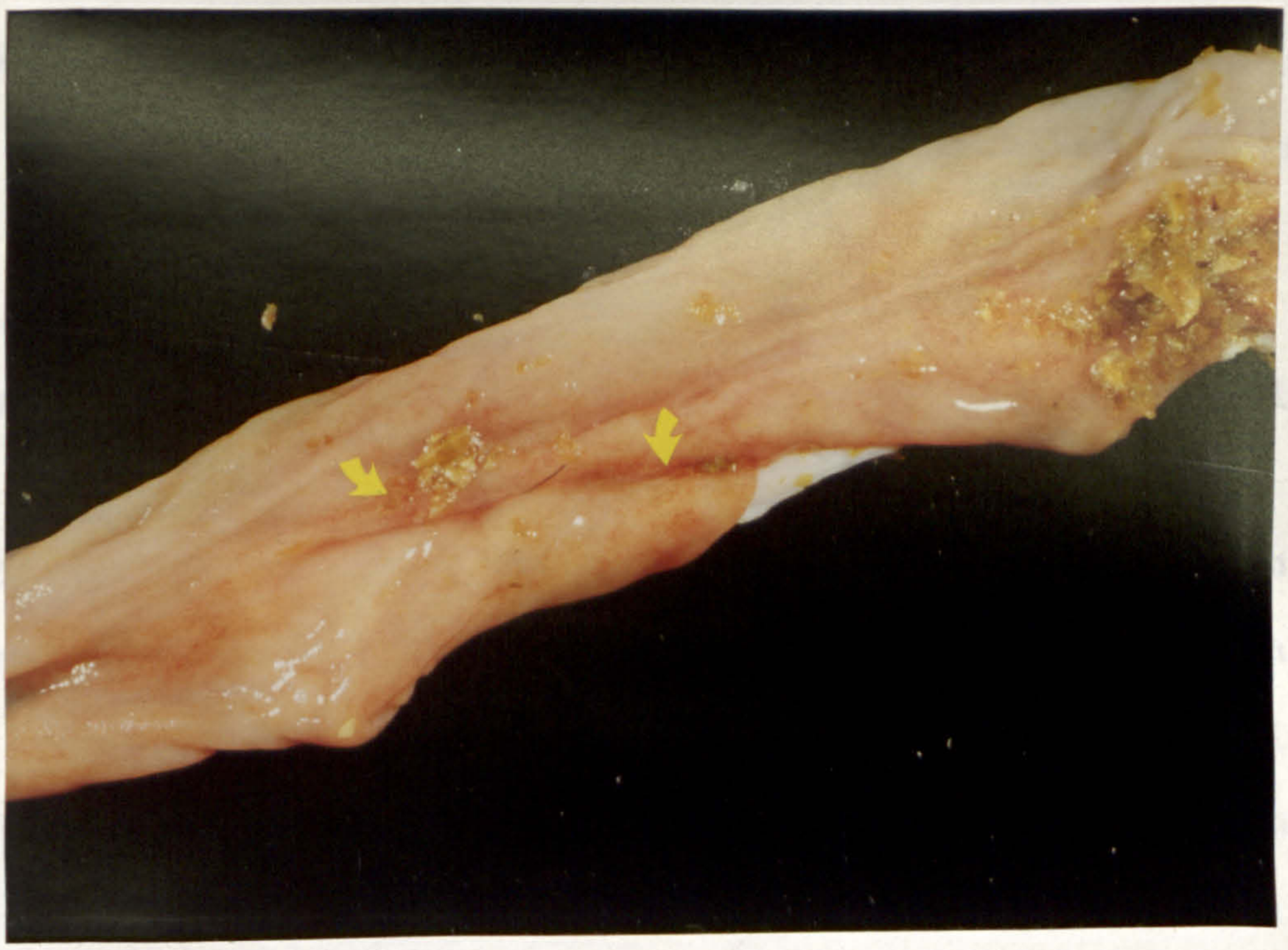


FIG. 6: Macroscopic appearance of the jejunal mucosa of Pig 121 at a site from which C. coli was isolated.
Note mild inflammation (arrow).

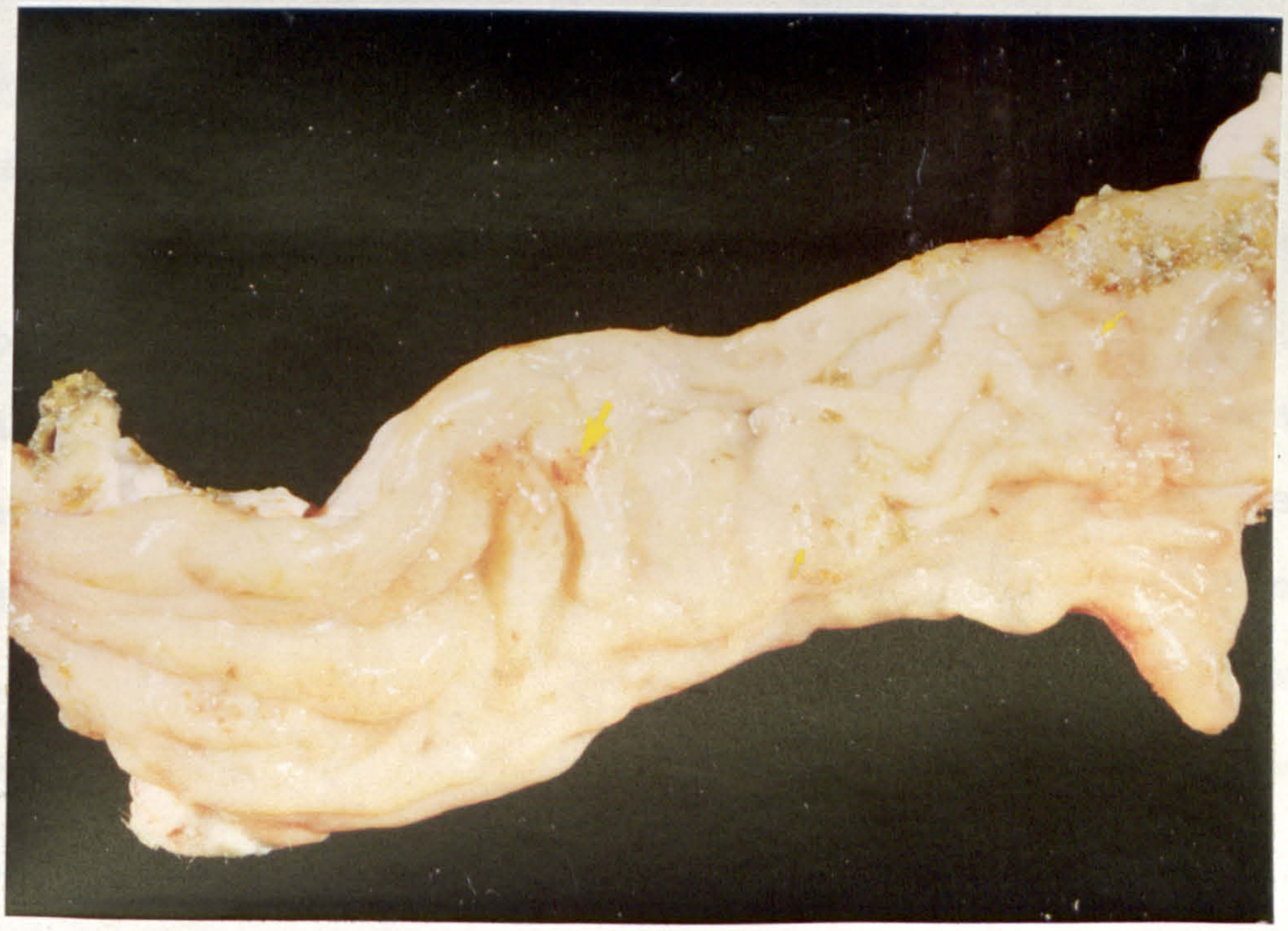


FIG. 7: Macroscopic appearance of the ileal mucosa of Pig 121 at a site from which C. coli was isolated.
Note mild inflammation and excess mucus (arrow).

was thickened, particularly in the terminal portion. The contents of the caecum and colon varied in consistency from fluid to pasty in all the animals from which C. coli was isolated. Some of the contents contained clear mucus. The mucosal surfaces of both the caecum and colon were mildly inflamed in some cases. The areas of inflammation were localised in other cases. The large intestinal mucosa in some cases had adherent contents (Fig. 8) and in one animal (Pig 51) necrosis of the mucosa was noted. The mesenteric lymph nodes were, in all cases, enlarged and pale. In the second group of 31 animals, these changes were not present in every case and, in some cases, changes referable to other organisms or conditions were also present.

Histological examinations were carried out on all those pigs in this group which were recently dead or which were killed. In all animals the height of the villi in the jejunum was reduced and fusion of the villi had taken place. In pigs 13 and 14 the villous epithelium was intact but in pig 4 the cells of the villous epithelium were flattened or absent and the crypts contained accumulations of inflammatory cells (Fig. 9). Changes seen in the ileum included shortening and fusion of the villi, the presence of dilated lacteals and infiltration of the lamina propria with polymorphonuclear leucocytes and eosinophils. Mitoses were commonly seen in the crypts. Lymphoid follicles were prominent in the submucosa, and some of them were reactive (Fig. 10).

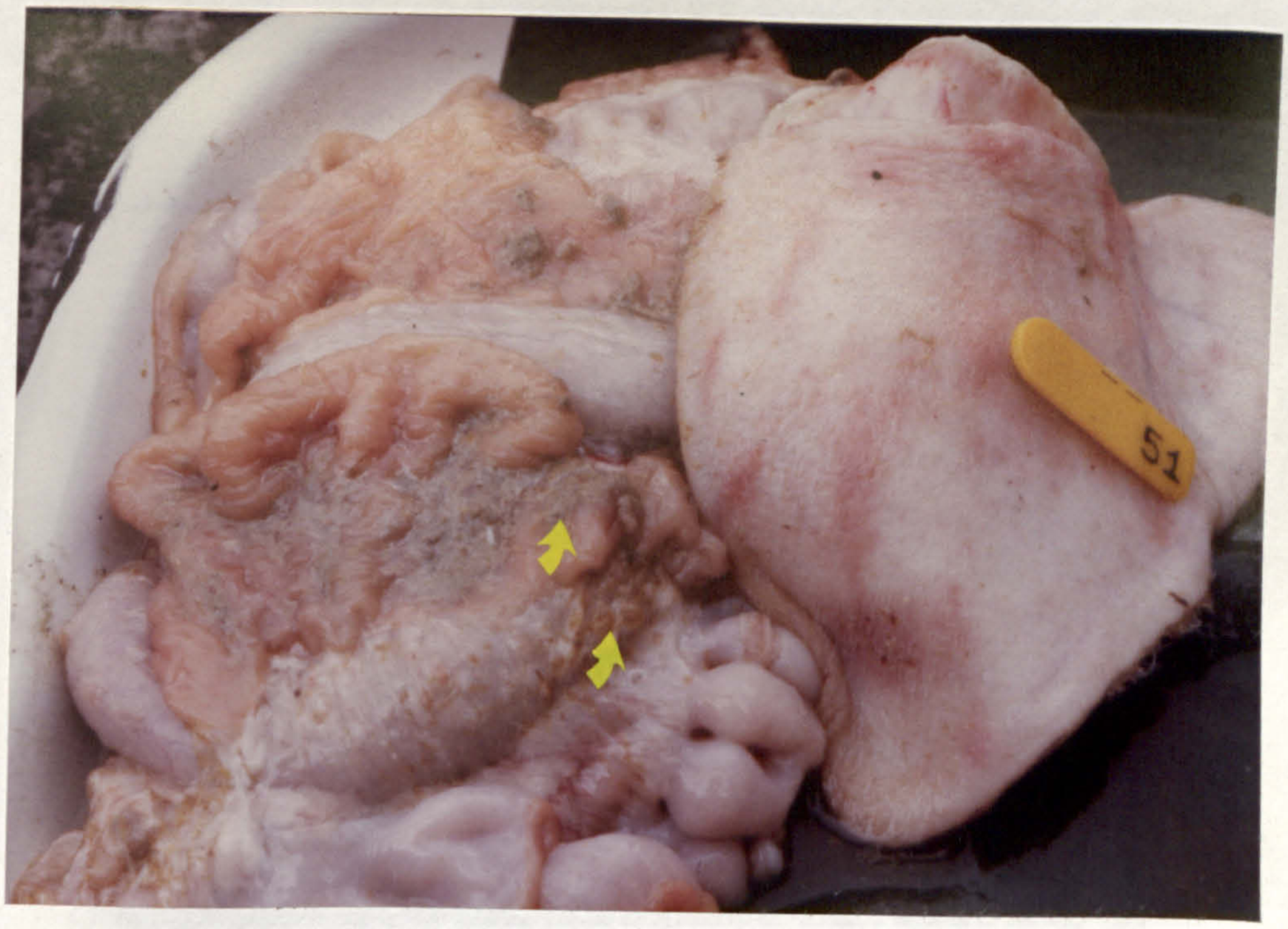


FIG. 8: Macroscopic appearance of the colonic mucosa of Pig 51 with adherent contents at a site from which C. coli was isolated.
Note the adherent contents (arrow).

FIG. 9: Histological section of the jejunal mucosa of Pig 36 from which C. coli was isolated.
Note the accumulated inflammatory cells in crypt (A), villous atrophy (V) and lowered villous epithelium (arrows). The lamina propria is hypercellular.
H & E x 120

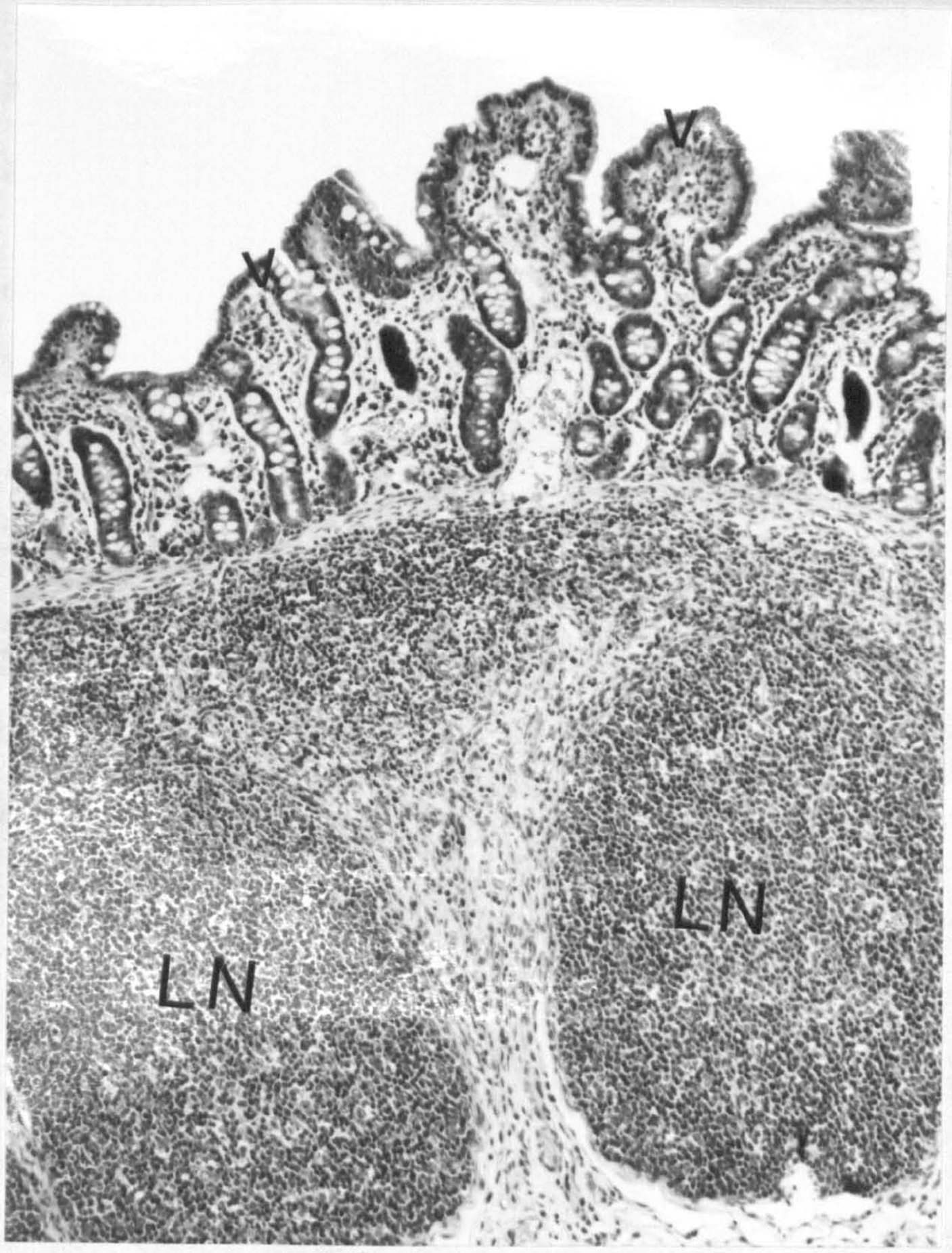


FIG. 10: Histological section of the ileal mucosa of Pig 36, at a site from which C. coli was isolated.
Note the lymphoid hyperplasia (LN), and stunting of villi (V).
H & E x 120

Dilated crypts were the major abnormality noted in most of the large intestinal sections examined (Figs. 11, 12 and 13). In some cases these were accompanied by dilated capillaries and inflammatory cells were seen in the lamina propria.

The mesenteric lymph nodes were enlarged and reactive in all cases.

Clostridium perfringens Type A

C. perfringens Type A was isolated from the intestinal mucosa of 23 pigs, from Farms 1, 2, 3 and 4. It was recovered from 12 sucking pigs of less than 3 weeks of age and from 11 weaned pigs and adults.

C. perfringens Type A was presumptively identified in primary cultures by its morphology and haemolysis pattern (Fig. 14). Double zones of haemolysis were characteristically produced on horse blood agar plates. The organism was further tested for its biochemical reactions, and the identity of the organism as Clostridium perfringens confirmed by the methods described in Chapter 2. The cells appeared as Gram positive, stout rods with truncated ends without spores in smears made from cultures (Fig. 15).

It was presumptively identified as belonging to Type A by its reaction on Egg Yolk Agar and the inhibition of the lecithinase produced using the Nagler reaction (Fig. 16).

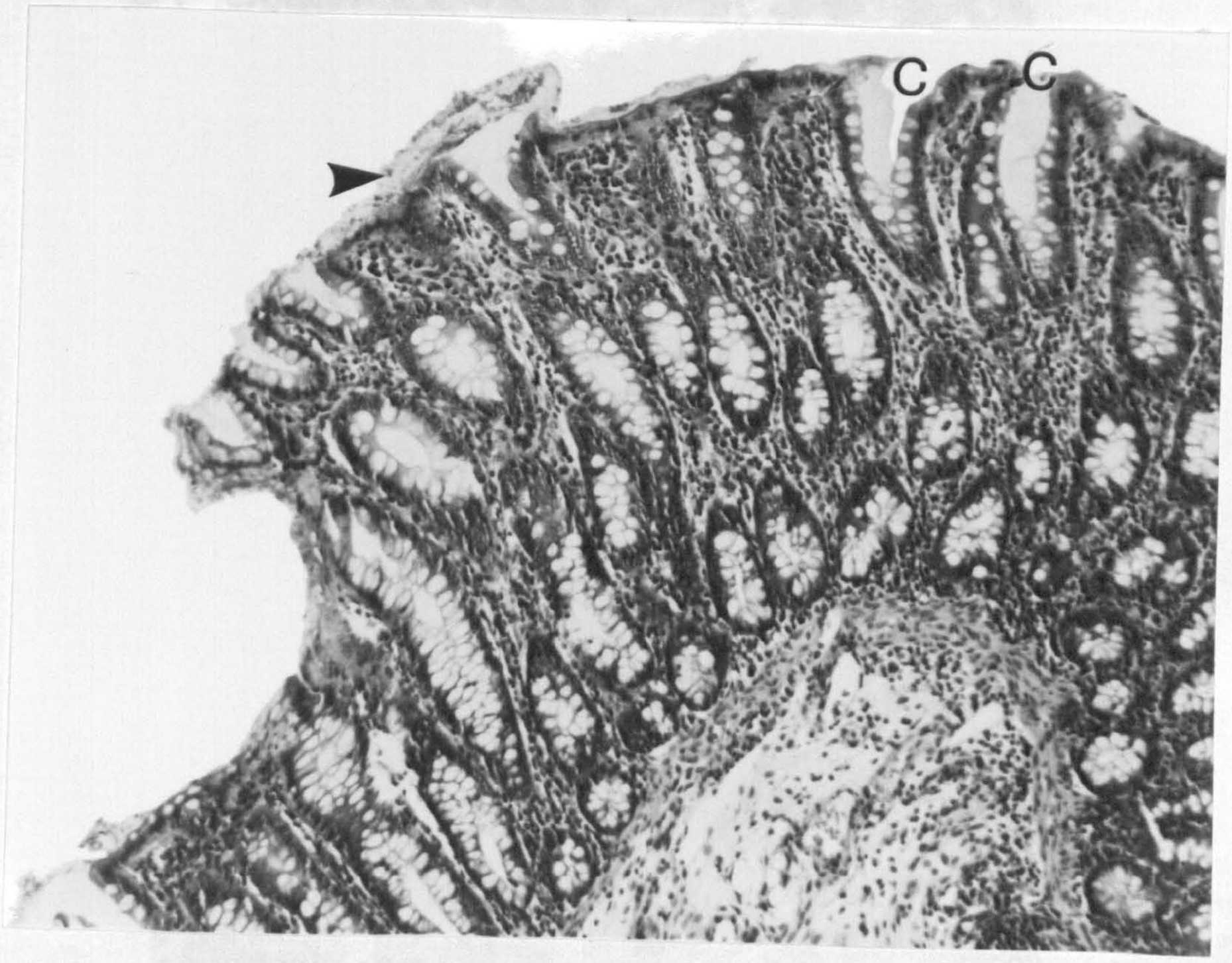


FIG. 11: Histological section of the colonic mucosa of Pig 36, at a site from which C. coli was isolated. Note the dilated crypts (C) and the presence of debris adjacent to the lumenal epithelium (arrow).
H & E x 120

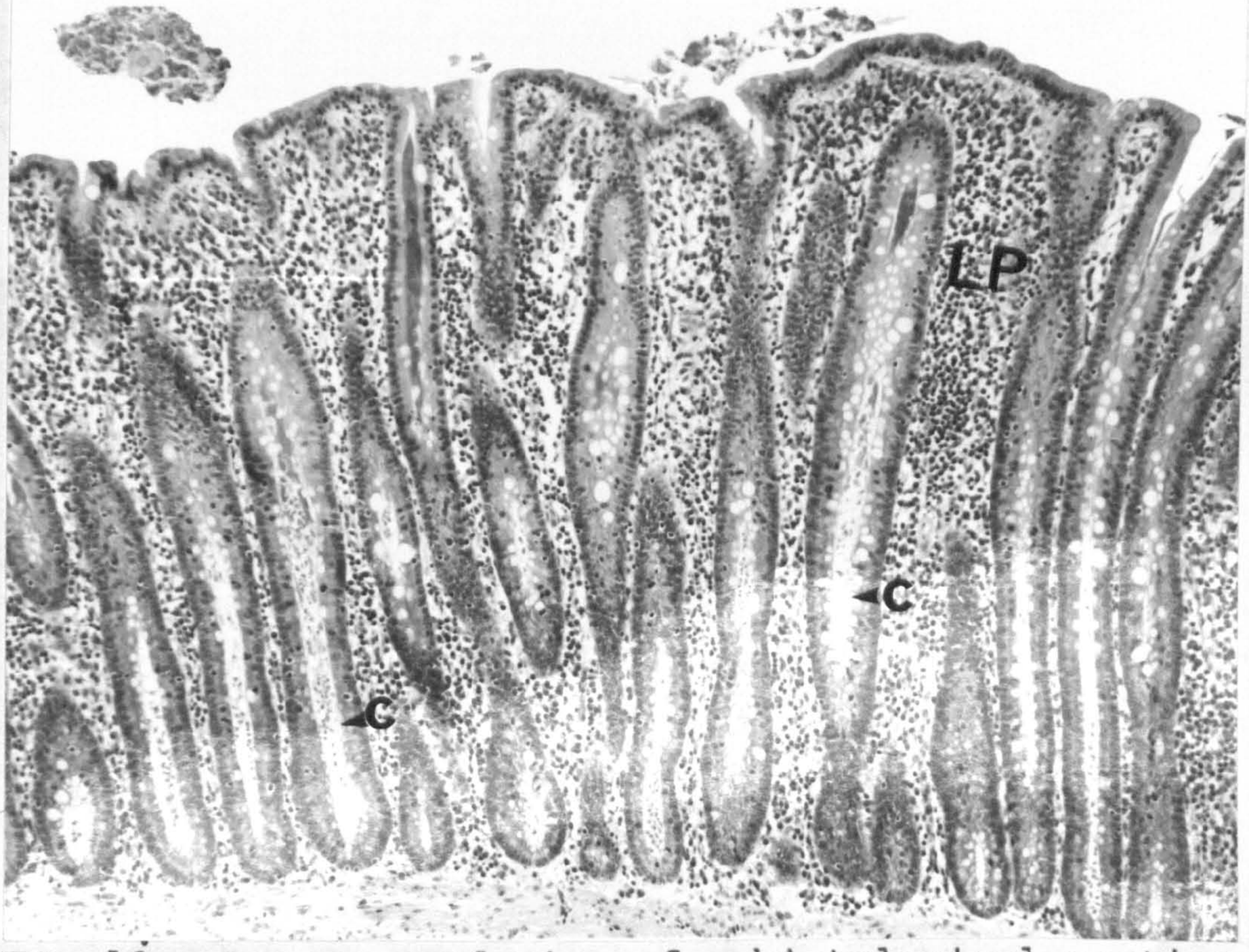


FIG. 12: Low powered view of a histological section of the colonic mucosa of Pig 51 from which C. coli was isolated. Note the thickened lamina propria (LP) and dilated crypts (C) containing organisms. Debris is present adjacent to the lumenal epithelium (arrows)
H & E x 120

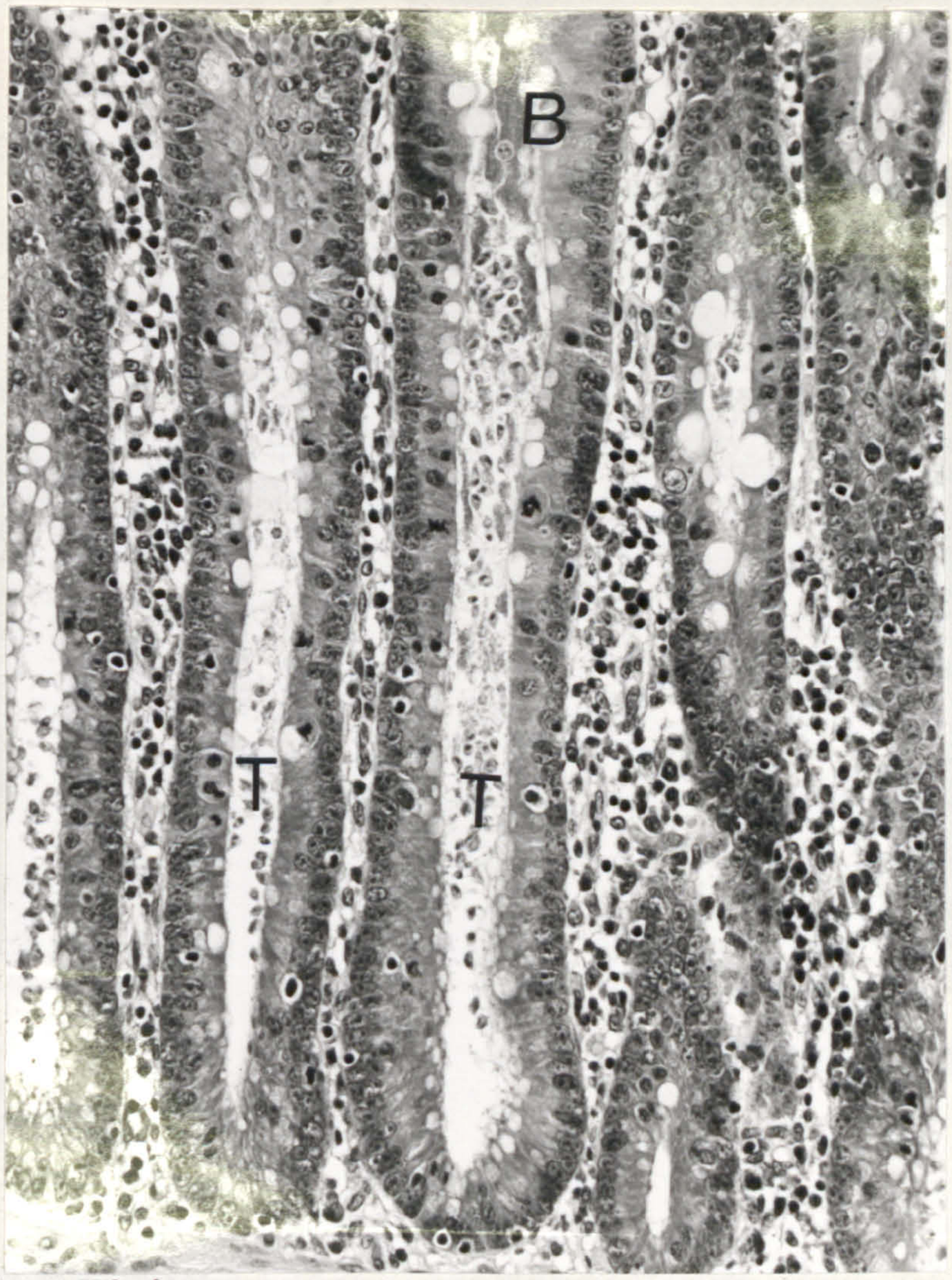


FIG. 13: Higher power view of a histological section of the colonic mucosa of Pig 51, shown in Figure 12. Bacteria are present towards the mouth of the crypt (B), and bodies resembling trichomonads (T) are present in the bases of the crypts.
H & E x 250

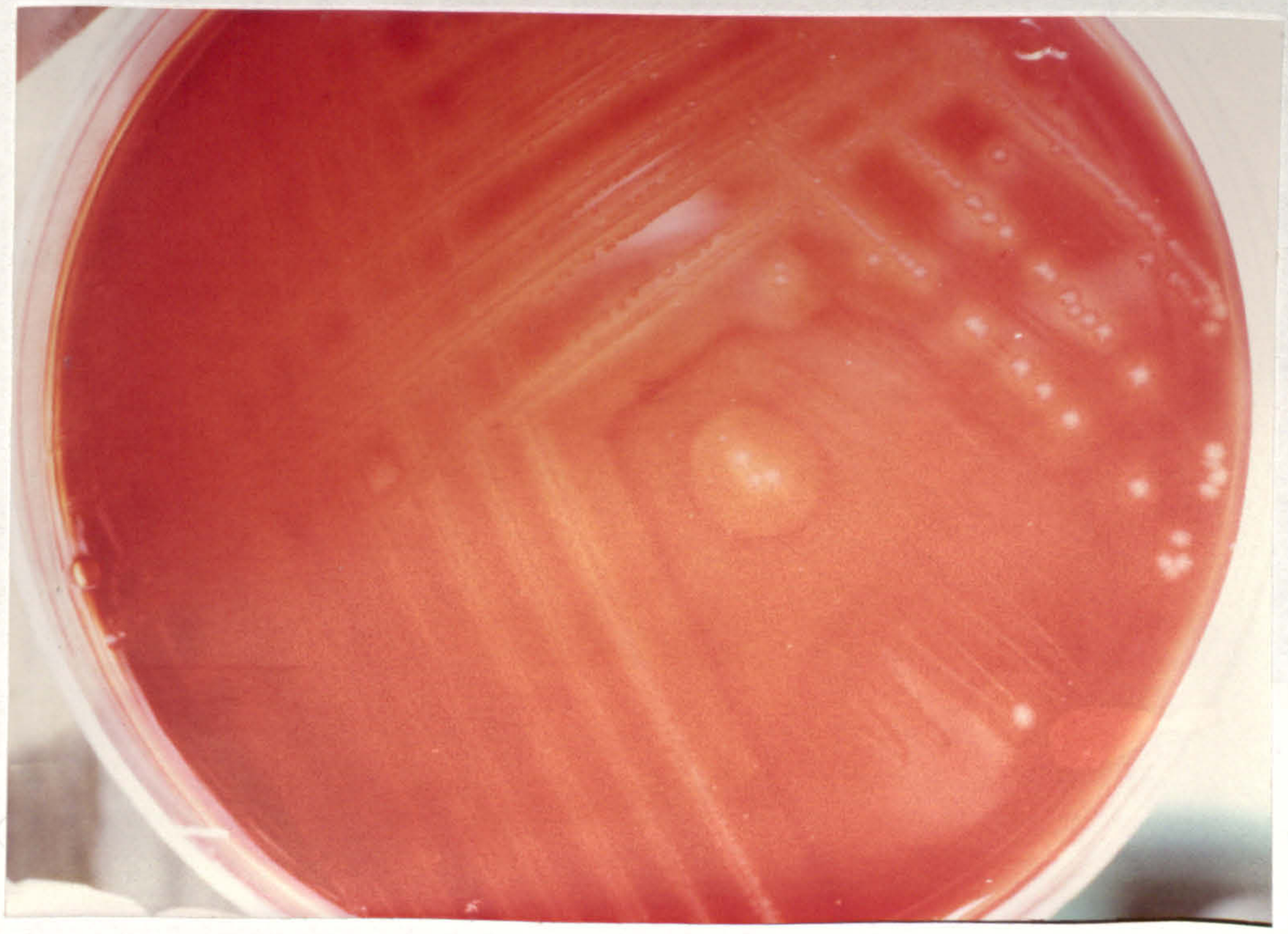


FIG.14: Colonial morphology of C. perfringens Type A isolated from Piglet 70, 24 hour culture on horse blood agar in anaerobic conditions at 37°C. Note the double zone of haemolysis around the colonies.

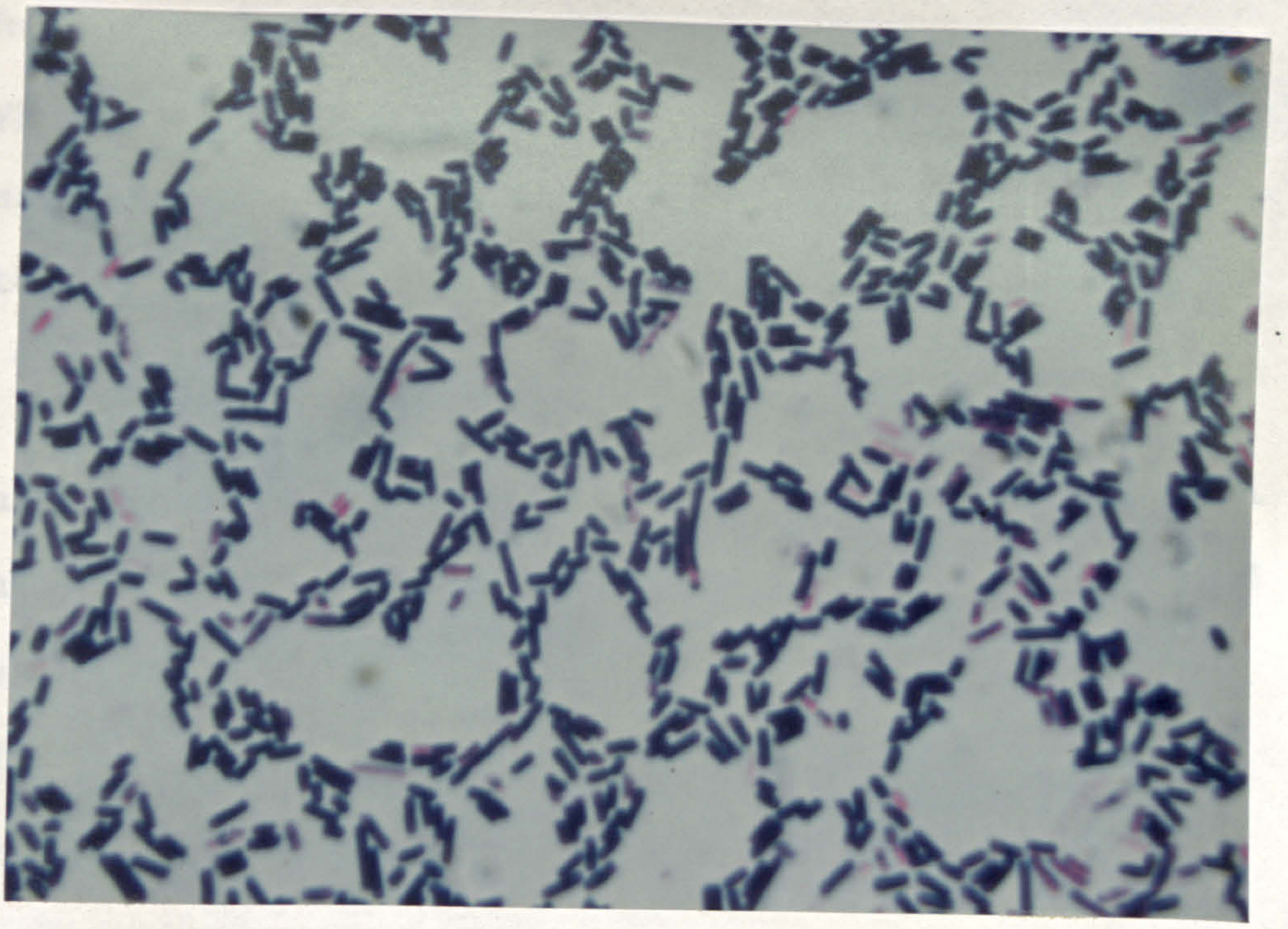


FIG. 15: Smear of a colony of C. perfringens Type A from the plate shown in Figure 14.
Gram x 1200

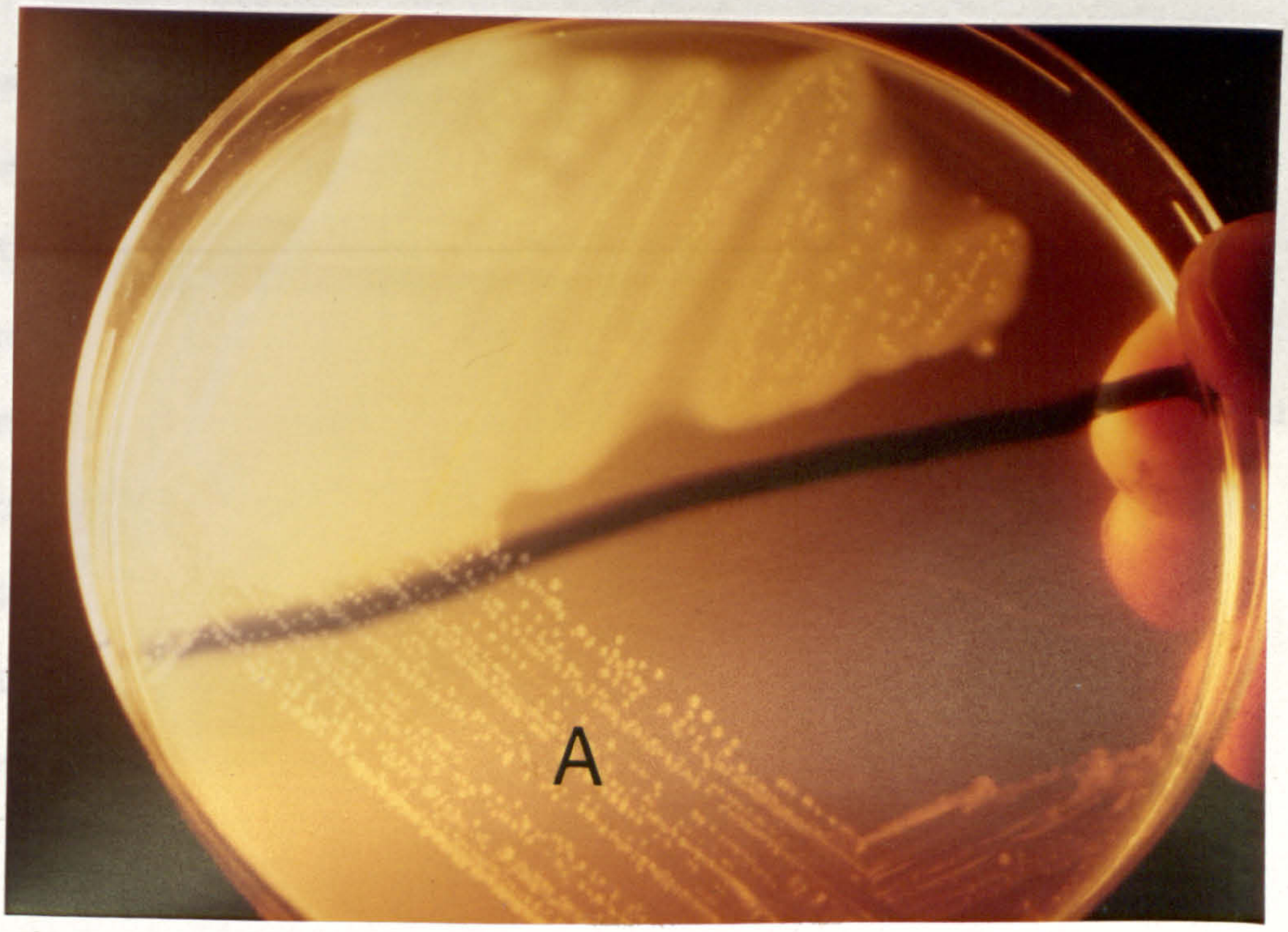


FIG. 16: Nagler reaction on egg yolk agar plate. Note the absence of the opalescent zone from the half of the plate (A) which had been treated previously with C. perfringens antitoxin A.

Where the lesions noted were particularly severe or when typical isolates were being defined on a farm, toxin testing was carried out. None of the isolates tested produced toxins lethal for mice and all were considered to belong to Type A.

Large numbers of colonies of C. perfringens Type A were isolated from the mucosa of the duodenum, jejunum and ileum of some animals. The organism was isolated less commonly from the gastric mucosa but was recovered in small numbers in the large intestinal mucosa of most animals in which it was present (Table 5). It was never recovered from sites outside the gastrointestinal tract.

In all cases C. perfringens Type A was accompanied by other bacteria in the lesions in which it was found. These bacteria included non-haemolytic E. coli, C. coli, faecal streptococci and Bacillus spp. in the small intestine and Bacteroides spp. and Lactobacillus spp. in the large intestine. In spite of this, certain features were noted in all of the 10 cases in which profuse or moderate cultures of the organism were isolated. In all sites from which it was recovered in profuse culture large numbers of Gram positive bacteria with the morphology of C. perfringens could be seen in smears (Fig. 17).

The description of the changes associated with the presence of C. perfringens Type A is based on the findings in 10 animals ranging in age from 2 days to 6 weeks. Two syndromes were associated with the presence of C. perfringens Type A; the majority of animals had diarrhoea and were

TABLE 5. Sites from which C. perfringens Type A was isolated in 23 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation							Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	MLN	
1	+	++	+++	++	+	+	-	36
3	+	+++	+++	++	+	+	-	70,108,128
6	-	+	+	+	+	-	-	14,29,39,43, 91,55
4	-	+	+	+	+	+	-	24,32,53,100
2	-	+	++	++	+	+	-	11,117
1	-	+	+++	+++	+	+	-	110
2	-	++	++	+	+	+	-	35,37
1	-	++	+++	++	+	+	-	57
1	-	++	++	++	+	-	-	60
1	-	-	+	+	-	-	-	87
1	-	-	+	+	-	+	-	38

MLN = Mesenteric Lymph Nodes

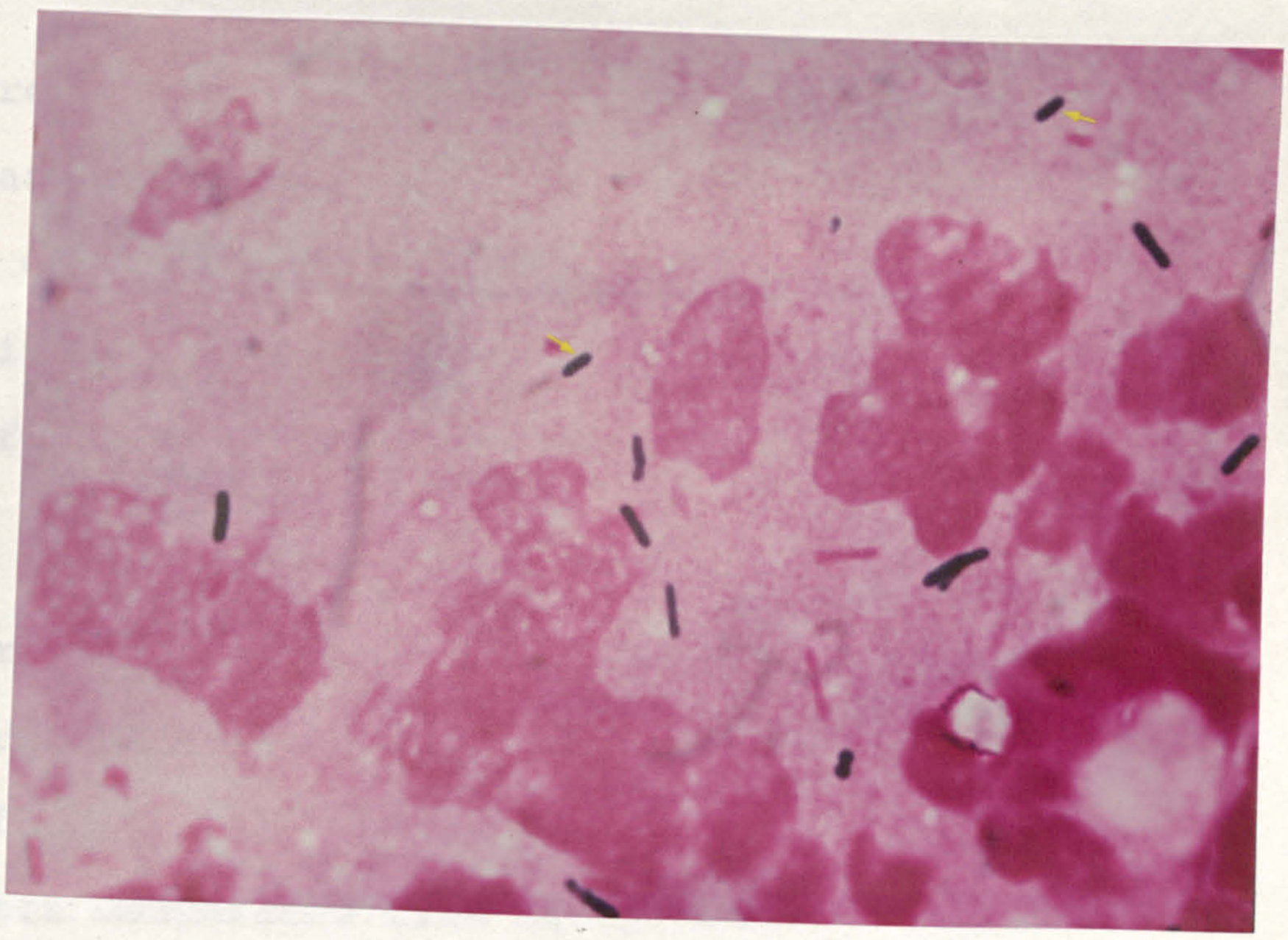


FIG 17: Smear of the small intestinal mucosa of Piglet 70 at a site from which C. perfringens Type A was isolated.
Note the presence of Gram-positive rods with the morphology of C. perfringens (arrow).
Gram x 1200

relatively young but a few (3 cases) had 'bloody gut'. The findings in non 'bloody gut' cases are described below as they formed the largest group of cases. The 'bloody gut' group will be described separately.

The findings in the cases in which profuse cultures of C. perfringens Type A were recovered from the small intestinal mucosa are as follows:

There was a history of diarrhoea in all cases when a history was available and the majority of animals were aged 9 days or less. The faeces in most of these cases were fluid in consistency and creamy in some. Blood was present as flecks in the faeces in 3 cases.

The bodily condition of the pigs ranged from very poor to fair, at the time of examination. The animals in poorest condition were piglets with bloody diarrhoea of the type described above. The eyes were sunken and the carcasses were dehydrated with hairy coats. The flanks were hollowed and the hindquarters were stained with the fluid faeces. The faeces were never the claret coloured faeces typically found in cases of acute C. perfringens Type C infection of the type described in Chapter 1. Minor changes such as the presence of clear straw coloured fluid in the pericardial and pleural cavities and some pallor of the myocardium and congestion of its vessels were seen in the thoracic cavity. The liver was pale in most cases and the spleen and liver were normal in size and appearance. The most obvious changes were in the gastro intestinal tract.

The stomach was either empty or half-filled with feed. The changes seen in the gastric mucosa ranged from slight localised hyperaemia to generalised congestion in some.

The small intestine was often distended with fluid contents, and the serosa was congested to a variable extent. This was most severe in piglet 70 in which the serosa was red in colour (Fig. 18). Changes were least obvious in the duodenum in which the contents were fluid and often normal in appearance and in which mild congestion of the mucosa was seen. The contents of the jejunum were fluid and contained small particles of necrotic material or were blood tinged (Piglet 55). The mucosal surface was congested in all cases and haemorrhagic in some. Villi were absent or reduced in height and covered with flakes of fibrinous or necrotic material. The ileum was similar in appearance although the contents differed slightly in that they contained gas bubbles and were foamy in appearance. The mucosal appearance resembled that of the jejunum.

The large intestinal contents were fluid and bulky in some cases while in others they were pasty and adherent to the mucosal surface. The mucosal changes varied from slightly generalised congestion to localised areas of hyperaemia and pinpoint haemorrhages.

The mesenteric blood vessels were engorged in some cases particularly when the gut was severely congested and a variable degree of enlargement and oedema was seen in the mesenteric lymph nodes.

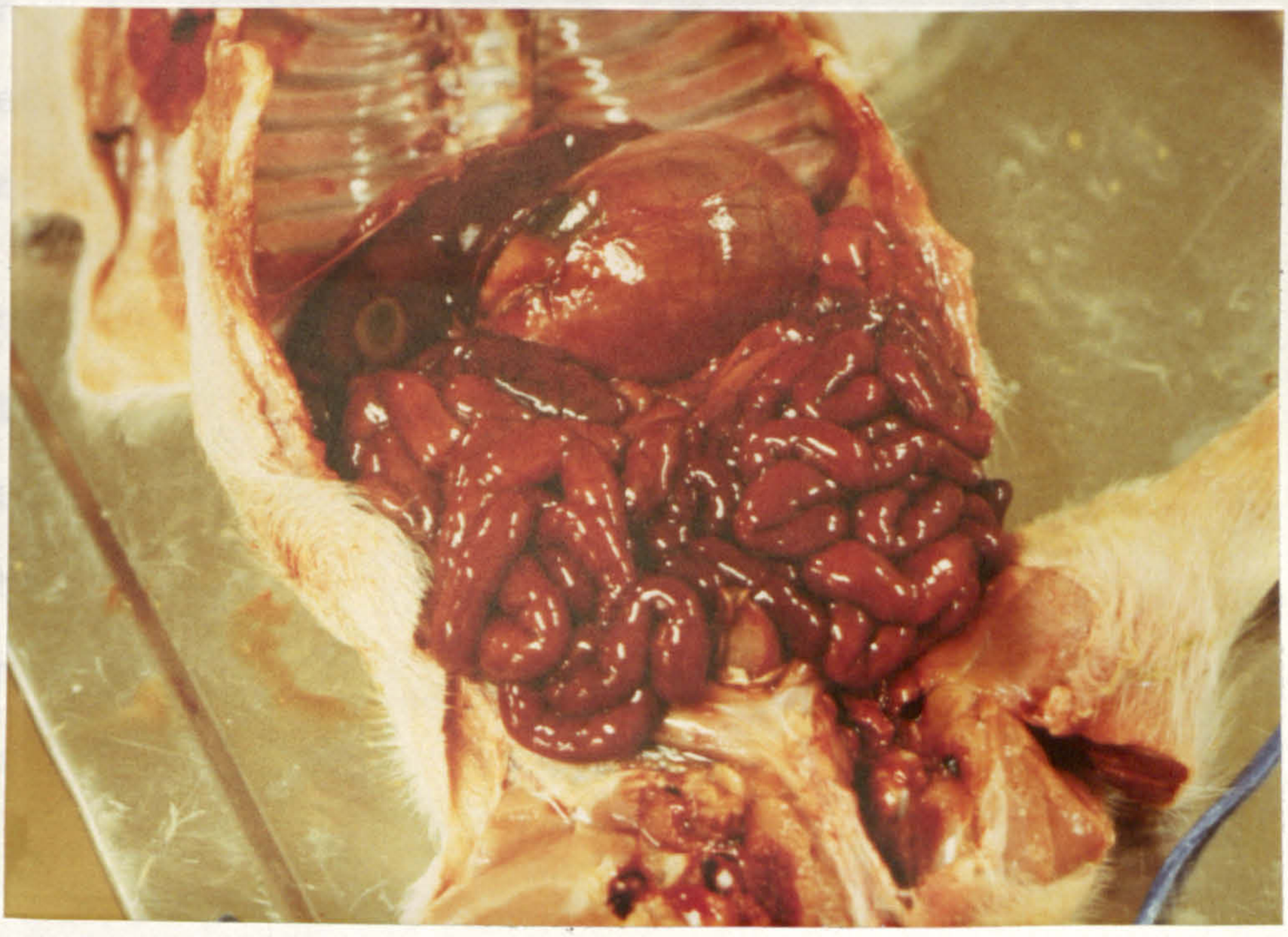


FIG. 18: Gross appearance of the intestines of Piglet 70, from which C. perfringens Type A was isolated.
Note the marked congestion of the small intestine.

Histological changes were most pronounced in the small intestine, particularly in the jejunum and ileum. In every case the mucosa was congested, there was villous atrophy with loss of the epithelial cells and the presence of red blood cells, shed epithelial cells, fibrin and inflammatory cells in the lumen even in animals which had been killed (Fig. 19). These changes were more marked in some cases than in others. Excess mononuclear cells and polymorphs were present in the lamina propria. Bacteria could be seen in clumps adjacent to the damaged villi.

The changes in the caecum and colon were less dramatic. The mucosal surface was still intact in most cases, even though signs of necrosis were seen in some. There was often a layer of cell debris on the lumenal surface of the epithelium. Some of the cellular debris could be seen in the crypts. The small blood vessels were prominent in the lamina propria.

The mesenteric lymph nodes were reactive and oedematous.

<u>3 cases of 'Bloody gut'</u>

In the 3 cases diagnosed as 'bloody gut' gross changes typical of that condition were noted. In particular the pigs were weaned or adult and had died suddenly in good condition. The abdominal cavity contained blood tinged fluid.

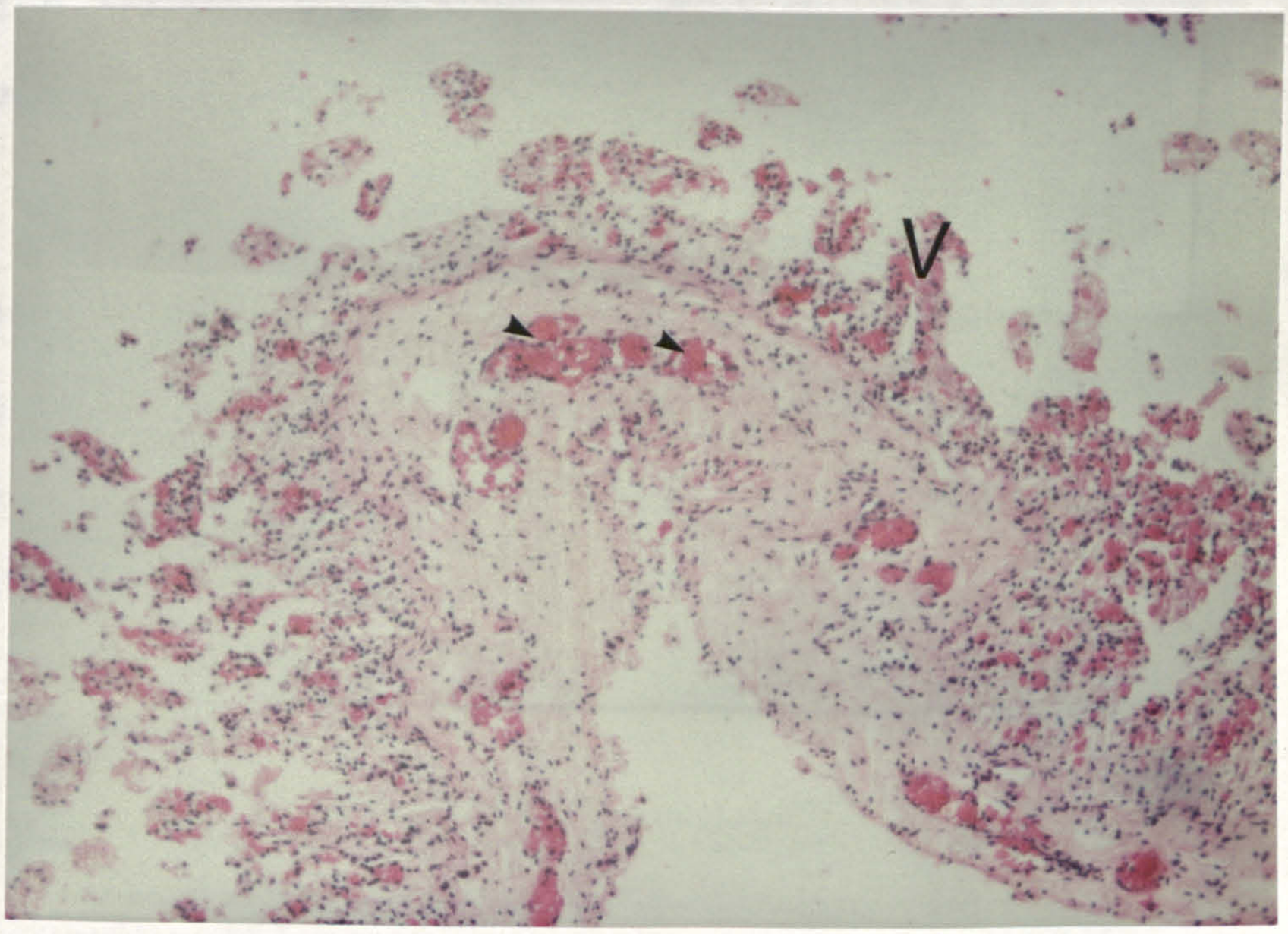


FIG. 19: Histological section of the jejunal mucosa of Pig 70, at a site from which C. perfringens Type A was isolated.
Note the destruction of the villi (V), and the congestion of the small blood vessels (arrow).
H & E x 40

The stomach was distended with fluid which contained blood clots in one adult animal. The gastric mucosa in the adult pig was haemorrhagic, but only mildly congested in others. The serosal surface of the small intestine was reddened (Fig. 20). The contents were fluid in consistency and were red in colour. The wall was thin and flaccid and the mucosal surface was haemorrhagic and velvety in texture throughout the length of the small intestine. The contents of the large intestine were blackish and in some cases tarry. The mucosal surface was only mildly inflamed or normal.

In histological sections, there was shortening of villi in the small intestine particularly in the jejunum. In all cases autolysis had occurred and the remnants of the villi present were completely denuded of epithelium. The lumen was filled with desquamated epithelial cells, red blood cells, inflammatory cells and clumps of bacteria and food debris (Fig. 21).

There was massive congestion of the blood vessels and accumulation of red blood cells in the lamina propria and submucosa.

The intact mucosa and largely normal layer of the large intestine was overlain by cell debris including free blood cells. The small blood vessels were heavily congested.

In Gram stained smears of the small intestinal mucosa, large numbers of bacteria were seen (Fig. 22). Many were Gram positive rods resembling C. perfringens and C. perfringens Type A was isolated from all 3 cases in profuse culture. Other bacteria such as B-haemolytic and

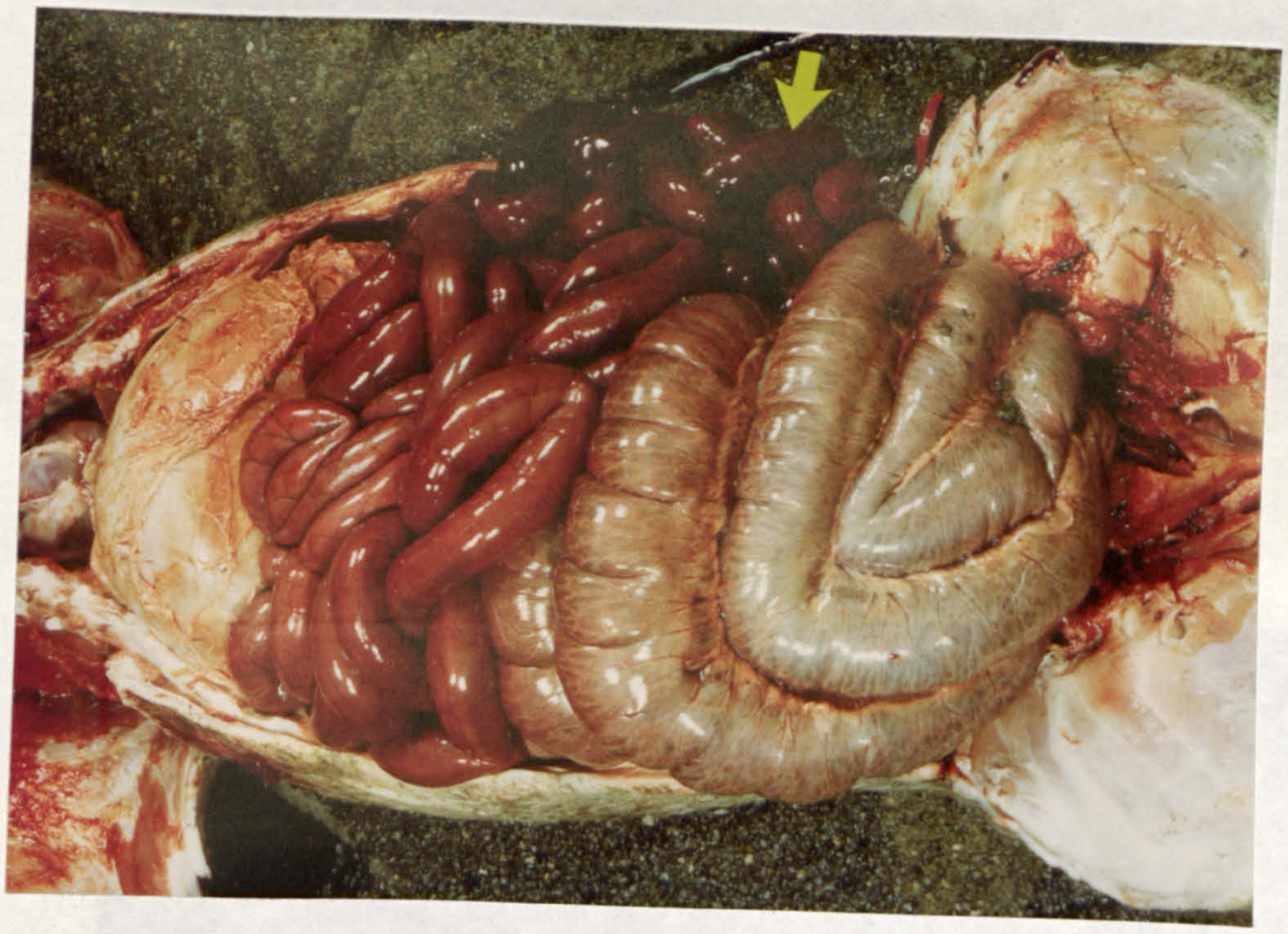


FIG. 20: Gross appearance of the intestines of Pig 96 with bloody gut.
Note the reddened small intestine (arrow).

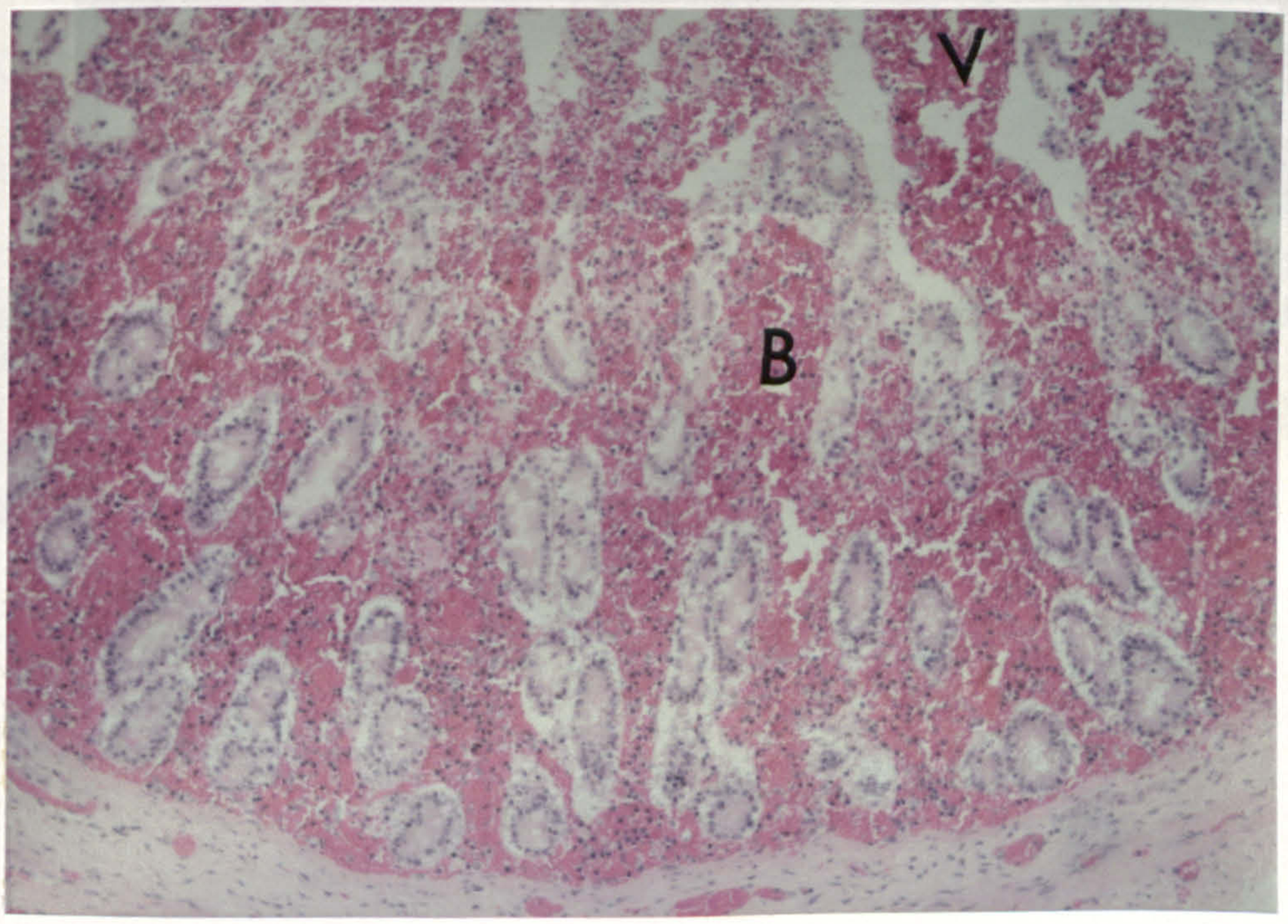


FIG. 21: Histological section of the jejunal mucosa of Pig 96 with bloody gut at a site from which C. perfringens Type A was isolated.
Note the congestion of the lamina propria with excess red blood cells (B) and the loss of villous architecture (V).
H & E x 40

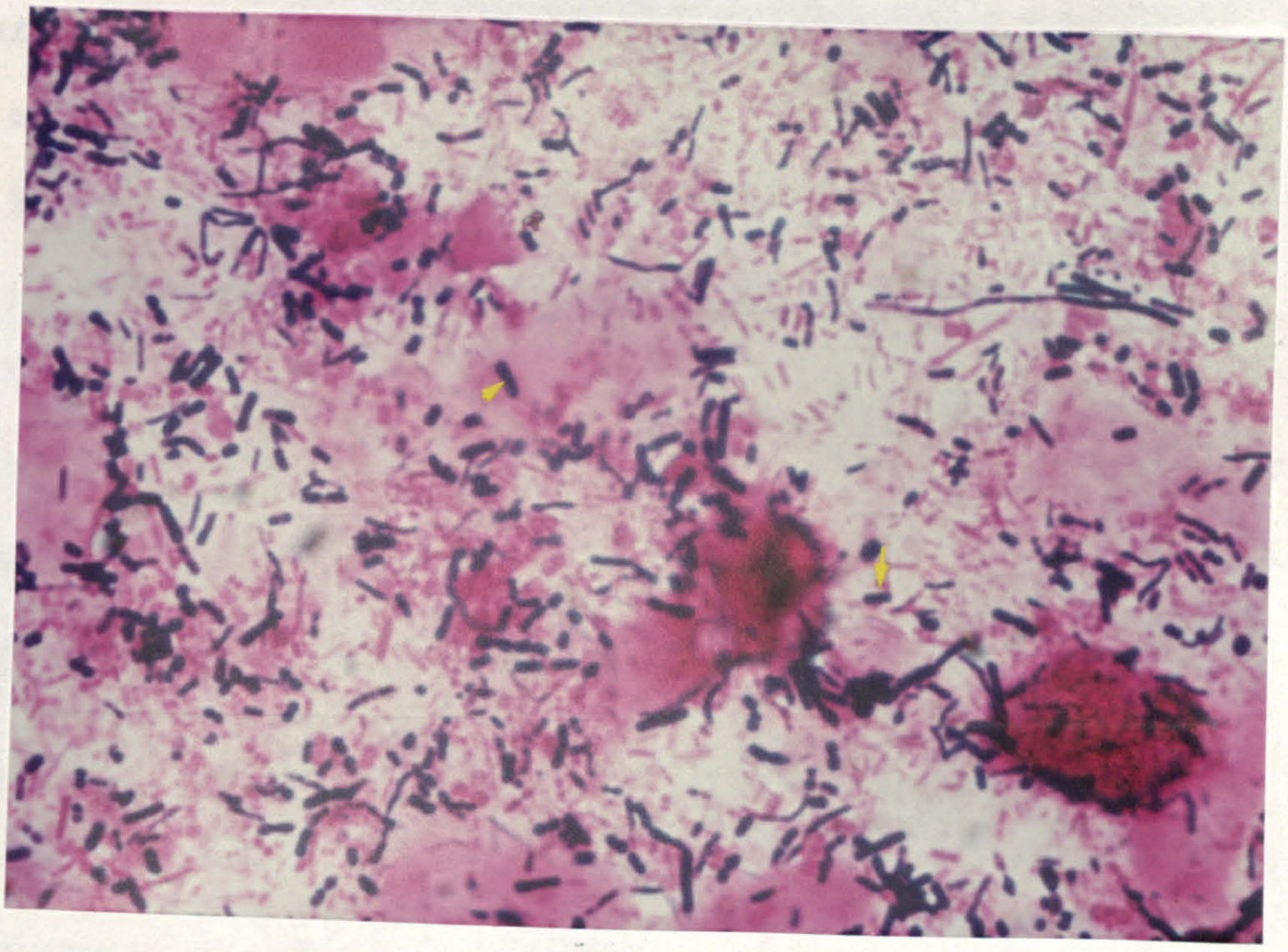


FIG. 22: Smear of the jejunal mucosa of Pig 96, with bloody gut at a site from which C. perfringens Type A was isolated.
Note the presence of large numbers of Gram-positive rods some of which (arrow) have the morphology of C. perfringens.
Gram x 1200.

non-haemolytic E. coli, C. coli amd faecal streptococci were also present.

Non-haemolytic C. perfringens

Non-haemolytic C. perfringens were isolated in small numbers from the gastrointestinal mucosa of 17 of the 129 pigs examined in the survey. These isolates differed in their cultural characteristics from C. perfringens Type A. No attempt was made to test the isolates for toxin production by mouse inoculation. Gram positive rods were seen along with other bacteria in smears made from sites from which these organisms were isolated. The locations from which they were isolated are shown in Table 6. Other bacteria were present at the sites from which non-haemolytic C. perfringens were isolated.

The organism was recovered from both normal and mildly inflamed mucosa; but there were no specific lesions which were common to the sites from which they were isolated.

C. sporogenes

C. sporogenes was isolated in small numbers from the gastro-intestinal mucosa of 3 of the 129 pigs examined (Table 6). Gram positive rods, but without characters of C. sporogenes were seen in smears made from sites from which they were isolated. Other bacteria were present at the site of isolation in all the 3 cases. The organism was isolated from mildly inflamed mucosa.

TABLE 6. Sites from which non-haemolytic Clostridia were isolated in cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	C. perfringens						
5	+	+	+	-	-	-	1,2,3,7,46
3	+	+	+	+	-	-	52,61,79
6	+	+	+	+	+	-	28,30,41,42,44, 59
3	-	+	+	+	+	+	
	C. sporogenes						
1	+	+	-	-	-	-	21
2	-	-	+	+	+	-	126,127
	Unidentified Clostridium spp.						
1	+	+	+	+	-	-	119
1	-	+	+	+	+	+	129

Unidentified Clostridium spp.

.These organisms were isolated in small numbers from the gastrointestinal mucosa of 2 of 129 pigs examined in the survey. They could not be identified because of their inconclusive biochemical reactions. Gram positive rods were seen with other bacteria in smears made from the sites from which the organisms were isolated. The organisms were isolated from normal mucosa in 119 and from inflamed mucosa in 129.

Treponema hyodysenteriae

Treponema hyodysenteriae was isolated from the large intestinal mucosa of 20 weaned pigs, with ages ranging from 6 weeks to 13 weeks. Two of these animals were field cases from Farm 2 (126 and 127). The remainder were animals from Farm 3 which had been experimentally infected with an isolate of T. hyodysenteriae.

T. hyodysenteriae was isolated as strongly B-haemolytic colonies with little or no surface growth on spectinomycin blood agar or as weakly haemolytic colonies which developed strong haemolysis on subculture. Its identity was confirmed as T. hyodysenteriae by specific immunofluorescence and pathogenicity testing in other laboratories.

Clinical signs varied from acute swine dysentery to complete clinical normality in the experimental animals many of which had been treated with drugs of varying efficacy. As the lesions of swine dysentery found were typical of that

TABLE 7. Sites from which T. hyodysenteriae and non T. hyodysenteriae spirochaetes were isolated in cases examined at post-mortem, and the relative abundance of colonies.

Number of cases	Site of isolation		Animal Number
	Caecum	Colon	
9	+	+	41,44,47,46,50 51,80,83,127
6	++	++	43,45,49,81,89, 85
5	+++	+++	48,78,79.87,126
Weakly haemolytic intestinal spirochaetes other than T. hyodysenteriae			
1	++	++	98

disease and were present only in the large intestine they will not be described further.

The other organisms isolated from the large intestinal lesions are shown in Appendix 1 under the case numbers given in Table 7. They included F. necrophorum, C. coli, L. catenaforme, B. vulgatus, B. fragilis, S. faecalis, C. sporogenes, C. perfringens, non haemolytic E. coli and S. epidermidis.

Non T. hyodysenteriae spirochaete

Weakly haemolytic spirochaetes were isolated on spectinomycin blood agar from one of the 129 pigs in this survey. It was isolated from an 8 week old pig from Farm 1 in moderate numbers and did not become strongly haemolytic on subculture. T. hyodysenteriae had never been isolated from Farm 1 in 10 years of monitoring and isolates of this type had never been identified as T. hyodysenteriae in a battery of tests. Non-haemolytic E. coli, C. coli and faecal streptococci were the only other organisms identified. The colonic mucosa was congested and the colon contents were fluid. The ileum of this animal was also inflamed and a diagnosis of uncomplicated spirochaetal diarrhoea could not be reached. It is possible that C. coli may have been the initiating agent of the small intestinal changes seen.

Campylobacter sputorum subspecies mucosalis

C. sputorum ss. mucosalis was isolated from 2 weaned pigs from Farm 7 but not from the samples from the other 70 animals from Farms 1, 3, 4 and 5 examined using selective

medium. The organism was isolated in moderate numbers from the terminal portion of the small intestine and in low numbers from the large intestinal mucosa. Both animals were weaners with a history of swine dysentery. At post mortem examination they were found to have gross lesions of proliferative intestinal adenopathy and lesions of swine dysentery in the large intestinal mucosa. Campylobacters could be seen in small numbers with other bacteria in the smears of the mucosal epithelium at all sites from which C. sputorum ss. mucosalis was isolated with other bacteria. Other bacteria isolated from the small intestinal sites included moderate numbers of C. coli, non-haemolytic E. coli, non-haemolytic C. perfringens and, from the large intestinal mucosa, T. hyodysenteriae and lactobacilli.

Streptococcus species

Streptococcus spp. were isolated from 30 piglets less than 3 weeks of age and from 66 pigs aged 3 weeks and above from Farms 1, 2, 3, 4, 5 and 7. The isolates were easily identified presumptively on routine media and their identity was confirmed as such by the methods described in Chapter 2. They were assigned to the following species:- S. faecalis (70); S. faecium (17) and S. suis (4). Isolates from 3 cases remained unidentified. Gram positive cocci with chains of different lengths characteristic of streptococci were seen in smears made from all the sites from which streptococci were isolated. The locations from which the streptococci were isolated and the species concerned are shown in Table 8.

TABLE 8. Sites from which Streptococcus spp. were isolated in 96 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation									Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Liver	Lung	Brain	
	S. faecalis									
10	+	+	-	-	-	-	-	N.D.	N.D.	27,28,33,34,61 62,68,98,99,102
7	+	+	+	-	-	-	-	N.D.	N.D.	1,91,96,100,103 106,112
6	+	+	+	+	-	-		N.D.	N.D.	12,20,110,118, 120,122
7	+	+	+	+	+	-		N.D.	N.D.	2,5,14,18,47,48, 51
12	+	+	+	+	+	+		N.D.	N.D.	16,23,37,39,42, 54,70,72,73,74, 75,123
10	-	+	+	+	+	+		N.D.	N.D.	4,43,44,45,46, 49,77,85,89,90
10	-	-	+	+	+	+		N.D.	N.D.	38,55,56,57,79, 80,83,86,87,125
8					+	+		N.D.	N.D.	6,11,13,17,21, 30,40,64
	S. faecium									
6	+	+	+	+	+	+		N.D.	N.D.	3,7,22,53,60,63
3	-	+	+	+	+	+		N.D.	N.D.	15,101,108
9	-	-	-	-	+	+		N.D.	N.D.	8,19,29,35,109,111, 117,121,127

TABLE 8 continued

Number of cases	Site of isolation									Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Liver	Lung	Brain	
	S. suis									
1	++	++	+	+	+	+	++	+	+++	58
1	+	+	+	+	+	+	+	+	+	59
2	++	++	+	-	+	+	+	+	+	92,93
	Unidentified streptococci									
1	+	+	+	-	-	-		N.D.	N.D.	67
2			+	+	+	+		N.D.	N.D.	50,107

N.D. = not done

Other bacteria were also present at the sites from which streptococci were isolated. General septicaemia and meningitis were present in all cases from which Str. suis was isolated. The mucosa of the intestinal tract was also congested. Other streptococci were isolated from both normal and congested mucosal surfaces.

Staphylococcus spp.

Staphylococcus spp. were isolated from 15 pigs of which 6 were older than 3 weeks of age. They were easily identified presumptively on routine media and their identity was confirmed as such by the methods described in Chapter 2. They were assigned to the following species - S. hyicus (1); S. epidermidis (13) and S. aureus (1). Gram positive cocci arranged in clumps characteristic of staphylococci were seen in smears made from all the sites from which staphylococci were isolated. The locations from which the staphylococci were isolated and the species concerned are shown in Table 9.

Other bacteria were also present at the sites from which staphylococci were isolated. Marked inflammatory lesions were present at the sites from which S. aureus was isolated. Both S. epidermidis and S. hyicus were isolated from both normal and mildly inflamed mucosa. S. hyicus was also isolated from the skin of the same animal.

Bacteroides spp.

Bacteroides spp. were isolated from 14 weaned and adult pigs from Farms 1, 3 and 7. The isolates were easily identified presumptively on routine media and their identity

TABLE 9. Sites from which Staphylococcus spp. were isolated in 15 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	S. epidermidis						
1	+	-	+	-	-	-	12
3	+	+	-	-	-	-	42,43,53
1	+	+	+				32
1	+	+	+	+	-	-	45
1	+	+	+	+	+	+	58
2	-	-	-	+	+	+	63,57
4	-	-	-	-	+	+	13,37,54,55
	S. aureus						
1	+	+	+	+	+	+	103
	S. hyicus						
1	+	+	+	-	- and skin	-	129

was confirmed as such by the methods described in Chapter 2. They were assigned to the following species :- B. fragilis (4); B. vulgatus (9) and B. melaninogenicus (1). Gram negative rods were seen in smears made from sites from which these organisms were isolated. The locations from which Bacteroides spp. were isolated and the species concerned are shown in Table 10.

Other bacteria were also present at the sites from which Bacteroides spp. were isolated. Inflammatory lesions were present at the sites from which all the 3 species of Bacteroides were isolated. These lesions varied from marked hyperaemia at sites from which B. fragilis was isolated to mild inflammation or localised areas of hyperaemia at sites from which B. vulgatus and B. melaninogenicus were isolated.

Fusobacterium species

Fusobacterium spp. were isolated from 2 piglets aged 1 and 3 weeks respectively from Farm 1; and from 8 pigs aged 12 weeks and above from Farm 3. The isolates were easily identified presumptively on horseblood agar plates incubated anaerobically and their identity was confirmed by the methods described in Chapter 2. Nine of the 10 isolates were assigned to the species Fusobacterium necrophorum and the remaining isolate resembled F. fusiformis in its biochemical reactions. Gram negative slender or filamentous rods with tapered ends with the characteristics of Fusobacterium spp. were seen with other bacteria in smears made from all the sites from which Fusobacterium spp. were isolated. The locations from which the Fusobacterium spp.

TABLE 10. Sites from which Bacteroides spp. were isolated in 14 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	B. fragilis						
1	-	-	-	-	++	++	43
1	-	-	-	-	+	++	45
1	-	-	-	-	+	+	110
1	-	-	-	-	++	+	118
	B. vulgatus						
3	-	-	-	-	++	++	41,44,50
1	-	-	-	-	+	++	46
1	-	-	-	-	++	+	51,126
3	-	-	-	-	+	+	56,73,81
	B. melaninogenicus						
1	-	-	-	-	+	+	98

were isolated are shown in Table 11.

In all cases, Fusobacterium spp. were isolated in small numbers and other bacteria were present at the sites of isolation (Appendix 1).

Pig 103 (3 weeks old from Farm 1) had peritonitis with necrotic enteritis. Similar lesions were present in sites from which Fusobacterium necrophorum was isolated and sites from which the organism was not isolated. Pig 108 (1 week old) had mildly inflamed colonic mucosa and Fusobacterium fusiformis was isolated only from the colon. Other pathogens were also present. The remaining 8 pigs were animals used in swine dysentery drug trial experiments. The lesions varied from normal to mildly inflamed mucosa and intestinal spirochaetes were always present.

Pasteurella spp.

Pasteurella spp. were isolated from 5 pigs aged 3 weeks and above from Farms 1, 3 and 4. The isolates were easily identified presumptively on routine media and their identity confirmed as such by the methods described in Chapter 2. They were assigned to the following species - P. haemolytica (1) and P. multocida (4). Gram negative coccobacilli resembling Pasteurella spp. were seen in smears made from all sites from which they were isolated. The locations from which Pasteurella spp. were isolated and the species concerned are shown in Table 12.

TABLE 11. Sites from which Fusobacterium spp. were isolated in 10 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	F. necrophorum						
4	-	-	-	-	+	++	42,57,60,78
2	-	-	-	-	++	+	47,87
1	-	-	-	-	+	+	103
2	-	-	-	-	++	++	55,73
	F. fusiformis						
1	-	-	-	-	+	+	108

TABLE 12. Sites from which Pasteurella spp. were isolated in 5 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation								Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Lungs	Liver	
	P. multocida								
1	+	+	-	-	-	-	++	-	11
1	+	+	+	-	-	-	++	-	20
1	++	+	+	-	-	-	++	-	57
1	-	+	+	-	-	-	++	-	107
	P. haemolytica								
1	++	++	++	++	++	++	++	++	101

Other bacteria were also present at the sites from which Pasteurella spp. were isolated. Marked inflammation was present at all sites from which P. haemolytica was isolated. There was reddening of the serosal intestinal surface of Pig 101 (Fig. 23) with petecchial haemorrhages of the mucosa, stunted villi, bleeding points and heavy congestion of the remainder of the mucosa (Fig. 24). Large numbers of organisms were recovered from all levels of the intestinal tract (Table 12).

Changes seen at sites from which P. multocida was isolated varied from normal to mildly inflamed mucosa.

Pseudomonas aeruginosa

P. aeruginosa was isolated from 6 pigs from farms 1 and 3. Two of the pigs were aged 2 and 3 weeks respectively, while the remaining four were aged 4½ weeks and above. The isolates were identified presumptively on routine media by their colonial morphology, pigment production and odour. Their identity was confirmed by the methods described in Chapter 2, and they were assigned to the species P. aeruginosa. Gram negative rods were seen with other bacteria in direct smears taken from sites from which P. aeruginosa were isolated. The locations from which P. aeruginosa were isolated are shown in Table 13.

Small numbers of P. aeruginosa were isolated in all cases. Other bacteria were present at the sites from which it was isolated. P. aeruginosa was isolated from both normal and mildly inflamed mucosa.

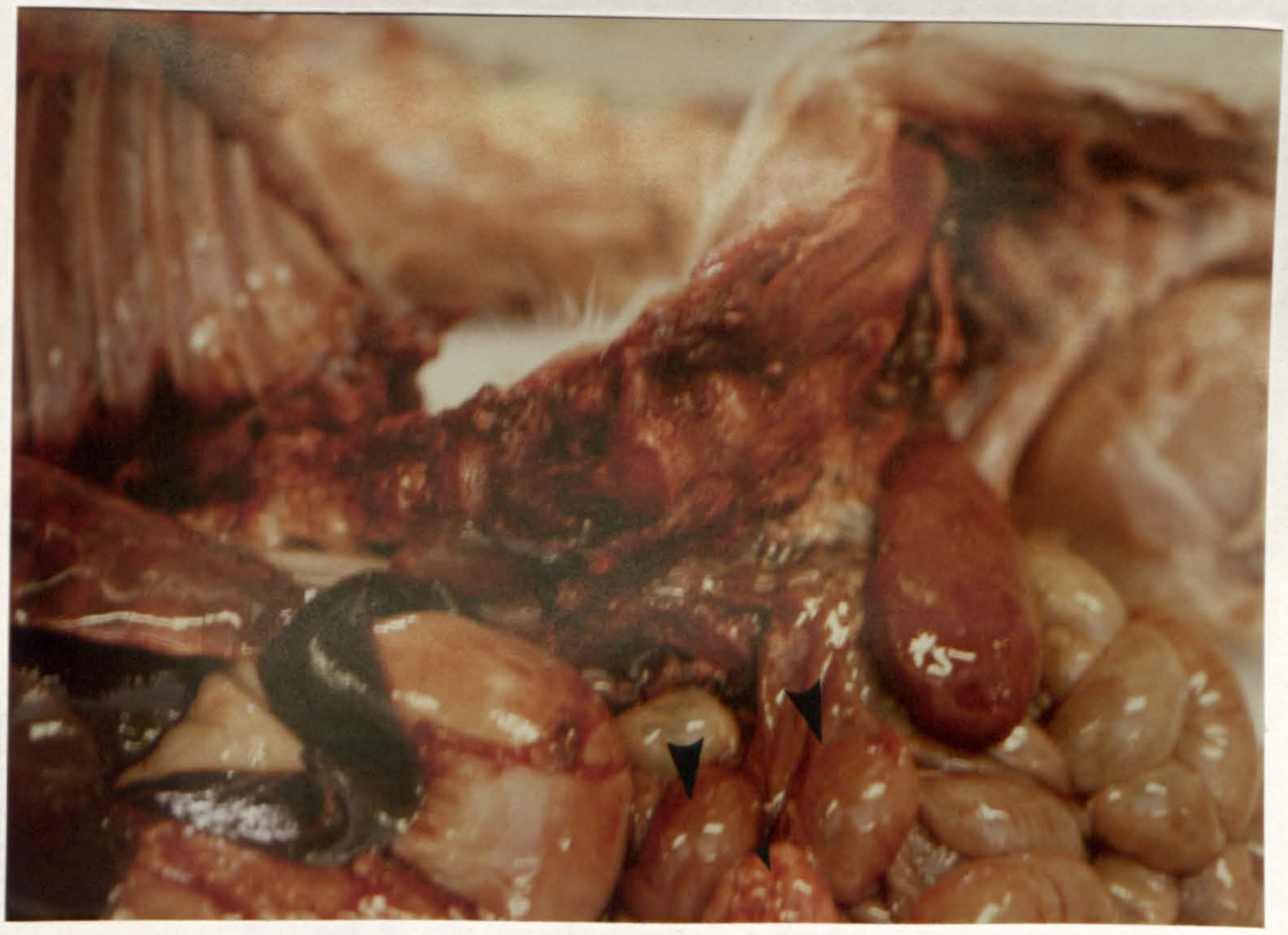


FIG. 23: Gross appearance of the abdominal viscera of Pig 101 from which P. haemolytica was isolated.
Note the congested intestines (arrow).

FIG. 24: Microscopic appearance of the villi of the small intestine of Pig 101 at a site from which P. haemolytica was isolated.
Note the haemorrhagic tips of the shortened villi (V).
x 110

TABLE 13. Sites from which Pseudomonas aeruginosa was isolated in cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
1	-	-	-	+	+	+	41
2	-	-	+	+	+	+	68,85
1	-	+	-	+	-	+	96
1	-	-	+	+	-	+	103
1	-	-	-	-	++	+	109

Corynebacterium species

Corynebacterium spp. were isolated from 8 pigs aged 3 weeks and above from Farms 1, 2, 3 and 4. They were not easily identified on routine media on primary culture as growth was always sparse after 24 hours; and after 48 hours the colonies resembled those of streptococci. The identity of the isolates were established and confirmed by the methods described in Chapter 2. All the 8 isolates were assigned to the species C. pyogenes.

Gram positive rods with the characteristics of Corynebacterium spp. were either not seen or present in very small numbers with other bacteria in direct smears taken from sites from which C. pyogenes was isolated.

The locations from which C. pyogenes was isolated and the frequency of isolation are shown in Table 14. Other bacteria were also present at the sites from which it was isolated. C. pyogenes was isolated from both normal and diseased mucosa.

Erysipelothrix rhusiopathiae

E. rhusiopathiae was isolated from one adult (pig 34) from Farm 2. Growth on routine media was sparse after 24 hours but was apparent after 48 hours. The isolate was identified and its identity confirmed by the methods described in Chapter 2. Gram positive rods with the characteristic of Erysipelothrix were seen along with other bacteria in smears taken from the sites from which E. rhusiopathiae was isolated. The organism was isolated

TABLE 14. Sites from which Corynebacterium pyogenes was isolated in 8 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation							Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Lungs	
1	+	+	-	-	-	-	++	34
3	+	+	-	+	-	-	+	42,101,103
3	++	+	+	-	-	-	++	57,58,60
1	+	-	+	-	-	-	+	68

in small numbers from the mucosa of the stomach, duodenum, jejunum, ileum, caecum, colon and liver of the animal.

Bloody gut with pericarditis and septicaemia was seen in the pig from which E. rhusiopathiae was isolated. The sites in the small and large intestine from which it was isolated were congested. Other organisms such as C. perfringens Type A were also isolated.

Proteus spp.

Proteus species were isolated from the small duodenal mucosa of two pigs (Nos. 38 and 56) aged two and three weeks respectively from Farm 1, and from the jejunal mucosa of PIg 56. They were easily identified presumptively on routine media and their identity was confirmed as such by the methods described in Chapter 2. They were assigned to the species P. mirabilis. Gram negative rods were seen in smears from the sites from which Proteus species were isolated. The organism was isolated mainly from the duodenum of the two cases. Other bacteria isolated from the sites from which Proteus species were isolated include, B-haemolytic E. coli; and S. faecalis in P56; and non-haemolytic E. coli in P38.

No lesions were present at the site from which P. mirabilis was isolated in P38; but the mucosa was mildly inflamed in P56.

Peptostreptococcus spp.

Peptostreptococci were isolated from 10 pigs aged 1 week old to adult from Farms 1, 2 and 3. They were not easily identified on routine media except that they were obligately anaerobic cocci. Their identification as peptostreptococci was established and confirmed by the methods described in Chapter 2. In all cases they were isolated in small or moderate numbers and in no case were they isolated in pure culture. It was difficult to assign the isolates to species because of the inconsistencies in their biochemical behaviour. However, 3 isolates were assigned to Peptostreptococcus intermedius, while the remaining 7 isolates remained unidentified.

Other organisms were isolated from the sites from which peptostreptococci were isolated. These sites are shown in Table 15.

No specific lesions could be attributed to Peptostreptococcus spp.. The appearance of the mucosal surfaces varied from normal to mildly or moderately inflamed; and was heavily congested in one weaned pig (P34).

TABLE 15. Sites from which Peptostreptococcus spp. were isolated in 10 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	P. intermedius						
1	-	-	+	+	+	-	125
2	-	+	-	+	++	+	29,34
	Unidentified Peptostreptococci						
1	-	+	+	-	+	-	19
2	-	+	+	++	+	-	22,23
3	-	+	+	++	++	+	20,36,77
	-	-	-	++	++	++	47

Bacillus spp.

Bacillus spp. were isolated from 18 animals from Farms 1, 3 and 4. Eight of these animals were less than 3 weeks of age, while the remaining 10 were 3 or more weeks old. The isolates were presumptively identified on routine media, and their identity confirmed by the methods described in Chapter 2. They were assigned to the following species:- B. licheniformis (10, B. mycoides (3) and B. cereus (1). The remaining 4 isolates remained unidentified. Gram positive large rods with the characteristic of Bacillus spp. were seen with other bacteria in smears made from all the sites from which the organisms were isolated. The locations from which Bacillus spp. were isolated and the frequency of isolation are shown in Table 16.

Other bacteria were also present at the sites from which Bacillus spp. were isolated.

The mucosal surface from which B. cereus was isolated was mildly inflamed. The lesions seen at the sites from which B. licheniformis was isolated varied from mild inflammation to marked congestion. The appearance of the mucosal surfaces from which other Bacillus spp. were isolated varied from normal to localised inflammation. They were isolated in the same frequency from both normal and congested mucosal surfaces.

Lactobacillus spp.

Lactobacillus spp. were isolated from 16 pigs from Farms 1, 2, 3 and 7. Four of the pigs were less than 3

TABLE 16 . Sites from which Bacillus species were isolated in 18 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	B. cereus						
1	-	-	-	+	++	+	58
	B. mycoides						
1	-	+	+	-	+	-	48
2	-	+	-	+	+		114,119
	B. licheniformis						
3	-	+	+	-	-	-	17,61,62
2	-	++	+	+	-	-	20,70
1	+	++	-	+	+	-	63
3	-	-	+	++	+	-	21,63,69
1	-	-	+	+	+	+	119
	Unidentified *Bacillus* spp.						
1	+	+	+	-	-	-	53
1	-	+	-	+	+	-	80
2	-		++	++	+	+	75,78

weeks of age, while the remaining 12 pigs were 3 weeks or over. The isolates were presumptively identified on microaerophilic and anaerobic plates and their identity confirmed by the methods described in Chapter 2. It was difficult to assign the isolates to species because of their erratic biochemical behaviour. Two isolates were assigned to the species L. fermentum and one was assigned to L. catenaforme. Others could not be identified to species. Gram positive rods were seen with other bacteria in smears from the sites from which lactobacilli were isolated.

Lactobacilli were isolated only in small numbers in all cases (Table 17) and at no time were they isolated in pure culture. Other bacteria were also present at the sites from which they were isolated.

No specific lesions could be attributed to Lactobacillus spp. as the mucosal changes seen varied from normal to mild inflammation. In the latter case, they were isolated along with other possible pathogens.

Torulopsis glabrata

T. glabrata was isolated in large numbers from the mucosa of all regions of the intestine of one weaned pig with rectal stricture, enteritis and pneumonia from Farm 3 (pig 125). The mucosal surfaces of the stomach, duodenum, jejunum and ileum were congested (Fig. 25) and the contents were yellowish. The large intestine was flaccid with soft yellowish contents and its mucosal surface was locally inflamed.

TABLE 17. Sites from which Lactobacillus spp. were isolated in 16 cases examined at post-mortem and the relative abundance of colonies.

Number of cases	Site of isolation						Animal Number
	Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	
	L. catenaforme						
1	-	-	-	+	+	+	126
	L. fermentum						
2	-	+	+	-	+	+	59,123
	Unidentified Lactobacilli						
1	-	-	-	+	+	-	34
4	-	+	-	+	+	+	41,47,82,84
5	-	+	+	-	+	-	50,51,64,74, 77
2	-	+	+	+	+	-	54,88
1	+	+	++	+	+	+	90

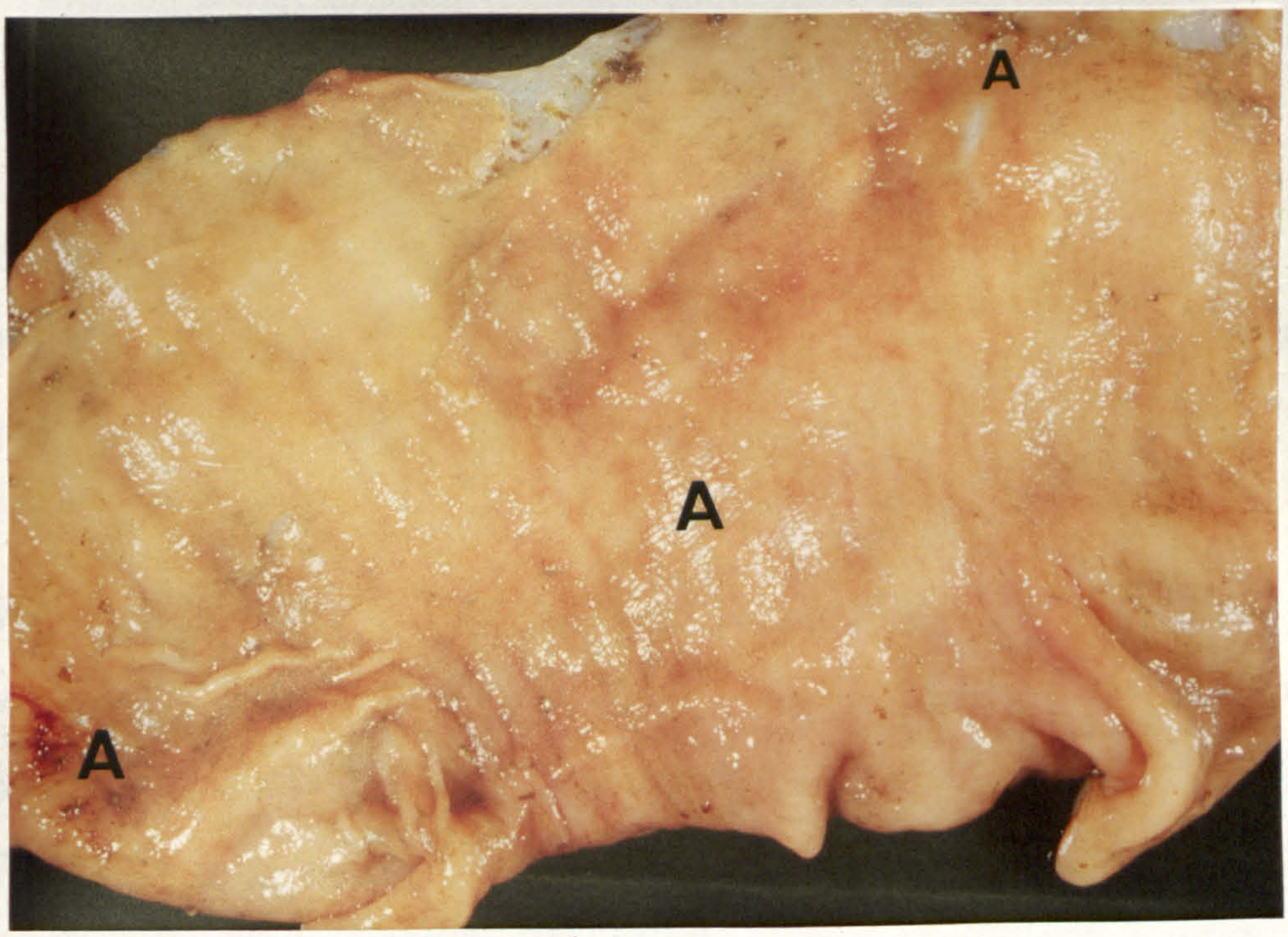


FIG. 25: Macroscopic appearance of the large intestinal mucosa of Pig 125 from which Torulopsis glabrata and C. coli were isolated.
Note the inflamed and granulomatous area (A).

Microscopic lesions were present throughout the length of the small intestine. The villi were stunted and inflammatory cells were present in the lamina propria (Fig. 26).

Other bacteria isolated along with the yeast included C. coli.

DISCUSSION

The examination of field cases showed that a wide variety of bacteria could be isolated from both normal mucosa and from lesions. More than one species of bacterium was often present in each site sampled. In addition, other agents such as coccidia, Cryptosporidium spp. or viruses were identified in the lesions or known to be present on the farms from which the animals came. In cases in which agents other than bacteria had not been demonstrated at the time of examination, it cannot be taken for granted that they are not contributory to the lesions seen as they might have been present at a time prior to the time of examination. The lack of freshly killed animals in this survey limited the value of histological examination.

These factors made interpretation of the results difficult. In some cases the bacteria isolated were known to cause enteric lesions in pigs e.g. B-haemolytic E. coli and T. hyodysenteriae, but others had been considered to be normal inhabitants of the alimentary tract e.g. streptococci and lactobacilli. However, some of the bacteria isolated e.g. C. perfringens Type A have been described in literature as causes of enteric disease in other species, and may, by

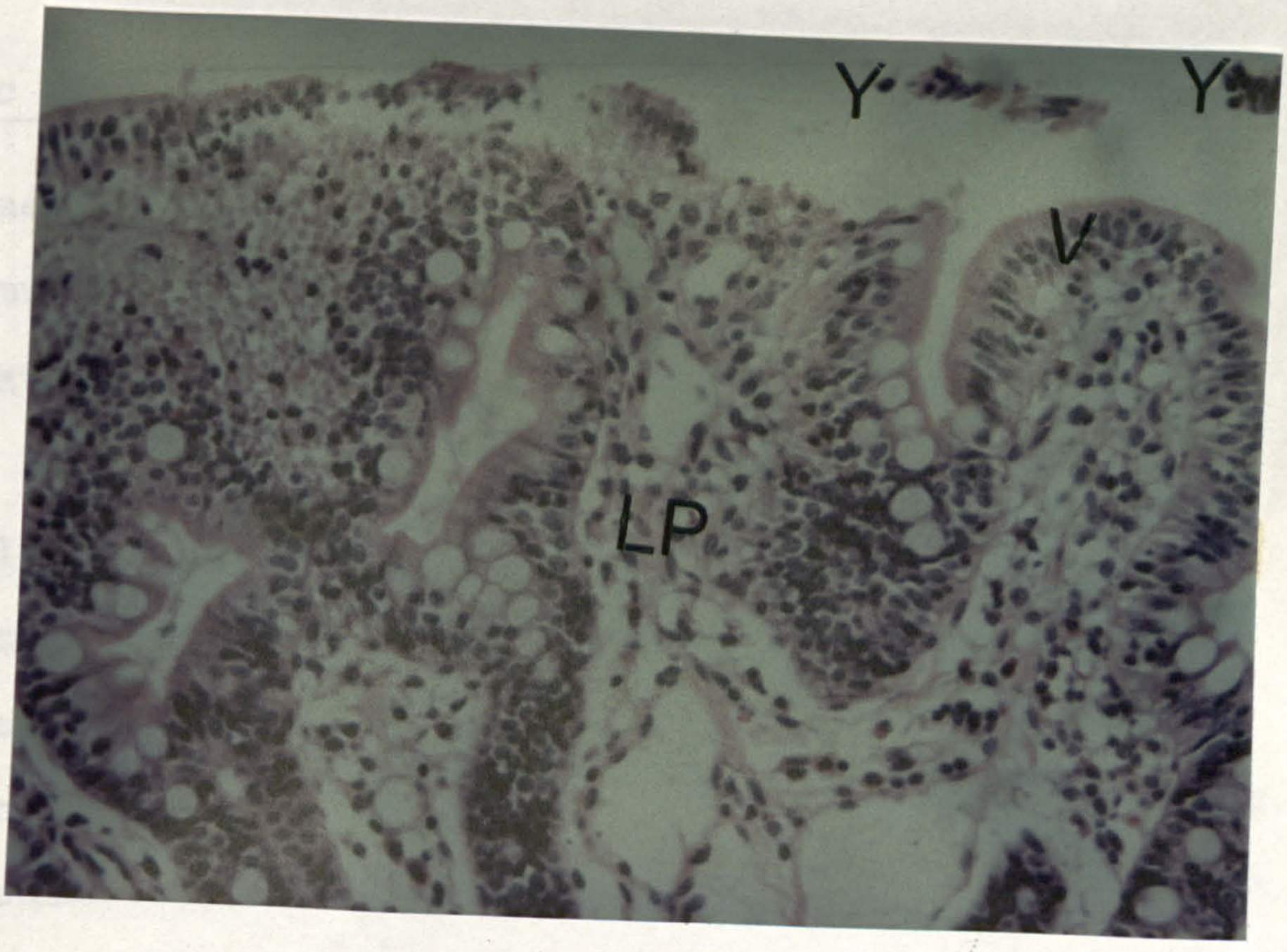


FIG. 26: Histological section of the jejunal mucosa of Pig 125, at a site from which T. glabrata and C. coli were isolated.
Note the stunted villi (V), and presence of inflammatory cells in the lamina propria (LP).
Cells which may be yeasts (Y) may be seen in the lumen.
H & E x 250

inference, be important in pigs. It was, however, possible to associate certain bacteria with particular types of lesions and the significance of these results is discussed below.

Detailed consideration of the significance of individual bacterial species

B-haemolytic *E. coli*

B-haemolytic *E. coli* were isolated from the intestinal mucosa of 25 of the 129 pigs examined. Fourteen of the isolates were obtained from piglets less than 3 weeks of age, and 11 were isolated from weaned pigs and adults. These animals were obtained from Farms 1, 2, 3, 4 and 6. B-haemolytic *E. coli* were isolated in significant numbers from the small intestine of 24 sucking and 13 weaned pigs. In the absence of any other major enteric pathogens at these sites, the animals were considered to have *E. coli* enteritis. The lesions seen at the sites of isolation were minimal and could be detected under the dissecting microscope in some of the cases. The histological changes seen were minimal in uncomplicated cases. Inflammatory reactions were prominent in 2 complicated cases with high degree of inflammatory reaction in the lamina propria and reduction of the height of the villi.

Non-haemolytic E. coli was isolated from the mucosa of 80 of the 129 animals examined. It was present only in small numbers in both the small and large intestines in all cases. The organism was isolated in the same frequency from both normal and abnormal mucosa and in the absence of serotyping

or pathogenic determination its significance in the lesions in which it was found cannot be further discussed.

Campylobacter coli

Campylobacter coli was isolated from both the small and large intestinal mucosa of 22 piglets less than 3 weeks of age and from 25 pigs aged 3 weeks and above. The isolation of C. coli in profuse culture from the small intestines of piglets as shown in Table 4 is of particular interest. Gram-negative curved rods or spirals were not seen in smears made from all sites from which Campylobacter coli was isolated. The isolation methods used may therefore have been a more sensitive indicator of the presence of Campylobacters than direct examination. All the piglets from which C. coli was isolated in profuse culture had enteritis, with mild to moderate degrees of inflammation on the mucosal surface of both the small and the large intestine. The thickening of the wall of the terminal ileum and the enlargement of the mesenteric lymph nodes appeared to be common to all cases. Stunted villi and histological evidence of inflammatory change such as lymphoid hyperplasia, and the accumulation of inflammatory cells in the dilated crypts were common (Figs. 9 and 10). Fusion of the villi was present in the ileum, there was dilatation of the lacteals, and cellular infiltration of the lamina propria. Mitosis were commonly seen in the crypts.

C. coli was also isolated from the large intestinal mucosal of all the infected pigs. Some of them had colonic lesions either of generalised mild inflammation or localised

hyperaemia of the mucosa (Pigs 36, 120 124, 125).

Few specific histological changes were seen other than, perhaps, dilatation of the crypts and the presence of organisms within them (Figs. 11 and 12). In some cases there was dilatation of capillaries and inflammatory cells were seen in the lamina propria.

These results indicated that C. coli is common in enteric lesions in both the small and large intestines of pigs. These findings agree with the report of Deas (1960) who demonstrated C. coli in the terminal ileum of weaned pigs with diarrhoea. The absence of T. hyodysenteriae or other spirochaetes from cases of mucoid diarrhoea observed in some cases from which C. coli were recorded in profuse culture, and the presence of changes of mild colitis are of interest. These findings may suggest that previous authors (Doyle, 1944; Roberts, 1956a,b) may have been correct in attributing to C. coli (V. coli) a causal role in large intestinal disease in pigs.

Clostridium perfringens Type A

C. perfringens Type A was isolated from the gastrointestinal mucosa of 12 suckling pigs of less than 3 weeks of age and from 11 weaned pigs and adults from Farms 1, 2, 3 and 4. None of the haemolytic isolates of C. perfringens obtained in this survey produced toxins lethal for mice under the conditions recommended for strain identification by the Anaerobe Laboratory Manual. All produced large quantities of lecithinase and were therefore identified as C. perfringens Type A. The test used (mouse

inoculation) would not have detected the production of enterotoxins by these strains. In spite of the fact that other bacteria were isolated along with C. perfringens Type A, certain features were noted in all of the 10 cases in which the organism was isolated in profuse to moderate amounts. The majority of the animals had diarrhoea and three pigs had bloody gut. The small intestinal mucosa was congested to a variable extent. There was sloughing of villi or reduction in the height of the villi, particularly in the jejunum and ileum. Cell shedding and cellular accumulation were present in the small intestine lumenal surface of 3 cases with bloody gut. The changes were milder in the large intestinal mucosa. There have been a number of reports of the isolation of C. perfringens Type A from intestinal lesions of pigs (Amtsberg et al., 1976), but the role of the organism in porcine enteric lesions remains a matter for speculation (Moon and Dillman, 1972). These results however suggest that C. perfringens Type A can be found in diarrhoeic syndromes in sucking piglets in particular. It may however be difficult to assess the significance of the isolates because of the number of other agents which were present at the same sites in clinical and post mortem material.

Non-haemolytic C. perfringens

Non-haemolytic C. perfringens was isolated in small numbers from gastrointestinal mucosa of 17 pigs. The organism was isolated from both sucking and weaned pigs. It was also isolated in the same number from both normal and

slightly inflamed mucosa. Smith and Jones (1963) recorded the isolation of non-haemolytic C. perfringens from both normal and diseased pigs. In the absence of any test of toxin production of these isolates, it may be difficult to assess the importance of their presence in any enteric lesions seen.

C. sporogenes

No specific lesions could be attributed to the isolation of C. sporogenes from the 3 cases in this survey as they were present in both normal and abnormal mucosa. Its role in enteric lesions is not clear.

Unidentified Clostridium species were isolated in small numbers from both normal and abnormal mucosa. Their role in the lesions seen at the sites from which they were isolated in Pig 129 was unclear. Other bacteria were also present at the site from which they were isolated.

Treponema hyodysenteriae

T. hyodysenteriae was isolated from the large intestinal mucosa of 20 pigs from the survey. Two of these pigs were obtained from Farm 7 with a history of swine dysentery in the herd. The remaining 18 animals were from Farm 3, and the animals had been involved in experimental drug trials for swine dysentery. The lesions found in the sites from which T. hyodysenteriae was isolated were typical of the disease. It is well established that T. hyodysenteriae is the initiating agent of swine dysentery (Taylor and Alexander, 1971). Other bacteria isolated

along with T. hyodysenteriae such as B. vulgatus and F. necrophorum were shown to contribute to the typical lesions of swine dysentery by Harris et al., (1978). The significance of T. hyodysenteriae in the aetiology of the lesions in which they were found is beyond doubt but the exact role of the other organisms found is not yet clear.

Non-T. hyodysenteriae spirochaete

Weakly haemolytic spirochaetes were isolated from the large intestinal mucosa of one of the 129 pigs in the survey. The animal was 8 weeks old and was obtained from Farm 1. The large intestinal mucosa of the animal was congested and the contents were fluid. The terminal ileal mucosa was also congested. Other bacteria such as C. coli were isolated from both the congested terminal ileum and large intestine. These other bacteria may be the initiating agent for some of the lesions seen particularly in the ileum. There is however an account in the literature which suggests that weakly haemolytic intestinal spirochaetes other than T. hyodysenteriae may initiate diarrhoea and some colitis in infected pigs (Taylor et al., 1980). It can be assumed that the presence of these spirochaetes in the large intestinal lesions may be of considerable importance in this case.

Campylobacter sputorum subspecies mucosalis

C. sputorum ss. mucosalis was isolated from the gastrointestinal mucosa of 2 weaned pigs out of 72 pigs examined specifically for this organism on the selective

medium described by Lawson and Rowland (1974). The organism was isolated from the terminal portion of the ileum and the caecum. Lesions of proliferative intestinal adenopathy were present at the sites from which the organism was isolated. These lesions were similar to those described in the literature (Rowland and Lawson, 1981). C. coli, non-haemolytic E. coli, non-haemolytic Clostridium spp. and L. catenaforme were also present in the small intestinal lesions and may have contributed to the picture seen.

Other bacteria such as T. hyodysenteriae and B. vulgatus were isolated from both the caecum and colon. Lesions of swine dysentery were also present in the large intestine. There is no doubt that these bacteria complicated the disease. This was evident from the complex nature of the lesions. In spite of all these facts, the presence of C. sputorum ss. mucosalis in the lesions was considered significant.

Streptococcus suis

S. suis was isolated from the gastro-intestinal mucosa of 4 pigs aged between 13 to 21 days from Farms 2 and 4. Lesions were present at the site at which the organism was isolated. It may be that it contributed to the lesions seen which consisted mainly of localised areas of hyperaemia. Septicaemia and meningitis were also present in these animals. Other bacteria were also present at the sites from which the organism was isolated. It is therefore difficult to assess the significance of the isolations in the enteric lesions seen but it is probably slight.

Streptococcus faecalis

S. faecalis was isolated from the gastrointestinal mucosa of 70 pigs in the survey from all the 8 farms. The animals ranged in age from one day to adult. The organism was isolated from both normal and diseased gastrointestinal mucosa. Other bacteria were also present at the sites from which the organism was isolated. Pesti (1962) and Smith and Jones (1963) recorded the presence of streptococci in both healthy and diseased pigs. The presence of the isolates is difficult to relate to the lesions which were present in some of the animals.

Streptococcus faecium

This organism was isolated from the gastrointestinal mucosa of 17 pigs of all age groups from all the 8 farms in this survey. The organism was isolated in the same frequency in both normal and abnormal mucosa. It was isolated along with other bacteria in all cases. The presence of the organism could not be related to any specific lesions.

Other streptococci

Unidentified Streptococci were isolated from 5 animals in this survey. These were isolated along with other bacteria. No specific lesions were noted at the sites of isolation.

Staphylococcus aureus

Staphylococcus aureus was isolated from one piglet aged 3 weeks from Farm 4. The piglet had a history of nervous disorder and respiratory distress. There were enteric lesions at the sites of isolation, but other bacteria were also present. It is possible that its presence in lesions represent opportunist invasion or is connected with other infections such as respiratory tract infection which may have formed the source of the organism.

Staphylococcus epidermidis

The organism is not usually considered to be pathogenic. It was isolated in small numbers from the intestinal mucosa of 13 pigs in the survey. The organism was isolated from both normal and abnormal mucosa and was also isolated along with other bacteria. It is commonly found in the faeces of pigs and its significance in these lesions is not clear but is likely to be slight.

Staphylococcus hyicus

Staphylococcus hyicus was isolated from one piglet aged 3 weeks with a clinical history of exudative epidermitis. The lesions at the sites of isolation in the gastrointestinal mucosa were mild. It is possible that the skin infection was the source of the organism. The significance of the presence of the organism in enteric lesions is not clear but is likely to be slight.

Bacteroides species

Bacteroides spp. were in no case isolated in pure culture. B. fragilis was isolated from 4 cases, B. vulgatus from 9 cases and B. melaninogenicus from one case. They were isolated from the large intestinal mucosa and varying degrees of enteric lesions were present at the sites. In all cases from which B. vulgatus and B. melaninogenicus were isolated, inflammatory change was present. Other bacteria were also present at the sites from which they were isolated. It is known that Bacteroides spp. are contributory to the lesions seen in swine dysentery (Whipp et al., 1979, 1980). Even though these species may not initiate the disease (Meyer et al., 1975) they are probably involved in producing the large intestinal lesions in weaned pigs. Their presence in the lesions is therefore of interest.

Fusobacterium species

Fusobacterium spp. were isolated in small numbers from 10 of the 129 animals in this survey. They were isolated from gastrointestinal mucosa along with other bacteria. F. necrophorum was isolated from Pig 103 aged 3 weeks with necrotic enteritis. Other bacteria such as C. perfringens Type A, C. coli and E. coli were also isolated. The remaining 8 isolates of F. necrophorum were obtained from sites with varying degrees of congestion and along with other bacteria, including spirochaetes. It has already been noted (Chapter 1) that F. necrophorum is widely distributed and normally occurs as a commensal in a variety of animal species such as pigs (Alexander et al., 1976).

It has however been recorded to aid intestinal spirochaetes in establishing typical lesions of swine dysentery (Whipp et al., 1979, 1980). The presence of this organism in enteric lesions is of uncertain significance as it has been shown to cause no changes in gnotobiotic pigs (Meyer et al., 1975) although it is capable of causing necrosis at any site in which it is found in conventional diseased pigs. Fusobacterium fusiformis was isolated from one pig (108). The presence of this organism was also associated with enteric lesions.

Pasteurella species

Pasteurella multocida was isolated in small numbers from the mucosa of anterior gastrointestinal tract of five pigs with pneumonia. The organism was also recovered from the pneumonic lungs of these animals. This finding may indicate colonisation of enteric lesions by respiratory tract pathogens passing through the gut. P. aerogenes has most frequently been isolated from the faeces of pigs (Carter, 1979) but it is of interest to note that none of the isolates in this survey was identified as such.

P. haemolytica was isolated from the gastrointestinal mucosa of one pig (101) with septicaemia and pneumonia. Enteric lesions were present at the sites of isolation of this organism. It is probable that the organism originated from a source other than the gastrointestinal tract and became established in the enteric lesions when passing through the gut.

Pseudomonas aeruginosa

P. aeruginosa was isolated in small numbers from the mucosa of the gastrointestinal tract of 6 out of 129 animals examined in this survey. It is possible that the Pseudomonas isolated in the survey was involved in the lesions seen although other bacteria were present in each case (see Appendix 1).

Corynebacterium pyogenes

C. pyogenes was isolated from the mucosa of the anterior part of the gastrointestinal tract of 8 out of the 129 animals in this survey. All the pigs from which the organism was isolated had pneumonia and this organism is frequently found in pneumonic lesions. Its significance in the initiation and maintenance of enteric lesions in the pig is unknown. It is therefore difficult to assess its role in the lesions present at the sites of isolation.

Erysipelothrix rhusiopathiae

E. rhusiopathiae was isolated from the mucosa of the whole of the gastrointestinal tract of an adult pig with septicaemia and bloody gut. Even though the organism is regarded as a pathogen of pigs, it is not regarded as initiating agent for any enteric disease. It is therefore difficult to relate the isolation to the lesions seen at the sites and its presence may merely indicate septicaemia.

Bacillus species

Bacillus spp. were isolated from 18 of the 129 pigs in this survey. They were assigned to B. licheniformis (10); B. mycoides (3) and B. cereus. The remaining 4 isolates remained unidentified. They were recovered from both normal and abnormal mucosa. B. cereus was isolated only from inflamed mucosa. There are reports of isolation of Bacillus spp. in pigs' faeces but no accounts of their role in enteric lesions. It is therefore difficult to relate their presence to any particular lesions.

Lactobacilli

Lactobacillus spp. were isolated from the gastrointestinal mucosa of 16 of the 129 pigs in this survey. Alexander et al. (1976) reported the isolation of lactobacilli from the colonic mucosa of healthy weaned pigs. Two of the isolates were assigned to the species L. fermentum and one to L. catenaforme and others remained unidentified. No specific lesions could be attributed to the presence of lactobacilli in the pig's intestine. Their role in the lesions in which they were present is unclear.

Proteus species

P. mirabilis was isolated from 2 of the 129 pigs in this survey. The organism was isolated in both cases along with other bacteria. Proteus spp. are widely distributed and may often contaminate clinical materials. It is not clear how relevant is the isolation of P. mirabilis in relation to the enteric lesions seen.

Peptostreptococci

.Peptostreptococci were isolated from the gastrointestinal tract of 10 pigs in this survey. Both P. intermedium (3 isolates) and unidentified Peptostreptococci (7) were recovered in the same amount from both normal and abnormal mucosal surfaces. The presence of Peptostreptococci in both healthy and diseased pigs has been recorded by Horvath et al. (1958), but no attempt has been made to relate their presence to any specific enteric lesion. Their role, if any, in initiating the lesions seen is therefore unclear.

Significance of the findings and further investigations

C. coli and C. perfringens Type A were the 2 bacteria most frequently isolated from enteric lesions in this survey. Since their role in porcine enteric lesions remains unexplained, they were selected for further studies which are described in Chapters 4 and 5 respectively.

CHAPTER 4

SECTION 1

Experimental infection with Campylobacter coli

INTRODUCTION

C. coli was isolated from animals in the survey described in Chapter 3. It was isolated both from the inflamed mucosa of the small intestines of piglets and from the inflamed large intestinal mucosa of the same pigs. It was also isolated from the large intestinal mucosa of pigs with colitis without any evidence of T. hyodysenteriae infection.

These findings and accounts in the literature of the production of diarrhoea or dysentery following the experimental inoculation of pigs with pure cultures of the organism (Doyle 1944, Roberts 1956a,b) suggest that C. coli could be capable of initiating enteritis in pigs.

In order to assess the importance of C. coli as a pathogen of the pig, pure cultures were fed to pigs in controlled experiments. They were given to milk-fed, hysterectomy derived, colostrum-deprived (HDCD) piglets, to conventional sucking piglets, to weaned HDCD pigs and to weaned conventional pigs. In addition to these experimental studies the distribution of infection was determined in the pig herd which formed the source of the experimental animals and the mechanism of infection was examined.

EXPERIMENT 1

OBJECTIVE: To determine the pathogenicity of an isolate of C. coli for hysterectomy-derived, colostrum-deprived (HDCD) piglets.

MATERIALS AND METHODS

The HDCD piglets were produced by the methods described in Chapter 2. Ten piglets were reared of which 6 were used in this study. One animal, P4, had congenitally contracted tendons. In this study, the conditions of housing and feeding were those described in Chapter 2. Temperature control in the room housing the experimental pigs failed between days 4 and 5 of the piglets' lives and this change is reflected in the rectal temperatures shown in Fig. 29 below. The ambient temperature was 25°C during this period. No antimicrobials were administered during this experiment.

The piglets were housed in individual isolators as described in Chapter 2. Infected animals were housed in separate blocks of cages from the controls. Animals P1, P2 and P3 were infected while P4, P5 and P6 acted as uninfected controls. Prior to inoculation, rectal swabs were taken daily from each animal and examined for the presence of B-haemolytic E. coli, campylobacters and Clostridium perfringens using the methods described in Chapter 2. Faeces samples were examined for the presence of coccidial oocysts and nematode eggs by the flotation method described in Chapter 2.

The infected group was inoculated on the 4th day of life with an isolate of C. coli obtained from the enteric lesions in the small intestine of a 7-day old piglet (No.36) which had died from diarrhoea. It had been cloned twice and was stored freeze-dried by the method described in Chapter 2. It was identified as C. coli by the criteria described in Chapter 2. Cultures for inoculation were prepared after one passage from this freeze-dried source as described in Chapter 2 and inoculated orally by the method described. 10ml of inoculum containing 2.1×10^{10} organisms was given.

The observations made are described in Chapter 2. Whole rectal faeces samples from infected piglets P1 and P2 were negatively stained and examined for the presence of viral particles by electron microscopy on day 3 post inoculation.

Clotted blood samples were taken at post mortem from all the piglets but none were taken at the beginning of the experiment. The serum was stored according to the methods described in Chapter 2. The serum samples were tested for agglutinating antibody to the inocular strain by the method described in Chapter 2.

The period of observation lasted 11 days and the piglets were killed on the 12th day post-inoculation with the exception of piglet P4 which was killed on day 4 post inoculation on humane grounds because of its congenital condition. The animals were killed by intracardiac barbiturate injection and examined post mortem.

Post mortem examination, histological and bacteriological examinations were carried out on all 6 piglets by the methods described in Chapter 2.

RESULTS

Clinical findings

All 6 piglets remained clinically normal during the period prior to inoculation with the exception of piglet P4 described above. Their faeces was normal and no campylobacters, C. perfringens Type A, or B-haemolytic E. coli were isolated. No parasites were demonstrated in their faeces. Faecal cultures revealed the presence of non haemolytic E. coli and a non haemolytic clostridium which could not be identified to species.

Changes in faecal consistency were noted on the 2nd day following inoculation in 2 of the 3 inoculated animals. The 3rd inoculated piglet developed diarrhoea on the 3rd day following inoculation. The faeces were very soft and yellowish in colour (Fig.27). Obvious clear mucus was present in the soft faeces of all 3 inoculated piglets as from the 3rd day post-infection (Fig.28). The faeces remained soft for the remainder of the period of observation. The changes in the faecal consistency of the piglets were summarised in Table 18. Traces of blood were only seen on one occasion (Pig P2, day 11 post infection).

The inoculated piglets appeared depressed on the 3rd day post infection and remained so for the duration of the experiment. The flanks of the infected piglets became

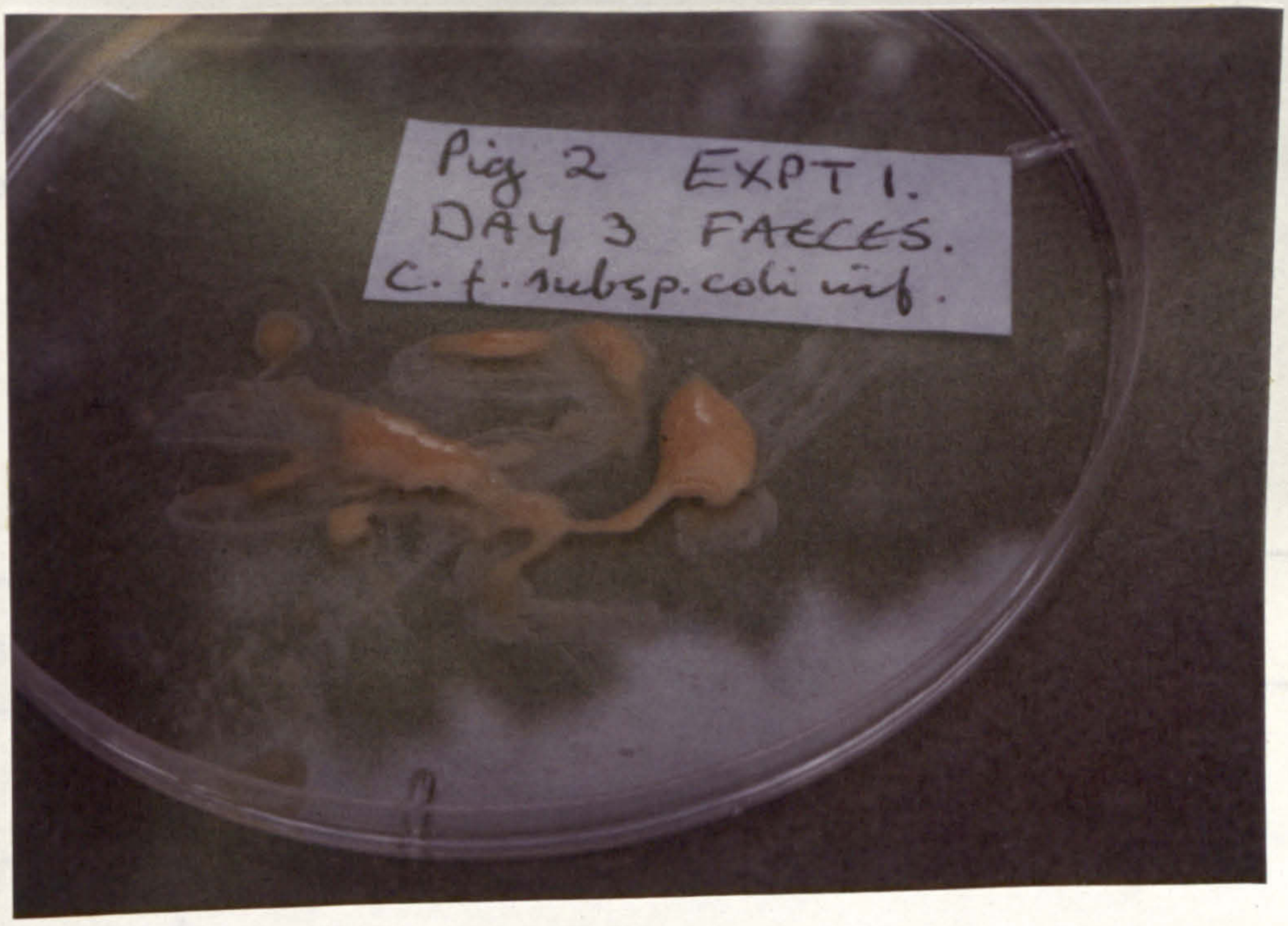


FIG. 27: Faeces of experimentally infected HDCD Piglet 2, 3 days post inoculation with C. coli.
Note the soft and mucoid nature of the faeces and its colour.

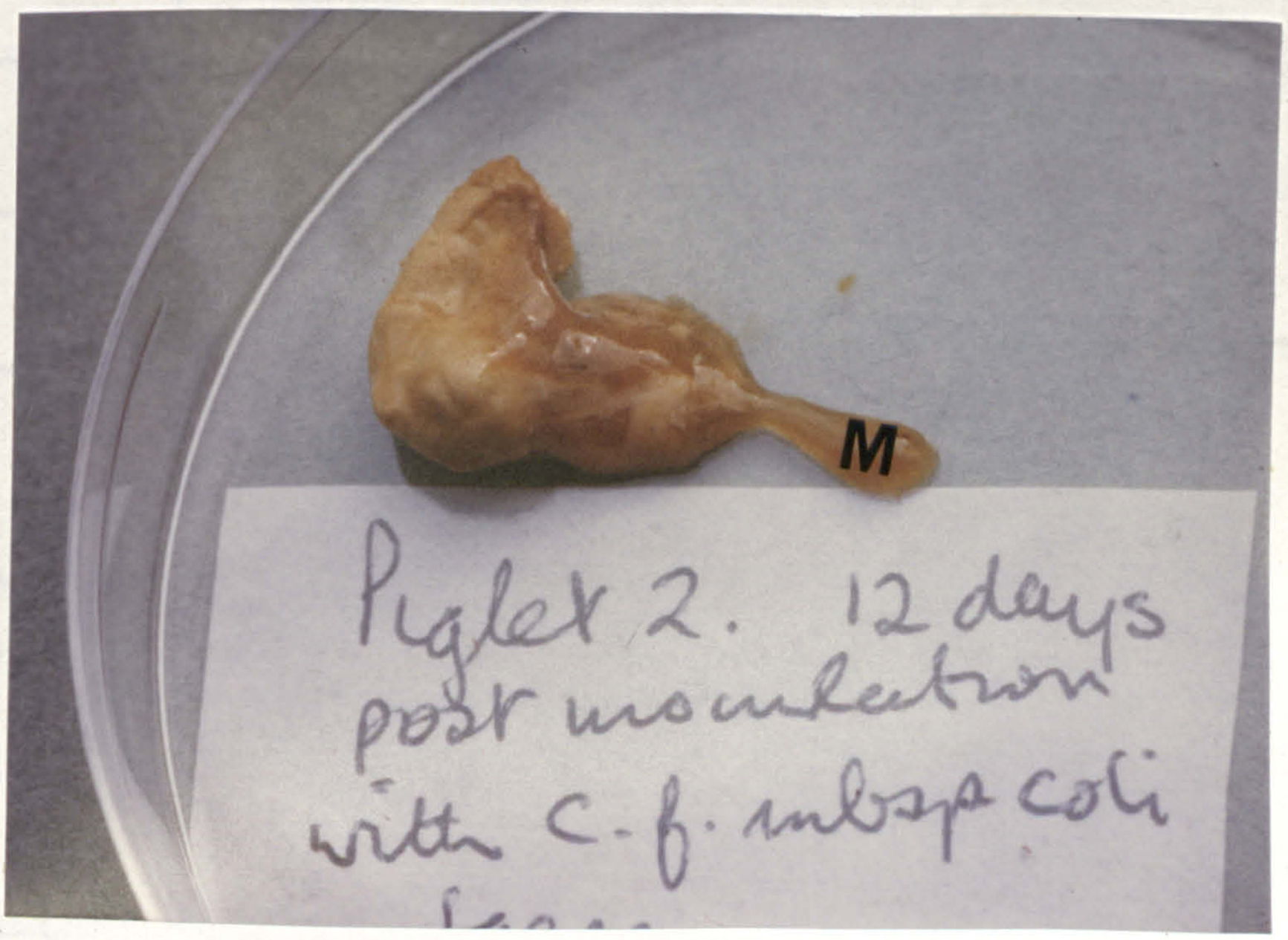


FIG. 28: Faeces passed by HDCD Pig 2, 12 days post inoculation with C. coli.
Note the tag of mucus (M).

TABLE 18

Changes in faecal consistency in hysterectomy derived, colostrum deprived piglets following infection with pure cultures of C. coli and isolation of the organism.

Pig No.	Infected	Day of Experiment 0	1	2	3	4	5	6	7	8	9	10	11	12
P1	+	N -	N +	N +	DM +	DM +	DM +	DM +	D +	D +	D +	D +	D +	K +
P2	+	N -	N -	D +	DM +	DM +	DM +	DM +	D +	D +	D +	D +	DB +	K
P3	+	N -	N -	D +	DM +	DM +	DM +	DM +	D +	D +	D +	D +	D +	K
P4	-	N -	N -	N -	N -	K								
P5	-	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	K
P6		N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	K

N = Normal faeces

D = Diarrhoea/soft faeces

M = Presence of mucus in faeces

B = Presence of blood in faeces

K = Killed

\+ = C.coli isolated from faeces

\- = C.coli not isolated from faeces

hollow, their coats hairy, their eyes sunken and their bodily condition deteriorated. Soiling of the perineum occurred in all the 3 inoculated piglets. These changes were not observed in the uninoculated controls.

Inoculation of the HDCD piglets was followed by a rise in rectal temperature to 41.1°C within 3 days and the high rectal temperatures were maintained for the remainder of the period of observation. The changes in rectal temperatures are shown in Fig.29.

Faecal cultures

C. coli was isolated from the faeces of one inoculated piglet (P1) 24 hours post inoculation and daily from the faeces of all inoculated piglets from day 2 post inoculation to the end of the experiment (Table 18). The organism was not isolated from the faeces of the inoculated animals prior to infection and was never isolated from the faeces of the control animals.

Non-haemolytic E. coli and the non-haemolytic Clostridium spp. were isolated from the faeces of both infected and control groups.

Salmonellae and B-haemolytic E. coli were not isolated from any of the animals. No nematode eggs or coccidial oocysts were demonstrated in the faeces of the animals in this experiment.

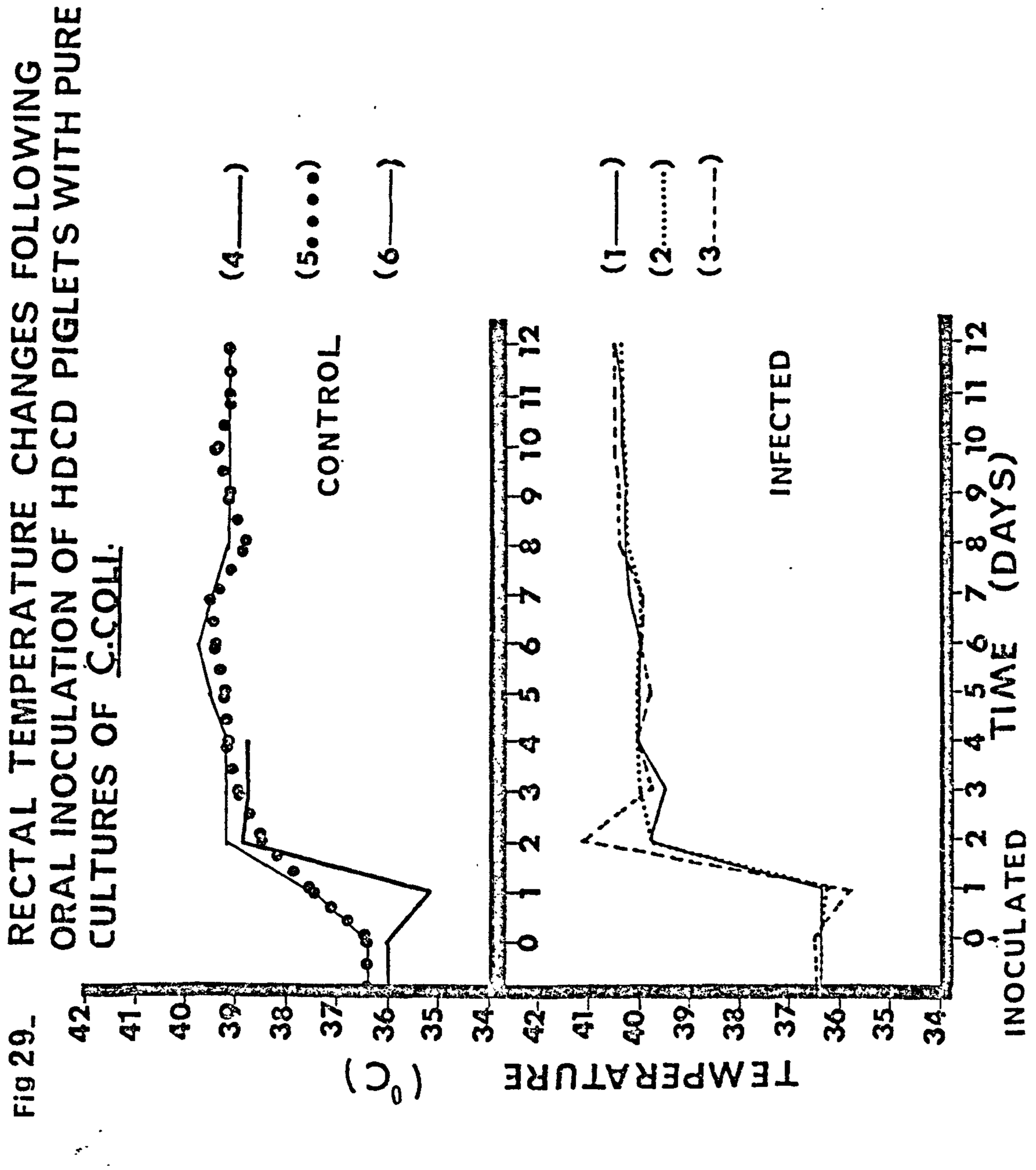

RECTAL TEMPERATURE CHANGES FOLLOWING
ORAL INOCULATION OF HDCD PIGLETS WITH PU
CULTURES OF C.COLI.
(4——)
(5• • •)
(6——)
CONTROL
(1——)
(2.....)
(3- - -)
INFECTED
TEMPERATURE (°C)
42
41
40
39
38
37
36
35
34
0 1 2 3 4 5 6 7 8 9 10 11 12
INOCULATED TIME (DAYS)

Fig 29.

No virus particles were seen in negatively stained preparations from the faeces of pigs P1 and P2 when viewed by electron microscopy on day 3.

Pathological findings

At post mortem examination, the infected piglets were in poor bodily condition with soiled hindquarters, sunken eyes, hollowed flanks and hairy coats (Fig. 30). Gross changes were confined to the abdominal viscera. The livers of all 3 piglets were slightly pale and the mesenteric lymph nodes were pale and enlarged. The jejunum and ileum appeared pale and thickened (Fig. 31). The serosal surface of large intestine and the other abdominal organs appeared grossly normal. The mesocolon was oedematous in pig P1.

The jejunal contents were yellowish in colour and contained excess clear mucus and the mucosa was reddened locally. The villi were reduced in height when observed under the dissecting microscope.

The ileal contents resembled those of the jejunum but the mucosa was more extensively hyperaemic (Fig.32) and the wall of the ileum was thickened and fleshy particularly in the terminal portion. Shortened villi were seen under the dissecting microscope. The contents of the caecum and colon were yellowish, pasty and contained obvious mucus which in pig P1 was streaked with fresh blood. The contents were adherent to the mucosa which was locally hyperaemic in that animal.

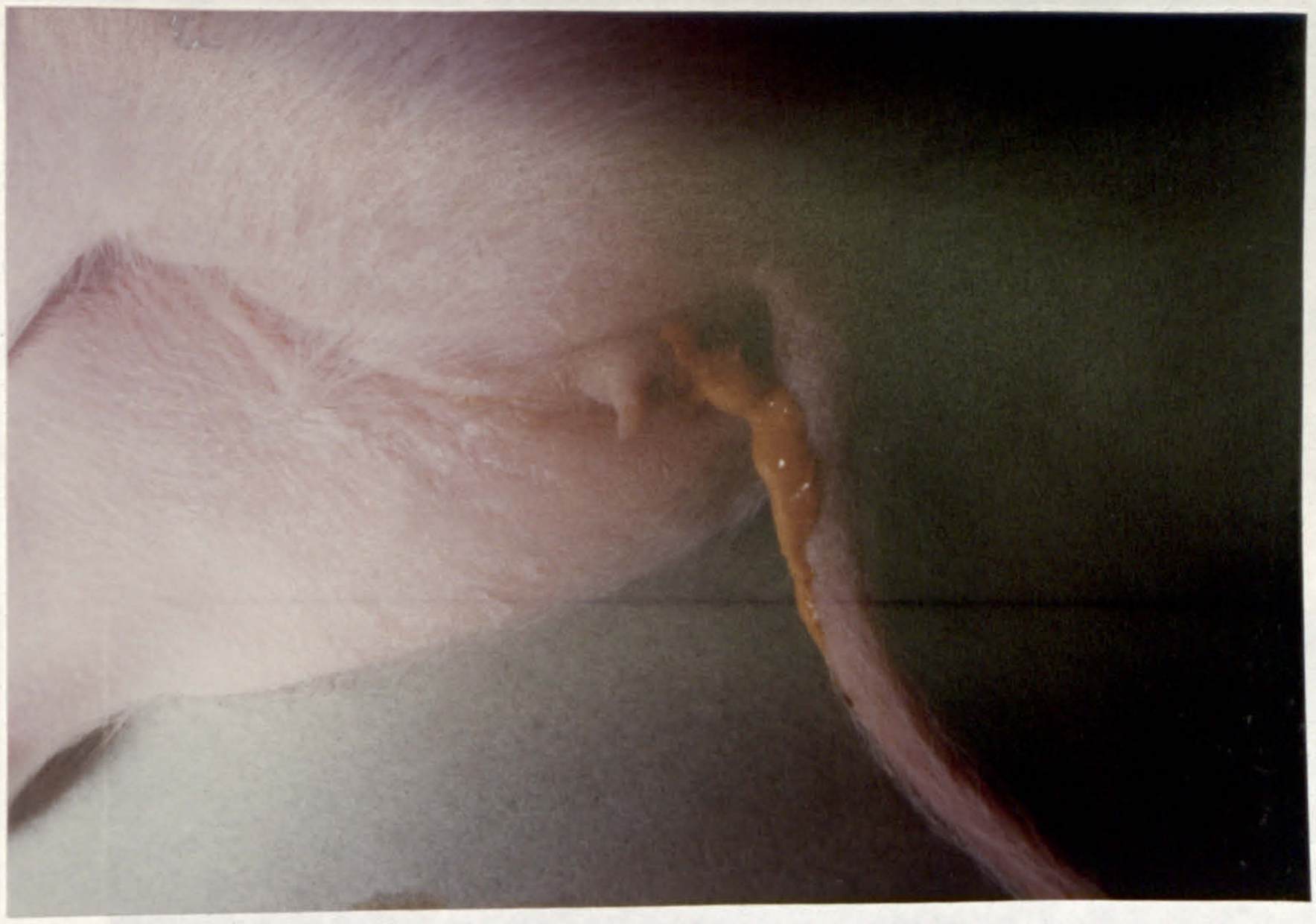


FIG. 30: Perineal region of HDCD Piglet P2, 12 days post inoculation with C. coli.
Note the texture of a typical motion.

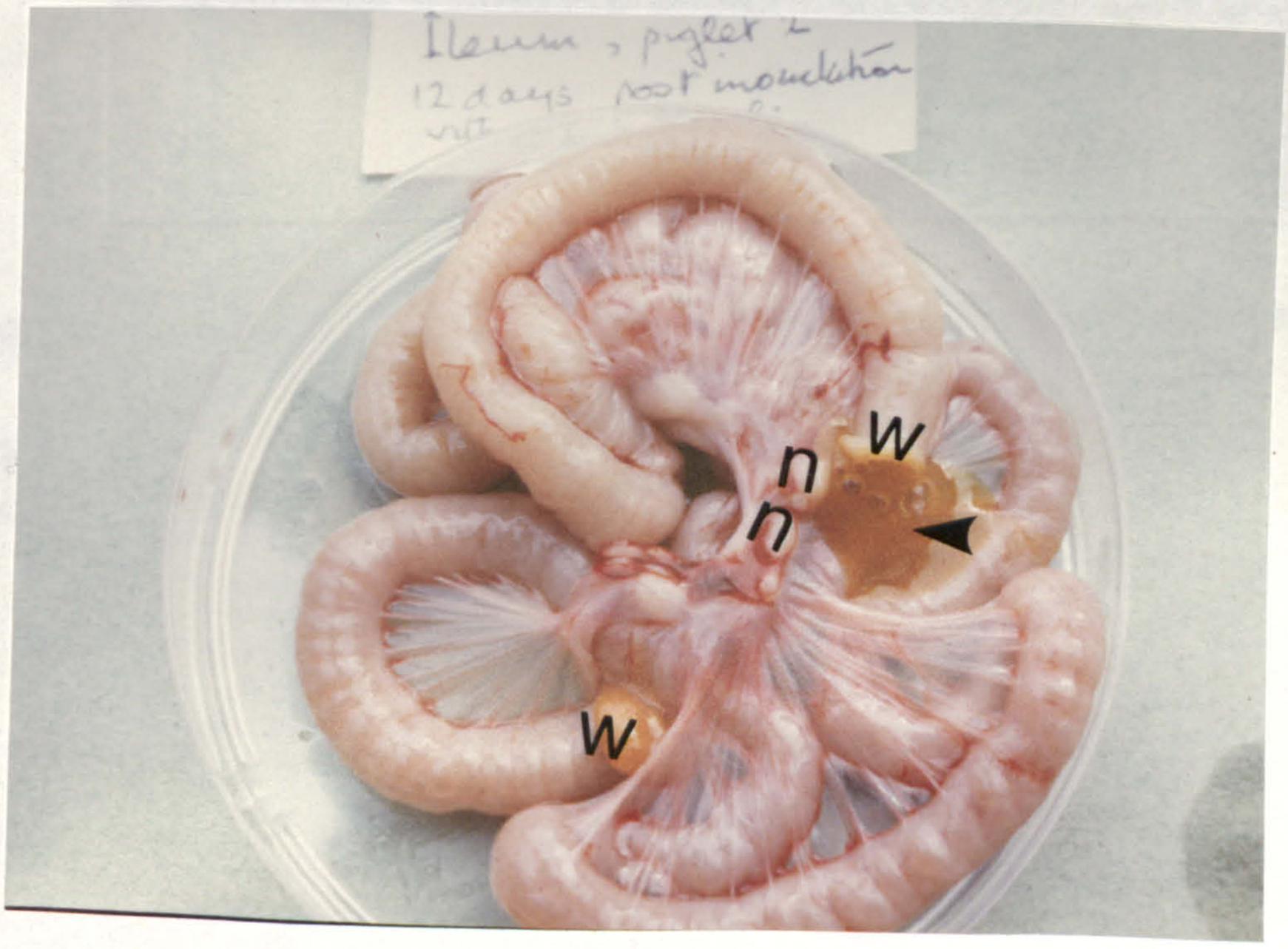


FIG. 31: Ileum and contents of HDCD Piglet P2, 12 days post inoculation with C. coli.
Note the thickened wall of the ileum (W), and the mucoid and fluid contents (arrow).
The mesenteric lymph nodes are enlarged and pale (N).

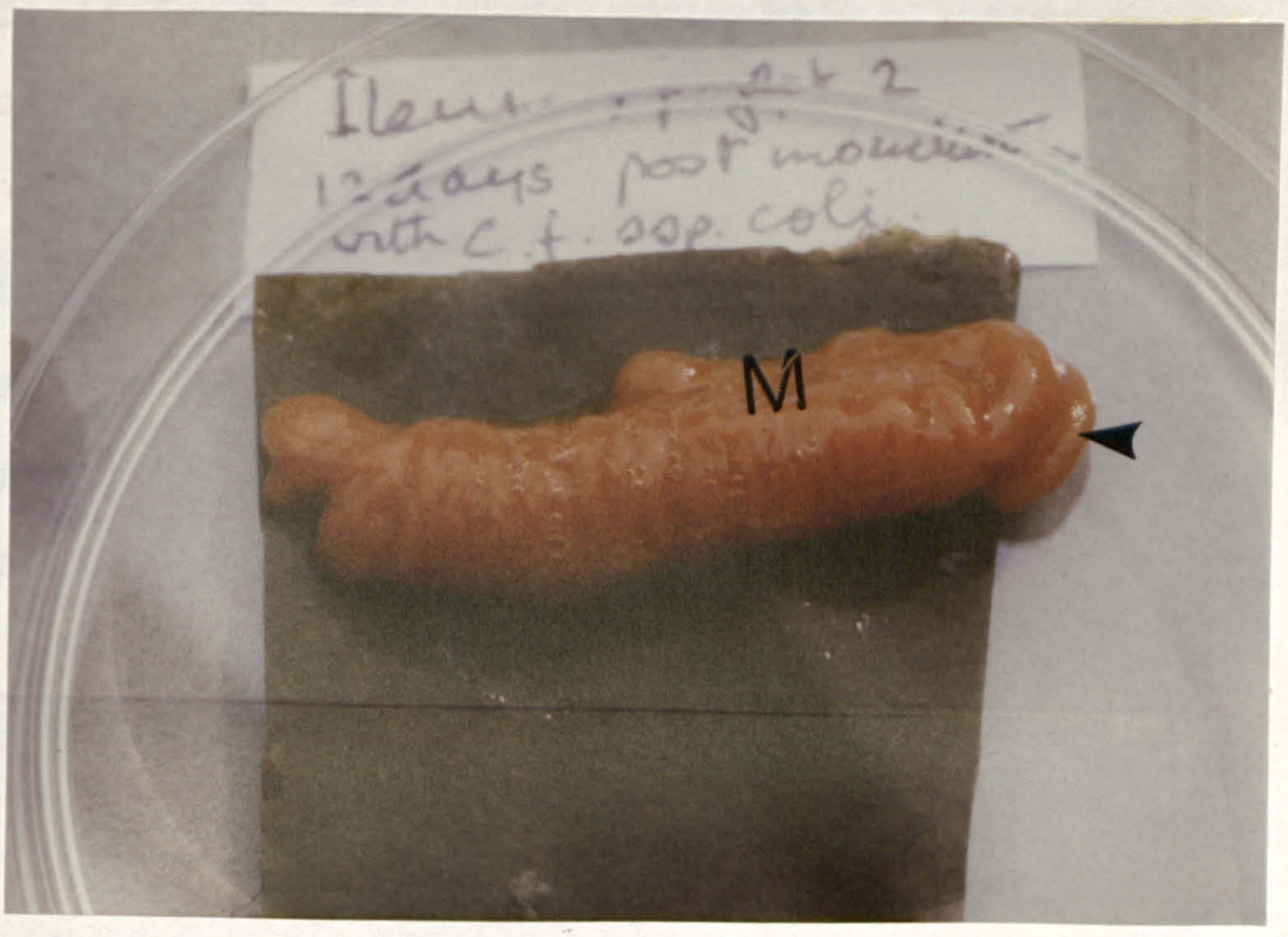


FIG. 32: Macroscopic appearance of the ileal mucosa of HDCD piglet, P2, 12 days post inoculation with C. coli.
Note the thickened and fleshy wall (arrow) and the excess mucus on the mucosa (M).

The control animals were in good bodily condition. Pig P4 had contracted tendons of the hindlimb and erythema of the tail and limbs consequent upon this and was killed on day 4 of the experiment on humane grounds. Pericarditis was present in 2 pigs (P4 and P5) but the abdominal contents were grossly normal with the exception of the mesocolon in pig 5, umbilical oedema in pig P4 and excess clear peritoneal fluid in pig P6. The gastrointestinal tract, mucosa and contents were all normal in appearance.

Histological findings

Mild histological changes were noted in the small intestine of all the pigs. In the control animals these were restricted to vacuolation of the ileal mucosal epithelium, the presence of some lymphoid tissue in the ileum and eosinophils in the lamina propria of the ileum.

In the infected animals stunting of the villi was noted in the duodenum of all 3 animals and in the jejunum of 1 (Pig P2, Fig.33). In that pig polymorphonuclear leucocytes were present in some crypts and some small lymphoid foci were seen.

Submucosal lymphoid tissue was prominent in the ileal mucosa of all the infected pigs and a mononuclear cell infiltration was present in the lamina propria (Fig.34). In Pig P1 some eosinophils were present in the lamina propria of the ileum.

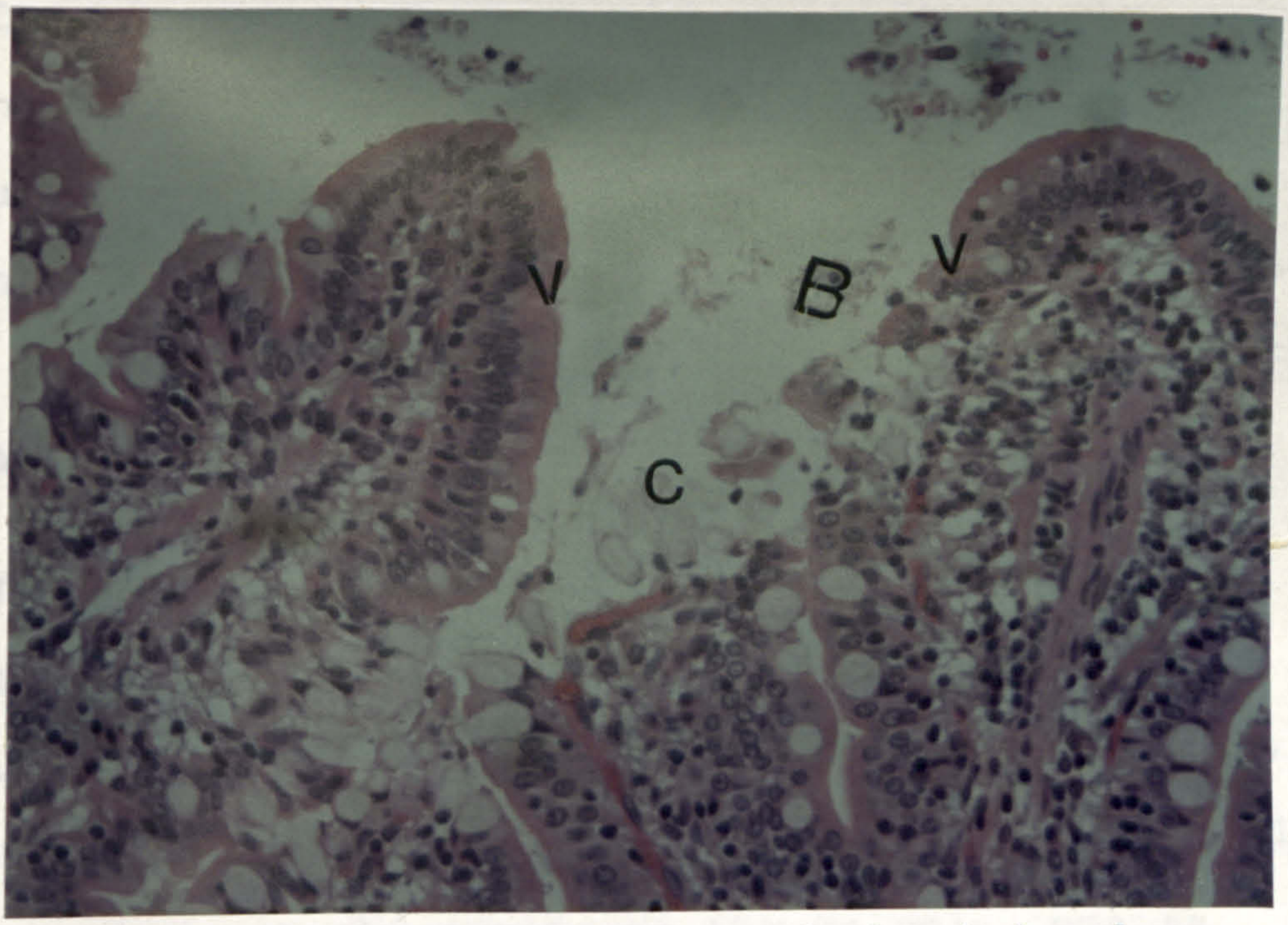


FIG. 33: Histological section of the jejunal mucosa of HDCD piglet, P2, killed 12 days post inoculation with C. coli.
Note the stunted villi (V), the cell loss into the lumen (C) and the bacterial debris (B).
H & E x 250

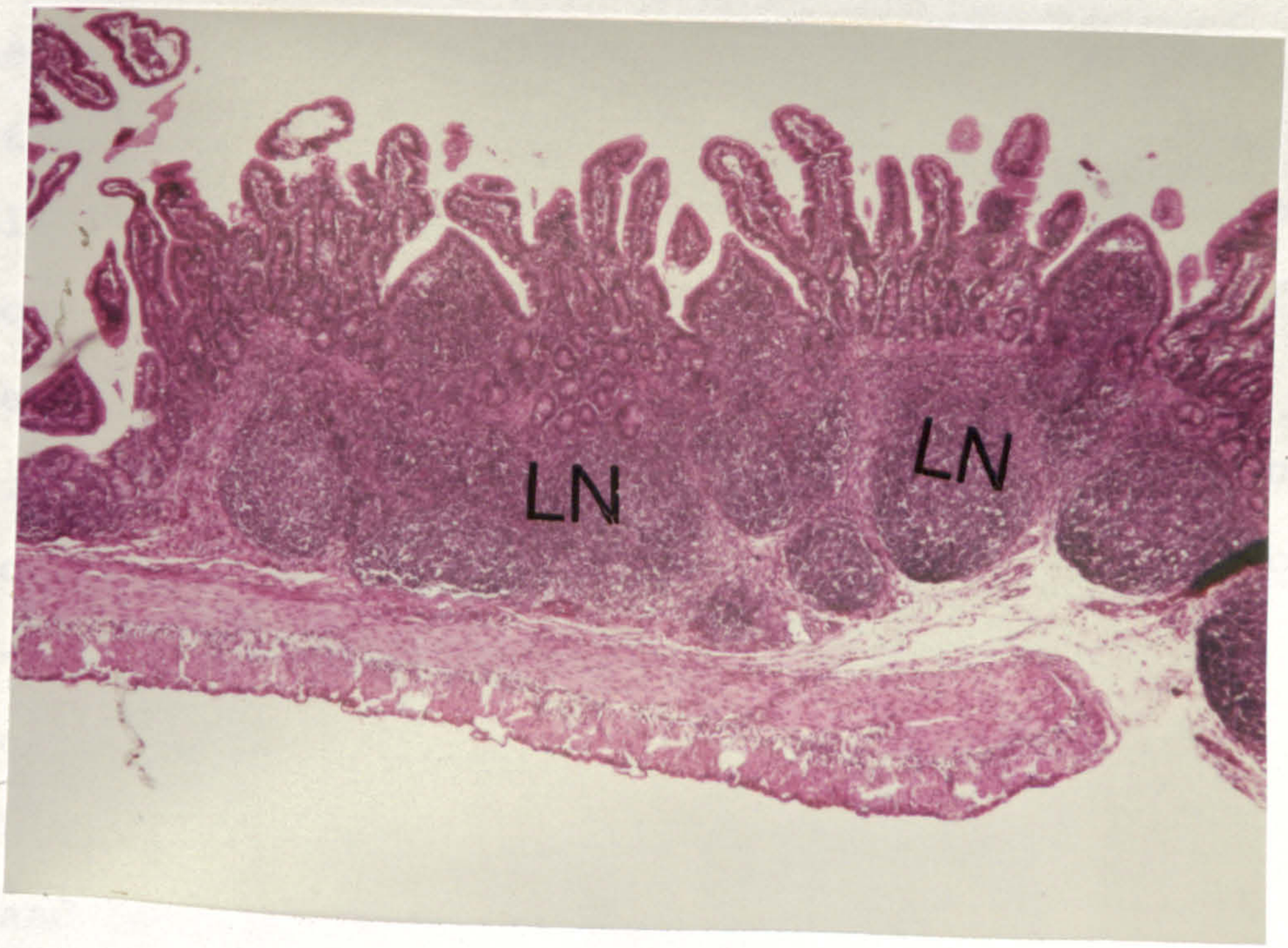


FIG. 34: Histological section of the ileal mucosa of HDCD piglet, P2, killed 12 days post inoculation with C. coli.
Note the submucosal lymphoid hyperplasia (LN).
H & E x 35

Changes were present in the large intestinal mucosa of all animals. They varied from lowering of the mucosal epithelium and bacterial adhesion to massive cellular exudation and polymorphonuclear cell infiltration (Fig.35). The only difference was a relative increase in cellularity in the lamina propria of the infected pigs.

In silver-stained sections from these animals no silver-stained bacteria were seen in the mucosa of the controls. Silver-stained bacteria, some of them curved, were seen in small numbers in the crypts of the ileum and in larger numbers in the crypts of the colonial mucosa (Fig.36). None were seen in the cytoplasm of the mucosal epithelium.

Colonies of C. coli were isolated in large numbers from the mucosa of the ileum, caecum and colon of all inoculated animals and jejunum of Pig P1. The organism was isolated in small numbers from the duodenum of one of the inoculated pigs. No campylobacters were isolated from the liver, gall bladder or gastric mucosa in any of the piglets. C. coli was only isolated from the mesenteric lymph nodes in Pig P2. The sites of isolation of C. coli at post-mortem examination are summarised in Table 19.

Gram negative curved bacteria with the morphology of campylobacters were seen in direct smears of the ileal, caecal and colonic mucosa. None were seen in similar preparations made from the controls. Non-haemolytic E. coli were isolated from the gastrointestinal tracts in all 3 inoculated piglets and a non-haemolytic clostridium resembling that found in the faeces was present in the

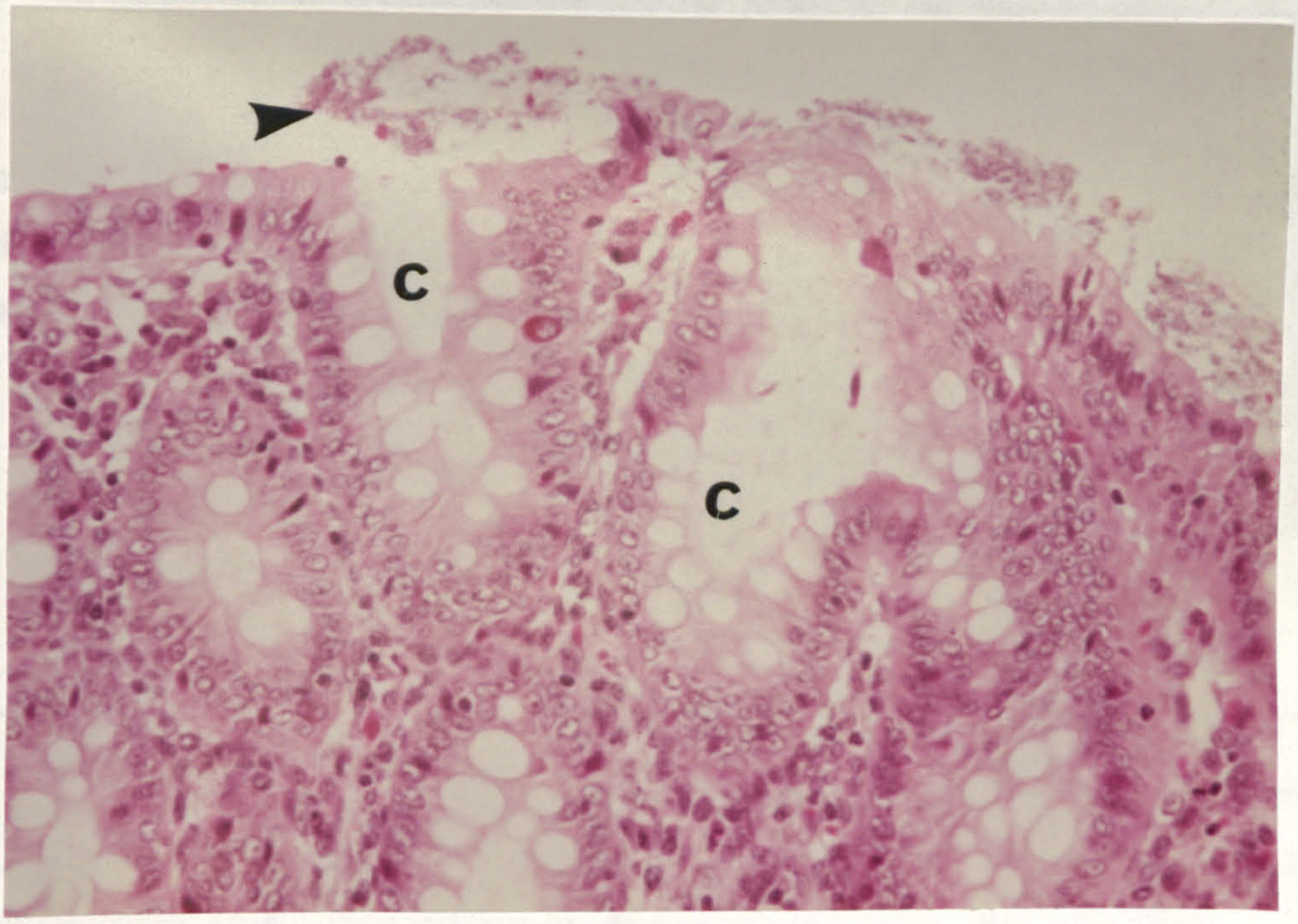


FIG. 35: Histological section of the colonic mucosa of HDCD piglet, P2, killed 12 days post inoculation with C. coli.
Note the dilated crypts (C) and the presence of debris adjacent to the lumenal surface (arrow).
H & E x 250

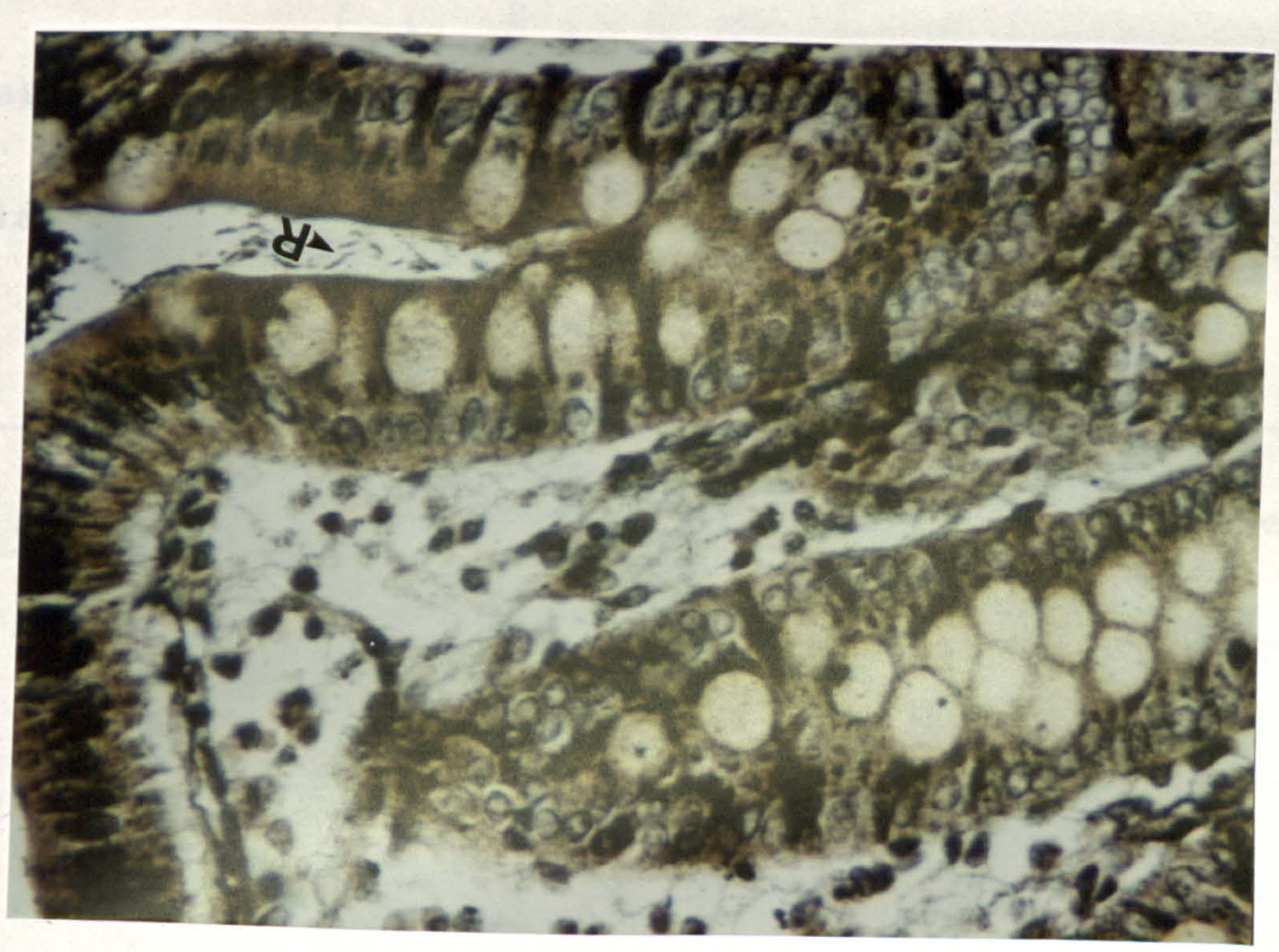


FIG. 36: Silver-stained histological section of the lumenal border and crypts of the colonic mucosa of HDCD piglet, P2.
Note the curved silver-stained rods present in the mouth of the crypts (R).
Young's x 1200

TABLE 19

Sites from which C. coli was isolated from experimental and control HDCD piglets killed 12 days following infection with pure cultures of the organism in Experiment 1.

Site of isolation	Infected			Control		
	P1	P2	P3	P4*	P5	P6
Stomach	-	-	-	-	-	-
Duodenum	+	-	-	-	-	-
Jejunum	++	+	+	-	-	-
Ileum	+++	+++	+++	-	-	-
Caecum	+++	+++	+++	-	-	-
Colon	++	++	++	-	-	-
Mesenteric Lymph Nodes	-	+	-	-	-	-
Liver	-	-	-	-	-	-
Gall bladder	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Kidney	-	-	-	-	-	-

\+ = C. coli isolated
\- = No C. coli isolated

\+ = less than 6 colonies seen
++ = 10 colonies seen
+++ = more than 10 colonies seen (or profuse culture)

* killed on day 4 of the experiment

ileum and large intestine of all 3 inoculated animals. The non-haemolytic E. coli and clostridium were also present in the alimentary tracts of the controls. In addition, non-haemolytic E. coli was recovered from the pericardial fluid in Pigs P4 and P5 and from the liver in Pig P4.

Serological findings

Agglutinating antibody to the inocular strain of C. coli was only demonstrated in the sera taken from the inoculated animals at the end of the experiment at titres of 1:320 (Pig P1) and 1:640 (Pigs P2 and P3). The results are shown in Table 20.

TABLE 20

Levels of agglutinating antibody to the inocular strain of C. coli in the sera of HDCD piglets of Experiment 1.

Animal No.	Infected	Titre present Day 12
P1	+	1:320
P2	+	1:640
P3	+	1:640
P4	-	0
P5	-	0
P6	-	0

EXPERIMENT 2

OBJECTIVE: To determine the pathogenicity and the pathogenesis of C. coli for conventional suckling piglets.

MATERIALS AND METHODS

A sow and a litter of 10 naturally farrowed piglets were used in this study as described in Chapter 2. The piglets were numbered 4207 to 4216. The piglets were monitored prior to infection by the methods described in Chapter 2 and in Experiment 1 for the presence of Salmonella spp., B-haemolytic E. coli, campylobacters and other bacteria, coccidial oocysts and nematode eggs. Faeces samples were not examined for the presence of viral particles.

One animal (Pig 4207) had splay leg and had difficulty in sucking, was killed on the 3rd day of life, and was examined post mortem. The remaining 9 piglets were inoculated with a pure culture of the isolate of C. coli used in Experiment 1 on day 4 of life.

Each piglet was inoculated orally with 10ml of the culture of C. coli and received approximately 3.2×10^{10} organisms.

The appearance of the piglets, the consistency of their faeces and their rectal temperatures were recorded daily. Rectal swabs were taken daily from all piglets and were examined for the presence of C. coli and other bacteria by the methods described in Chapter 2.

Colonies resembling those of Campylobacter spp. were confirmed as such by the methods described in Chapter 2.

Clotted blood samples were taken from all piglets at slaughter and the sera stored by the methods described in Chapter 2. These sera were examined for the presence of agglutinating antibody to C. coli by the methods described in Chapter 2.

The infected piglets were killed in the sequence shown below (Table 21) by the methods described in Chapter 2 and post mortem, histological and bacteriological examinations were also carried out using the methods described in Chapter 2.

RESULTS

Clinical findings

The faeces of all piglets in the infected group in this study were normal prior to infection.

No Salmonella spp., B-haemolytic E. coli, or campylobacters were isolated from the faeces of the piglets prior to infection. No coccidia or nematode eggs were present.

Soft, pale, faeces were passed by 2 piglets (4209, 4215) on the 3rd day after infection and clear mucus was seen on the poorly formed motions of 2 piglets (4209 and 4210) by the 4th day after infection. Faecal changes were noted in all inoculated piglets within 6 days of inoculation. The details of faecal changes are summarised in Table 21.

TABLE 21

Faecal changes in conventional suckling piglets following inoculation with pure cultures of C. coli and isolation of the organism from their faeces.

Pig No.	Infected	Day of Experiment -2	-1	0	1	2	3	4	5	6	7	8	9	10
4207		N −	K −											
4208	+	N −	N −	K −										
4209	+	N −	N −	N −	N −	N +	S +	SM_{+}^{K}						
4210	+	N −	N −	N −	N +	N +	N +	SM +	SM_{+}^{K}					
4211	+	N −	N −	N −	N −	N +	N +	S +	S +	SM +	SM_{+}^{K}			
4212	+	N −	N −	N −	N −	N +	N +	S +	S +	S +	S_{+}^{K}			
4213	+	N −	N −	N −	N −	N +	N +	S +	S +	S +	S +	S +	S +	S_{+}^{K}
4214	+	N −	N −	N −	N −	N +	N +	N +	SM +	SM +	SM +	S +	S +	S_{+}^{K}
4215	+	N −	N −	N −	N +	N +	S +	S +	S +	S +	S +	S +	S +	S_{+}^{K}
4216	+	N −	N −	N −	N −	N −	N +	N +	N +	S +	S +	S +	S +	S_{+}^{K}

N = Normal faeces
S = Soft faeces
M = Presence of mucus in faeces
\+ = C. coli isolated from faeces
− = C. coli not isolated from faeces
K = Killed

A slight rise in rectal temperature to a maximum of 40°C. occurred within 48 hours of infection and rectal temperatures fluctuated thereafter in the range 38.7-39.9°C. The rectal temperature changes following inoculation are summarised in Fig.37.

Faecal culture

Colonies of C. coli were isolated in large numbers from the faeces of 7 of the piglets on the 2nd day after inoculation and from those of all piglets from the 3rd day onwards. No B-haemolytic E. coli were isolated from the faeces of this group. -haemolytic streptococci, non-haemolytic clostridium and non-haemolytic E. coli were isolated from the rectal swabs of all animals.

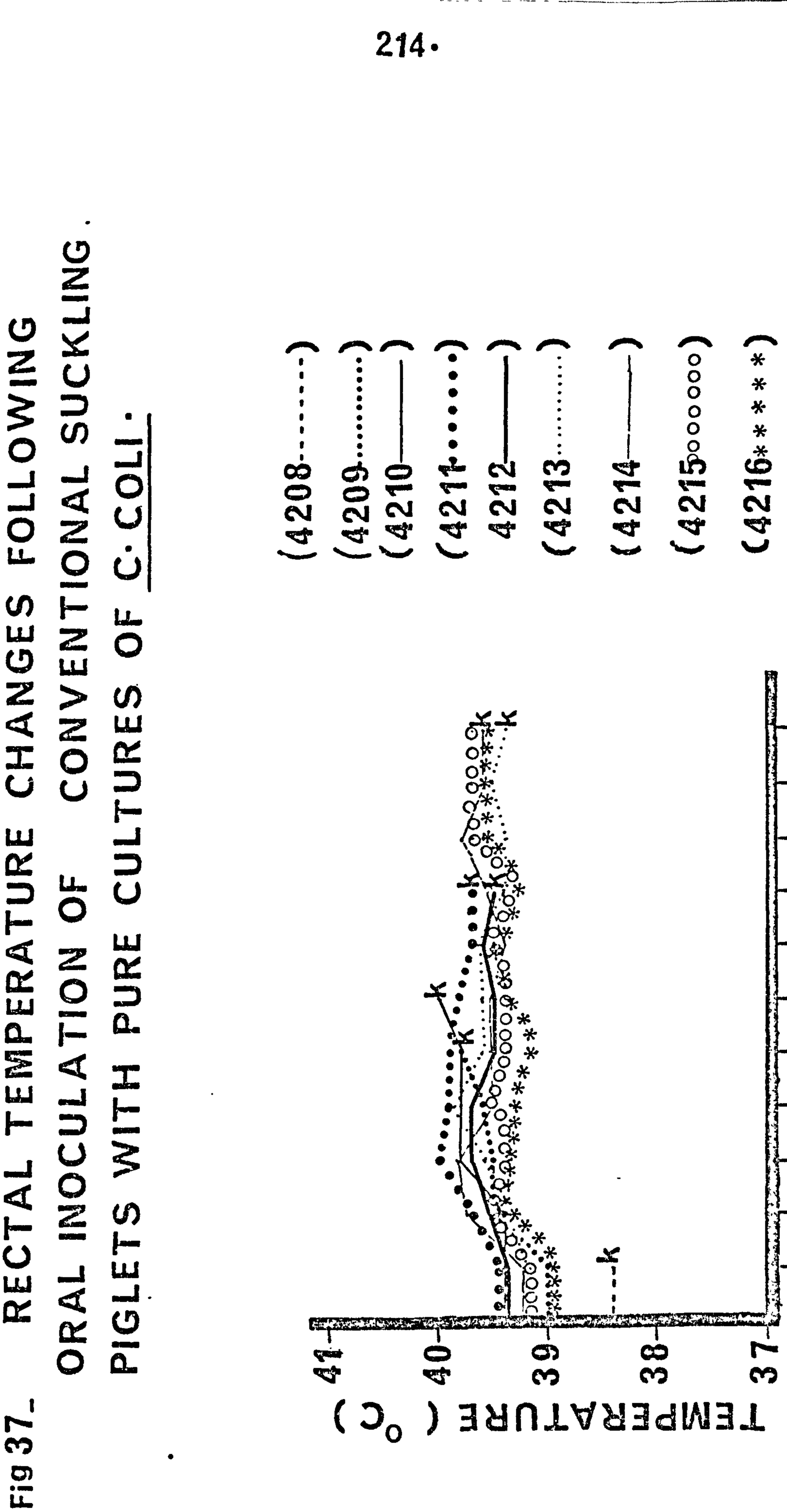
Pathological findings

No gross changes were seen in the piglets killed on the 3rd day of life and 4 hours after infection. The changes seen in the remaining piglets are described below in the order that they were killed.

Pig 4209, day 4

The general bodily condition was poor. The hindquarters were soiled by fluid yellow faeces. The stomach was normal as was the duodenum except for the presence of local hyperaemia of the mucosal surface. The jejunal content was fluid and yellowish. Clear mucus could be seen on the mucosal surface which was hyperaemic locally.

Fig 37- OBSERVED RECTAL TEMPERATURE CHANGES FOLLOWING ORAL INOCULATION OF PIGLETS WITH PURE CULTURES OF C. COLI.



The serosal surface of the ileum was pale in appearance and the terminal portion appeared thickened (Fig.38). The content was fluid in consistency. Clear mucus was present on hyperaemic areas of the mucosa.

The content of the caecum and colon was pasty and adhered to the mucosal surface which appeared grossly normal except for the presence of local 1-2mm patches of haemorrhage on the caecal mucosa. The mesenteric lymph nodes appeared pale and enlarged. The villi of the jejunum and ileum were stunted when examined under the dissecting microscope.

Pig 4210, day 5

The post mortem findings were similar to those for Pig 4209 (Figs.39,40). Differences included the bile staining of the ileal mucosa and the presence of tags of mucus on the mucosal surface of the colon which was also inflamed locally. The mesenteric lymph nodes were grossly enlarged and markedly pale.

Pigs 4211 and 4212, day 7

The changes noted were substantially those seen on days 4 and 5. Pig 4211 was in poor bodily condition with soiling of the perineum (Fig.41). Slight differences were seen in the lower intestinal tract. The serosal surface of the ileum was pale and the wall was thickened and fleshy. The content was fluid in consistency, with obvious clear mucus on the mucosal surface. The mucosal surface was bile stained and generally hyperaemic.

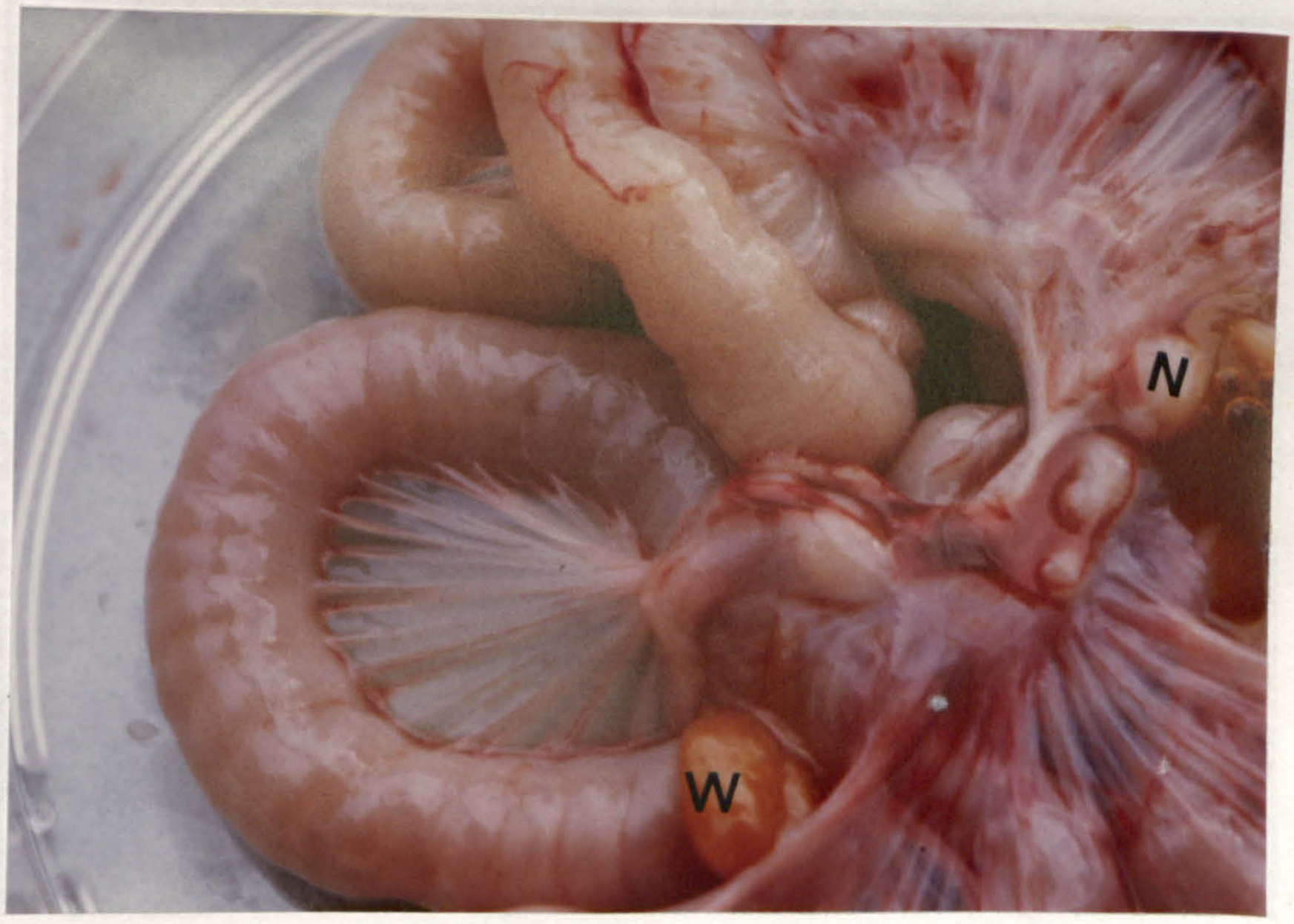


FIG. 38: Small intestine and contents of Piglet 4209, killed 4 days post inoculation with C. coli.
Note the thickened wall of the ileum (W); the enlarged mesenteric lymph nodes (N) and the mucoid contents.

FIG. 39: Perineal region of Piglet 4210, killed 5 days post inoculation with C. coli.
Note the texture of a typical motion.

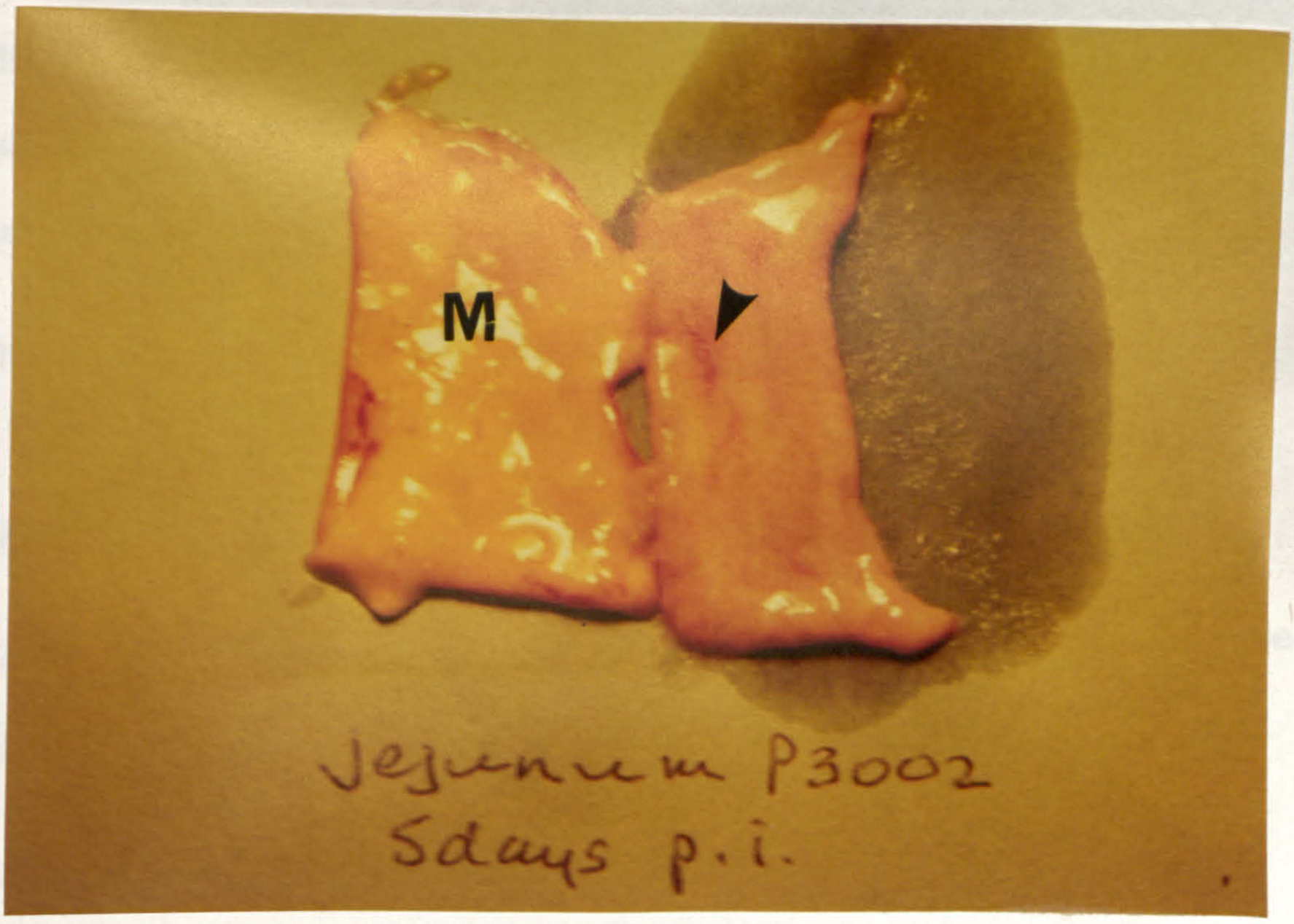


FIG. 40: Macroscopic appearance of the jejunal mucosa of Piglet 4210, killed 5 days post inoculation with C. coli.
Note mild inflammation (arrow) and the mucoid appearance of the mucosal surface (M).

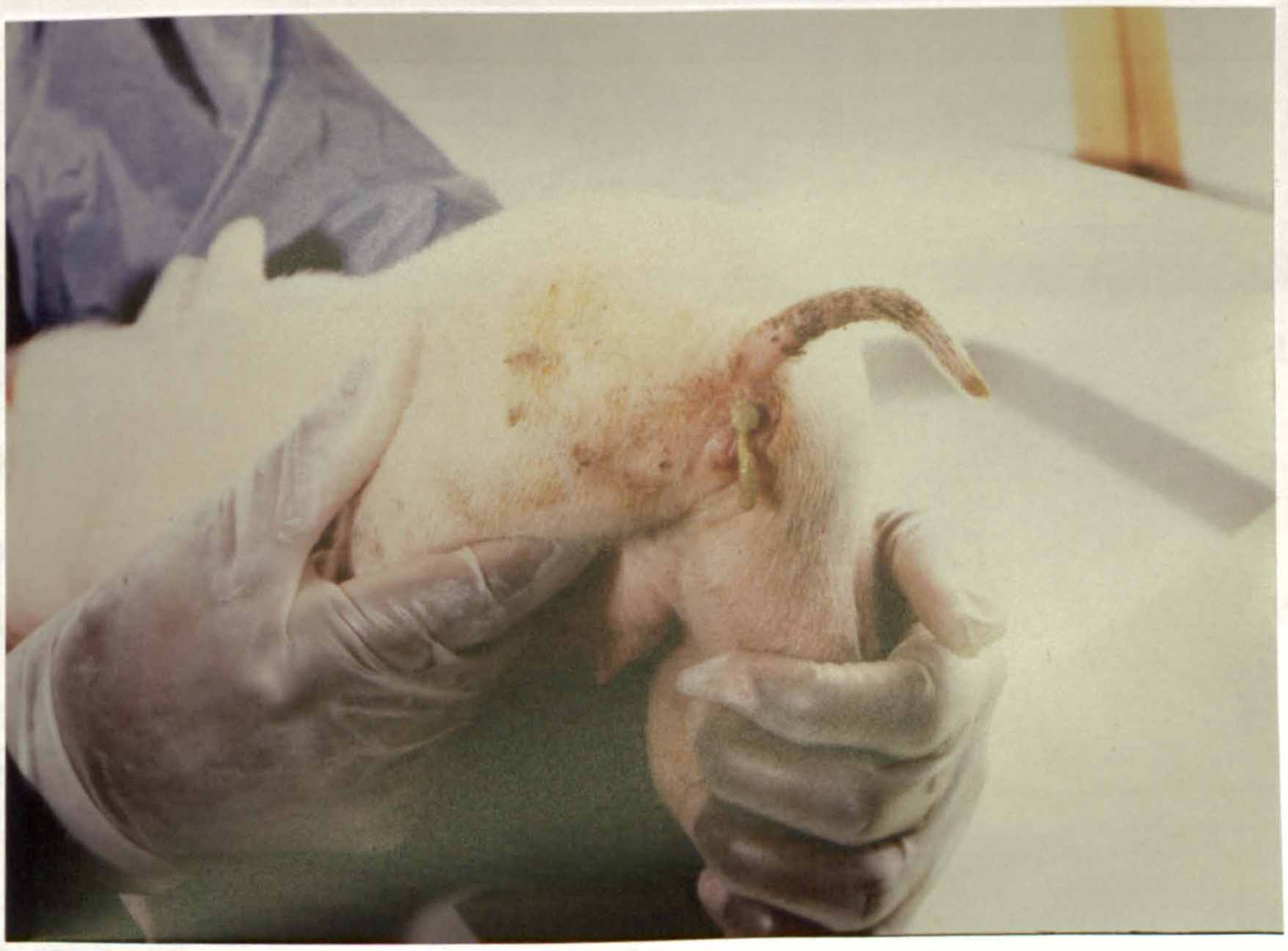


FIG. 41: Perineal region of Piglet 4212, killed 7 days post inoculation with C. coli.
Note the soiling of the perineum with soft mucoid faeces.

The large intestinal content was pasty with a few tags of mucus. The mucosal surface was grossly normal except for mild inflammation of the caecal mucosa.

The mesenteric lymph nodes appeared enlarged and were very pale. The liver was pale and had round edges. It had a brittle texture.

The height of the villi was slightly reduced in the lower jejunum and more markedly so in the ileum.

The general bodily condition of Pig 4212 was fair and there was no soiling of the perineum. The gastric, duodenal and jejunal findings resembled those in the pigs killed from day 4 onwards and the ileal findings were identical with those of pig 4211. The caecum contained greyish pasty content adherent to the petechiated mucosal surface.

The pasty content of the colon contained some tags of clear mucus but the mucosal surface was grossly normal. The mesenteric lymph nodes were enlarged and pale in appearance. One very near the ileum was particularly oedematous when sectioned. The liver was pale and enlarged.

Pigs 4213, 4214, 4215 and 4216, day 10

Many of the changes observed were seen in all 4 pigs. Pig 4213 was in poor condition unlike the others and the findings will be described separately.

Pig 4213: The general bodily condition was fair. The skin was hairy and rough. The stomach was filled with milk and the gastric mucosa was grossly normal. The duodenal content was mucoid and fluid. The mucosa was grossly normal except for local pinpoint haemorrhages.

The jejunal contents were fluid and bile stained. The mucosal surface was locally hyperaemic. The wall of the ileum was thickened and fleshy and its contents were fluid and brown in colour. The mucosal surface was hyperaemic and tags of obvious clear mucus were present at the hyperaemic sites. No gross changes were noticed in the caecum and colon except for the pasty content.

The mesenteric lymph nodes were grossly enlarged and the liver was pale in colour.

Pigs 4214, 4215 and 4216: These animals were in good condition, with normal gastric mucosa and contents, mucoid duodenal contents and pinpoint haemorrhages on the duodenal mucosa. In all cases the jejunal contents were bile stained and there was localised hyperaemia of the mucosa in the terminal portion.

The ileal wall was thickened and fleshy in all cases and obvious mucus was present in the fluid contents. Patchy hyperaemic areas were seen on the mucosal surface and were more extensive in Pig 4216. There was lowering of the height of the villi.

The contents of the large intestine were greyish in colour and pasty in consistency. The mucosal surface was grossly normal in all cases except for isolated areas of hyperaemia.

The mesenteric lymph nodes were grossly enlarged and pale in appearance. The livers were pale in appearance, otherwise normal.

Histological findings

Few histological changes were noted in the gastro-intestinal tracts of Pigs 4207 and 4208 killed prior to and early in the infected period. The most pronounced changes were seen in Pigs 4209 and 4210 killed 4 and 5 days after inoculation.

. Changes in the jejunum included shortening of the villi, the presence of cells in the lumen and the presence of neutrophils in the lamina propria.

In the ileum, submucosal lymphoid hyperplasia was present (Fig.42), eosinophilic and neutrophilic polymorphonuclear leucocytes were prominent in the lamina propria, there were mitoses in the bases of the crypts and bacteria were seen in large numbers adjacent to the mouth of the crypts. The crypts of the colonic and caecal mucosa were mildly dilated and contained some bacteria and eosinophilic debris. Inflammatory cells were present in the lamina propria. In silver-stained sections, no bacteria were seen in the cells of the mucosal epithelium

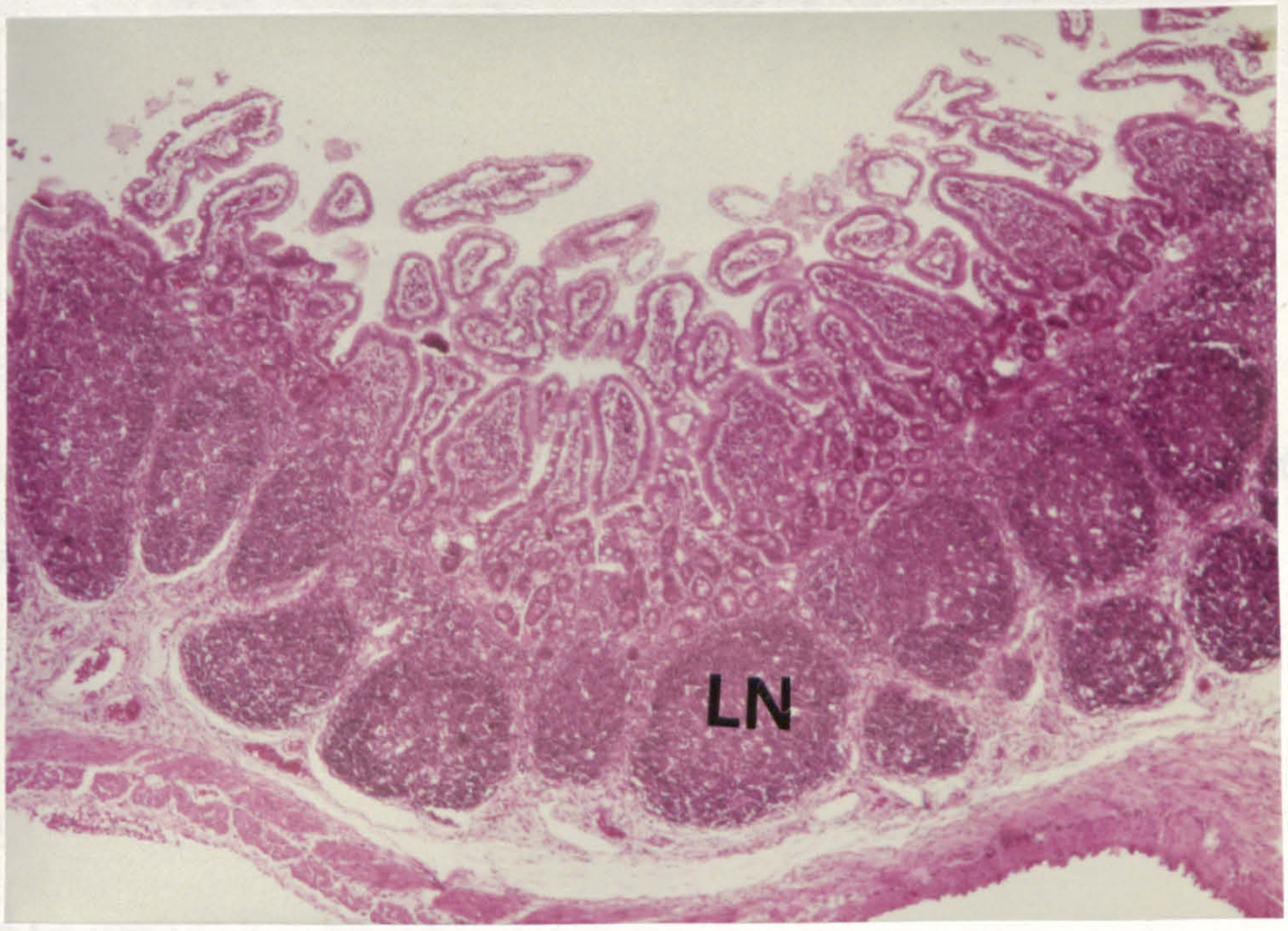


FIG. 42: Histological section of the ileal mucosa of Piglet 4209, 4 days post inoculation with C. coli. Note the submucosal lymphoid hyperplasia (LN). H & E x 35.

although in some cases silver-stained bacteria could be seen in the crypts of the ileal mucosa and between the villi (Fig.43).

In the animals killed at 7 and 10 days post infection, changes were most prominent in the ileum and were most marked in those killed 10 days post infection. They resembled those seen in the HDCD piglets of Experiment 1. Submucosal lymphoid hyperplasia was prominent and bacteria were seen in the crypts. Mild inflammatory changes were also noted in the colon and caecum in which dilated crypts and hypercellularity of the lamina propria were present (Fig.44). Silver-stained bacteria were present in the crypts of the ileal mucosa in small numbers and were present in similar numbers on the lumenal surface and in the crypts of the colonic mucosa.

Bacteriological findings

C. coli was not isolated from any part of the gastrointestinal tract of Piglet 4207 killed prior to inoculation (Table 22). It was only present in the stomach and duodenum of Piglet 4208 killed 4 hours after inoculation. Small numbers of colonies were isolated from the duodenum in all pigs killed on or prior to day 5 but only infrequently (Pig 4212 day 7 and Pig 4215 day 10, after that). Small numbers of colonies were present in the jejunal mucosa of all the pigs killed 4 or more days post infection.

Large numbers of colonies of the organism were recovered from the ileum, caecum and colon in all infected

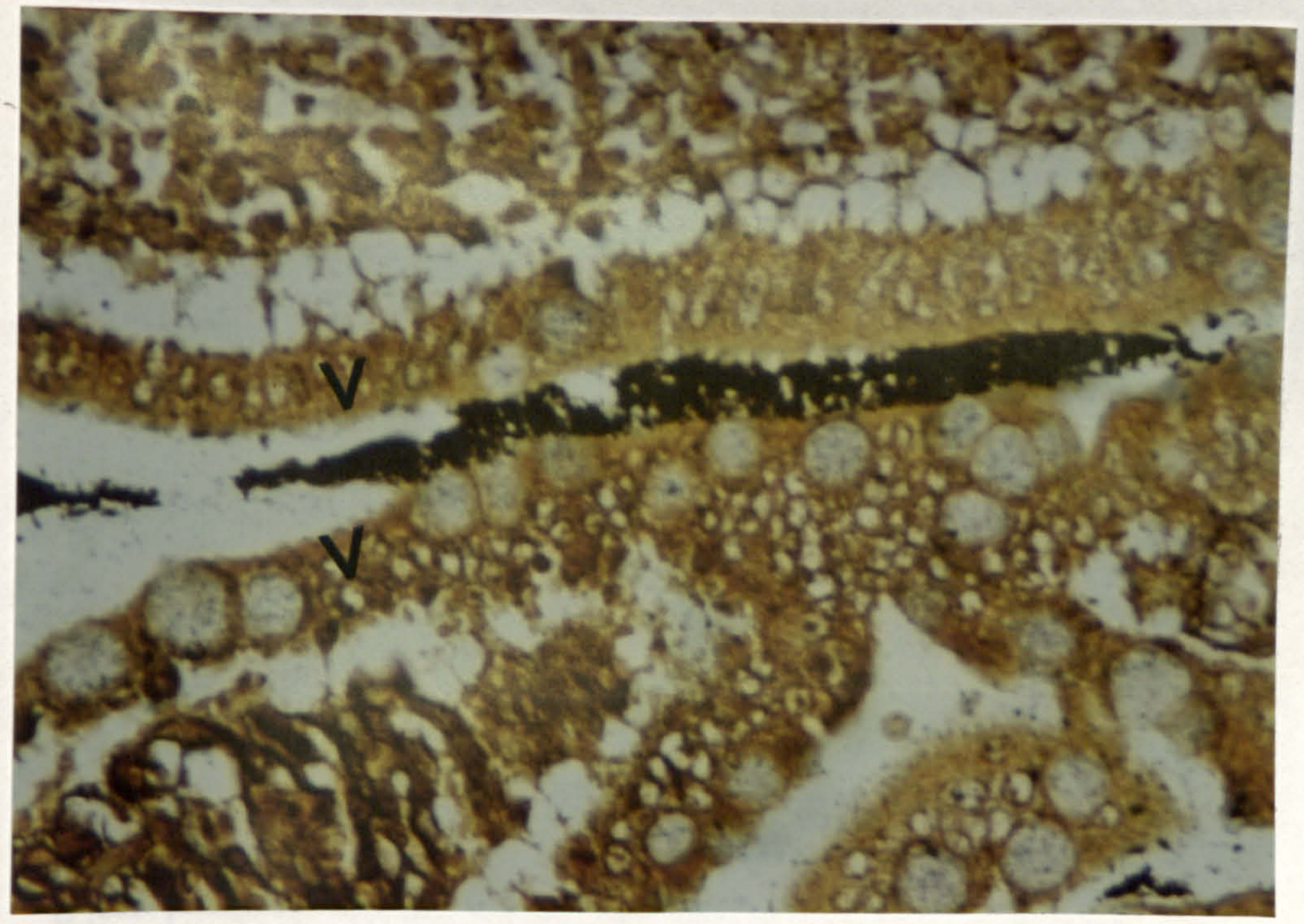


FIG. 43: Silver-stained histological section of the lumenal border and crypts of the ileal mucosa of Piglet 4210, 5 days post inoculation with C. coli. Note the silver-stained bacteria present in the crypt and between the villi (V).
Young's x 1200

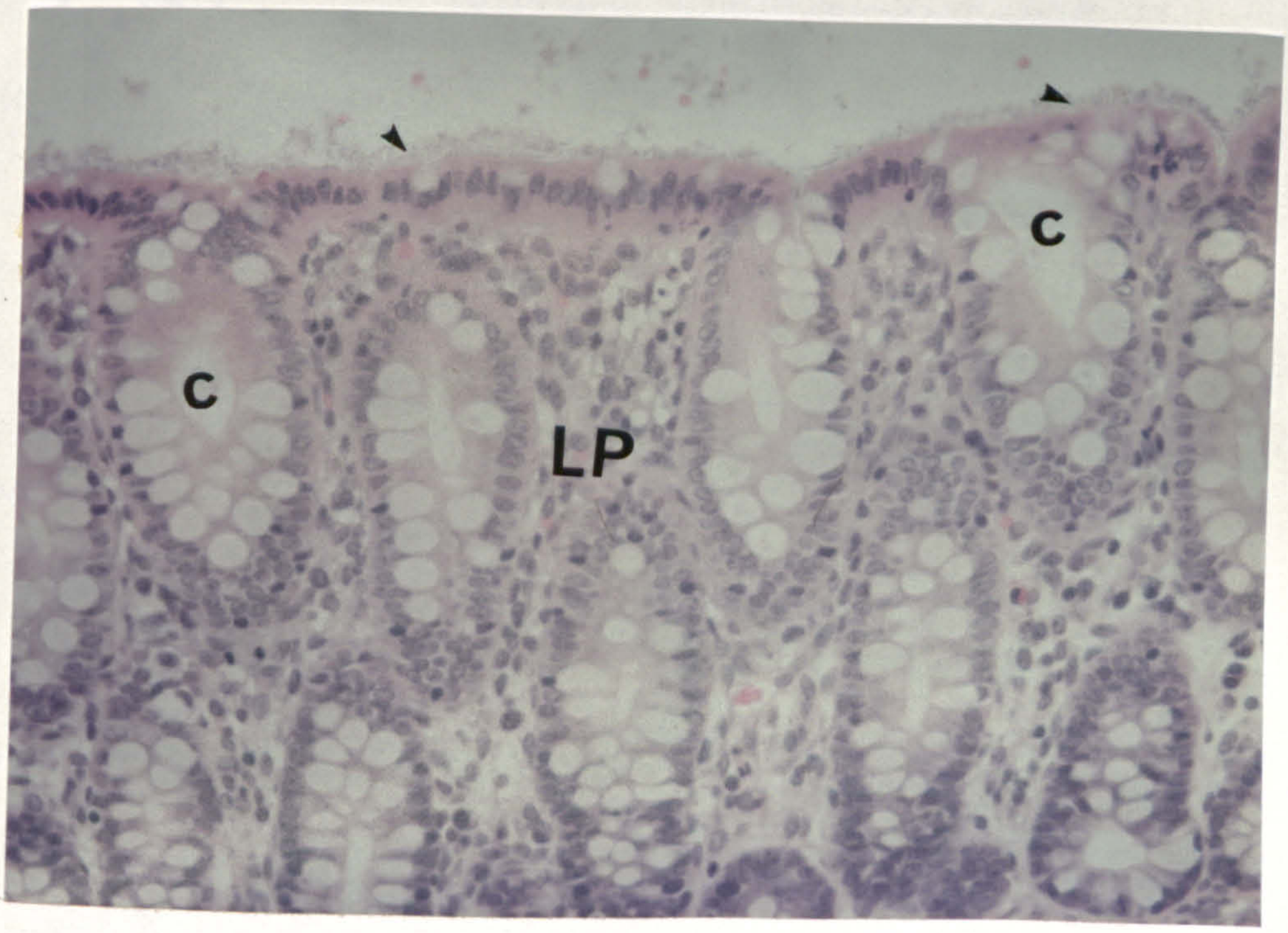


FIG. 44: Histological section of the colonic mucosa of Piglet 4219, 4 days post inoculation with C. coli. Note the dilated crypts (C), hypercellularity of the lamina propria (LP) and the organisms (arrow) attached to the lumenal border of the mucosal epithelium.
H & E x 400

TABLE 22. Sites from which C. coli was isolated from conventional sucking piglets killed at intervals following infection with pure cultures of the organism in Experiment 2.

Site of isolation	Day of experiment									
	-1	0*	4	5	7		10			
	4207	4208	4209	4210	4211	4212	4213	4214	4215	4216
Stomach	-	+	-	-	-	-	-	-	-	-
Duodenum	-	+	+	+	-	+	-	-	+	-
Jejunum	-		+	+	+	+	+	+	+	+
Ileum	-	-	+++	++	+++	++	++	++	++	++
Caecum	-	-	++	++	++	++	++	++	++	++
Colon	-	-	++	-	+	++	+	++	+	+
Liver	-	-	-	-	-	-	-	-	-	-
Gall bladder	-	-	-	-	-	-	-	-	-	-
Mesenteric Lymph Nodes	-	-	-		-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-	-

For key see Table 19.

* Killed 4 hours after inoculation

piglets with the exception of Piglet 4208 and 4210 from the colon of which C. coli was not isolated. A few colonies of the organism were recovered from the mesenteric lymph nodes of Pig 4210 (Table 22). The identity of the isolates was confirmed by the methods described in Chapter 2. C. coli was not isolated from any site outside the gastro-intestinal tract and its associated lymph nodes.

Other bacteria isolated from the gastrointestinal tract included faecal streptococci, non-haemolytic E. coli, Bacillus spp. and non-haemolytic clostridia.

Serological findings

Agglutinating antibody to the inocular strain of C. coli was present at slaughter in the sera of inoculated pigs killed from day 4 post inoculation. The results are summarised in Table 23.

EXPERIMENT 3

OBJECTIVE: To determine the pathogenicity of C. coli for conventional weaned pigs.

MATERIALS AND METHODS

All pigs used in this study were obtained from the University of Glasgow Animal Husbandry Department. Ten weaned pigs of 8 weeks of age were housed in the conditions described in Chapter 2 and divided into 2 groups of 5 in separate pens. The animals were observed and monitored prior to inoculation as described in Chapter 2 for 8 days.

TABLE 23

Levels of agglutinating antibody to the inocular strain of *C. coli* in the sera of the piglets in Experiment 2.

Day of experiment	-1	0	4	5	7		10			
Animal number	4207	4208	4209	4210	4211	4212	4213	4214	4215	4216
Level of antibody	0	0	1:40	1:80	1:160	1:320	1:640	1:320	1:320	1:640

Animals numbered 20, 21, 22, 23 and 24 were infected while 25, 26, 27, 28 and 29 were maintained as uninfected controls.

The inoculum was prepared by the same method and from the same isolate as in Experiments 1 and 2, and 3.5×10^{10} organisms were used for each pig. The period of observation lasted for 20 days and the animals were killed on the 21st day following inoculation.

Animals were examined daily for the parameters described in Chapter 2 daily. Daily weight gains were measured in all 10 pigs and feed consumption was measured on a pen basis. Rectal faeces samples and rectal swabs were examined daily for the presence of campylobacters and other bacteria by the methods described in Chapter 2. Faeces samples were not examined for viral particles in this study.

Clotted blood samples were taken from all animals prior to inoculation and at the end of the experiment. The serum samples were stored according to the methods described in Chapter 2. These samples were examined for the presence of antibody to the inocular strain of C. coli as described in Chapter 2.

All pigs were killed and examined at post mortem on the 21st day following inoculation. Post-mortem examination, histological and bacteriological examinations were carried out on all 10 animals by the methods described in Chapter 2. Bacterial colonies which developed were identified by the methods described in Chapter 2.

RESULTS

Clinical findings

No nematode eggs, coccidial oocysts, salmonellae, B-haemolytic *E. coli* or haemolytic clostridia were demonstrated in the faeces of the pigs of Experiment 3 prior to inoculation. *C. coli* was isolated as single colony from tim to time. All pigs remained clinically normal. The clinical changes noticed in the infected group were minimal.

Faecal changes were minimal. There were few obvious changes in faecal consistency but clear mucus was noted on the surface of formed motions passed by the infected pigs from the 3rd day onwards. No such mucus was seen on the firm faeces of the controls. The detailed changes noted are recorded in Table 24. No depression of appetite or loss of condition was noted in the infected animals. Their feed conversion ratio (2.8) was similar to that of the controls (2.9) and their mean rate of daily liveweight gain (543g) was greater than that of the controls (489g) over the same period.

A transient rise in rectal temperature to 40.0 to 40.2°C occurred in all 5 inoculated animals within 24 hours of inoculation. Forty-eight hours after infection no difference between the rectal temperature of control and infected pigs could be detected. The changes in rectal temperature are shown in Fig.45.

TABLE 24 Changes in faecal consistency in conventional weaned pigs following infection with pure cultures of C. coli and isolation of the organism.

PIG'S No.	DAYS OF EXPERIMENT																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
20	N	N	N	SM	SM	SM	SM	NM	NM	NM	NM	NM	NM	N	N	NM	N	N	N	NM	NM	NM
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	K
21	N	N	N	SM	SM	SM	SM	NM	NM	NM	NM	NM	NM	NM	N	N	NM	NM	N	NM	N	
	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	K
22	N	N	N	NM	NM	NM	SM	SM	SM	NM	NM	NM	NM	NM	NM	N	NM	NM	N	NM	N	
	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	K
23	N	N	N	S	SM	SM	NM	NM	NM	NM	N	N	N	N	N	N	N	N	N	NM	NM	
	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	K
24	N	N	N	SM	SM	NM	NM	NM	SM	NM	NM	N	N	NM	N	N	N	N	N	N	N	
	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	K
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	+	-	-	-	+	-	+	-	-	+	+	-	-	-	+	-	-	+	+	+	+	K
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	-	-	-	+	+	+	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+	K
28	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	-	-	-	-	-	+	-	-	-	-	-	+		-	-	-	-	-	-	-	-	K
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	K

N = Normal faeces
S = Soft faeces
M = Presence of mucus in faeces
K = Killed

\+ = C.coli isolated from faeces
\- = C.coli not isolated from faeces

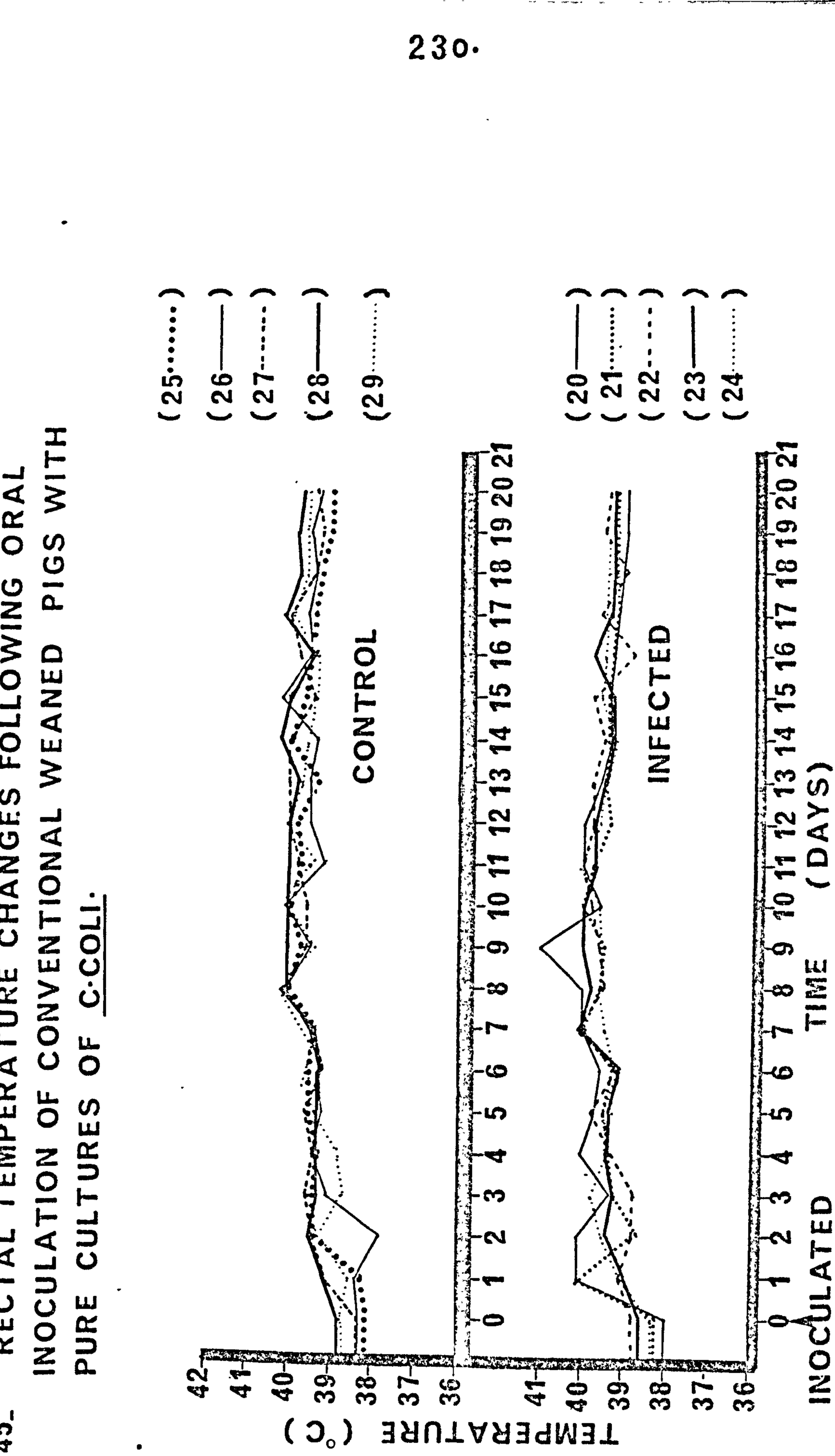


Fig 45. RECTAL TEMPERATURE CHANGES FOLLOWING ORAL INOCULATION OF CONVENTIONAL WEANED PIGS WITH PURE CULTURES OF C.COLI.

Faecal culture

C. coli was isolated from one pig in both groups prior to inoculation and intermittently from all pigs in the control group during the period of observation. The organism was isolated daily from the faeces of all in the infected group from day 2 post infection onwards (Table 24).

B-haemolytic E. coli, haemolytic clostridia and Salmonella spp. were not isolated from the faeces samples of any of the experimental animals. Nematode eggs and coccidial oocysts were reported from the faeces samples.

Pathological findings

At post-mortem examination all the pigs were in good bodily condition and, apart from some scarring of the liver, gross changes were restricted to the gastrointestinal tract and its associated lymph nodes. In all animals of the infected group, the serosal surface of the posterior jejunum and the ileum appeared pale and the organ appeared flaccid with the exception of the terminal ileum which appeared fleshy (Fig.46). The mesenteric lymph nodes were enlarged and pale. The jejunal and ileal contents were yellowish and fluid and the caecal contents were pasty and adherent to the mucosal surface.

Patchy hyperaemia was noted on the surface of the mucosa of the duodenum, jejunum and the whole of the ileum and the wall of the distal small intestine was thickened and fleshy. Changes in the terminal ileum were most pronounced in Pig 21 in which clear mucus was present on the

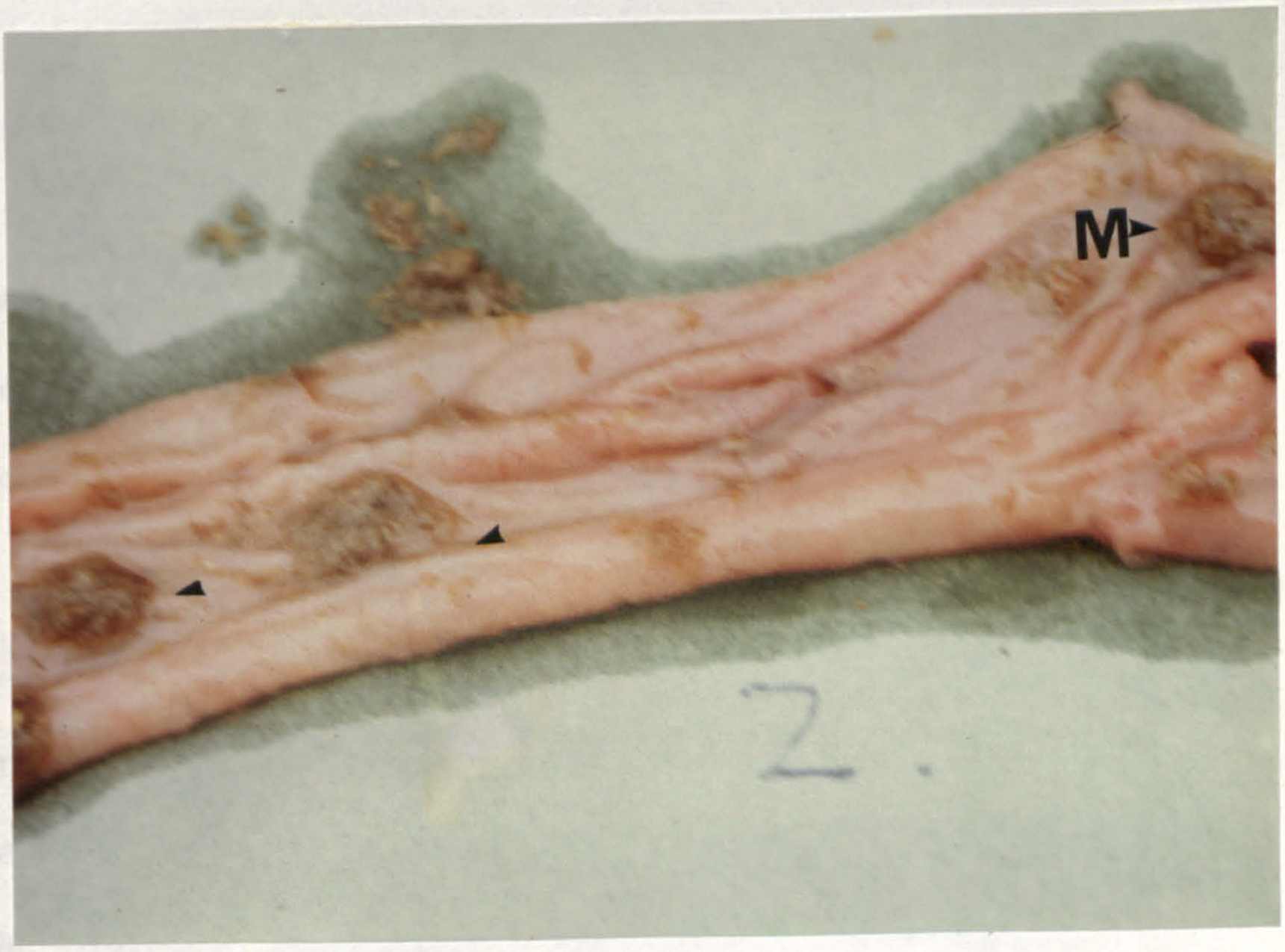


FIG. 46: Macroscopic appearance of the ileal mucosa of Pig 20, killed 21 days post inoculation with C. coli.
Note the mucoid contents (M).

mucosal surface of the terminal ileum. The villi of the distal part of the small intestine were lowered when viewed using the dissecting microscope and compared with those of the same region of the controls. The remainder of the gastrointestinal tract and its contents resembled those of the remainder of the group. The 5 control pigs appeared grossly normal with the exception of the jejunum of Pig 28 in which slight congestion and reduction of height of the villi was noted and the ileum of Pig 27 in which pin-point haemorrhages were seen on the mucosa. The thickening of the ileal wall and the enlargement of the mesenteric lymph nodes so prominent in the infected animals was not seen in these controls.

Histological findings

Changes seen in histological sections from the inoculated animals were most marked in the ileum and colon. In the ileum changes included stunting of the villi, cellular infiltration of the lamina propria with eosinophils and neutrophil polymorphs, mild capillary dilatation and lymphoid hyperplasia (Fig.47). In Pigs 20 and 23 inflammatory cells were seen in the crypts.

Colonic changes included marked dilatation of the crypts (Fig.48) and the presence of organisms within them. Mild capillary dilatation was also seen. Silver-stained bacteria were seen on the lumenal surface of the colonic mucosa.

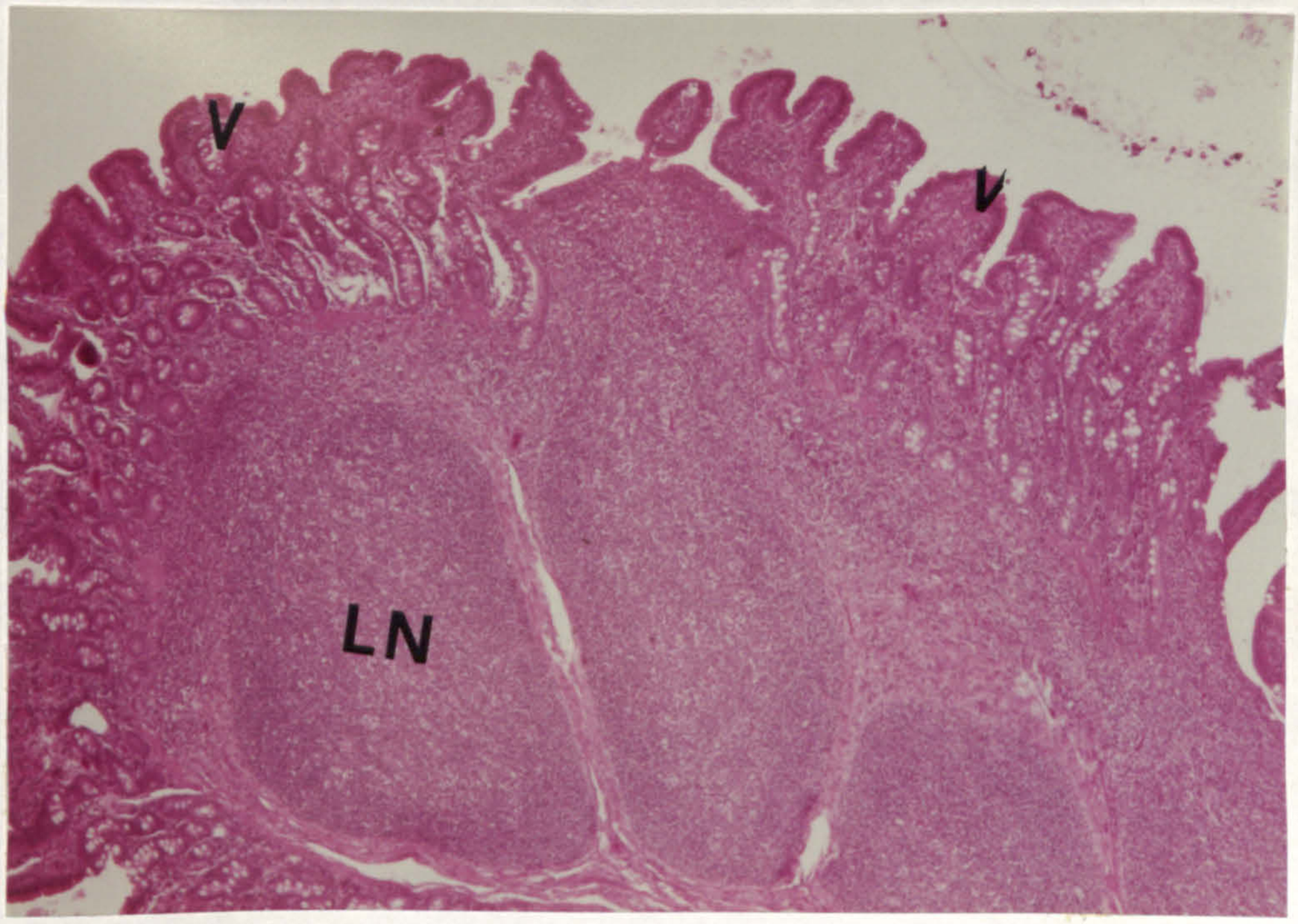


FIG. 47: Histological section of the ileal mucosa of Pig 21, 21 days post inoculation with C. coli. Note stunted villi (V), cellularity of lamina propria (LP) and submucosal lymphoid hyperplasia (LN). H & E x 35.

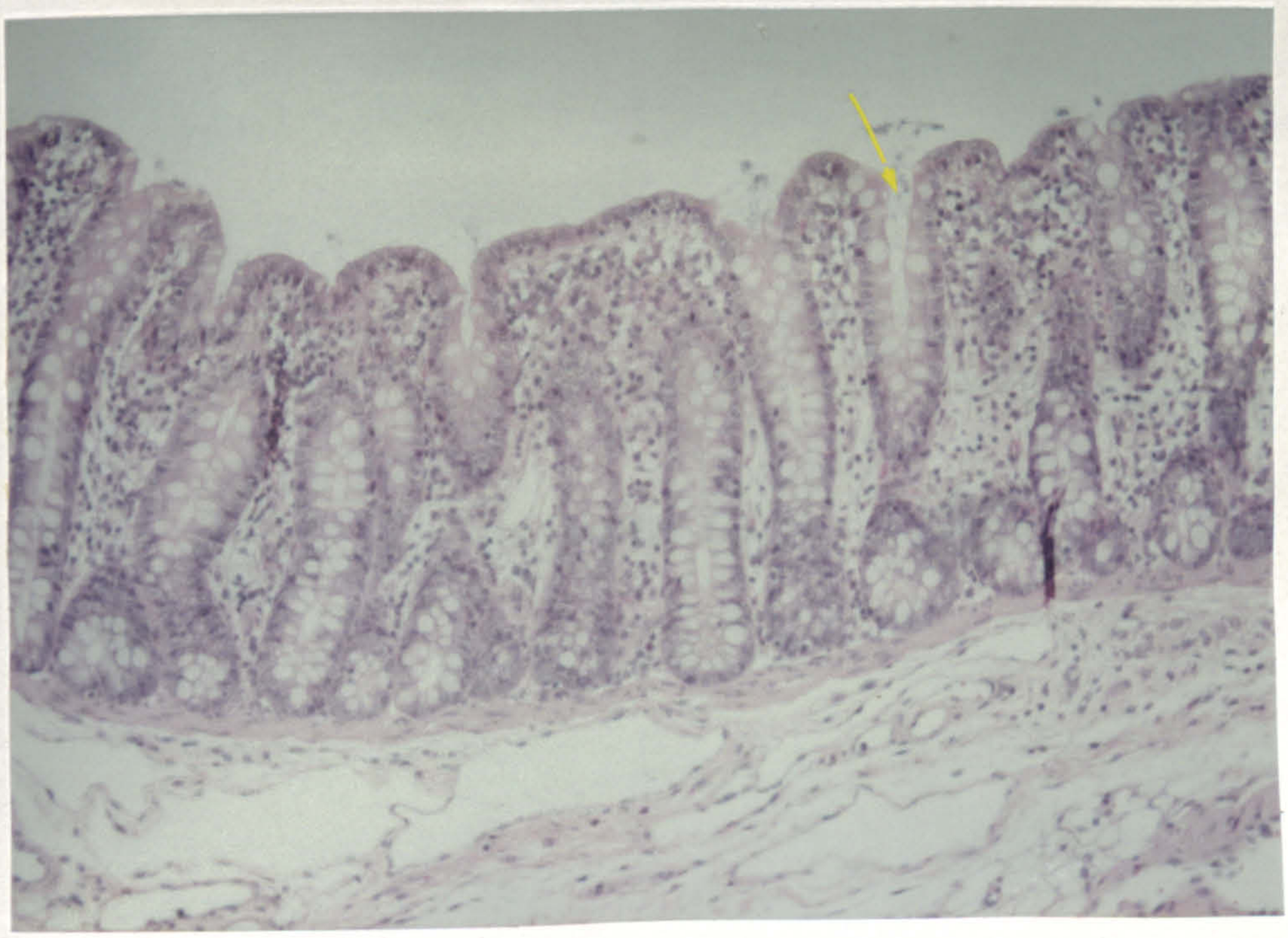


FIG. 48: Histological section of the colonic mucosa of Pig 21, 21 days post inoculation with C. coli. Note dilated crypts and the presence of organisms at the mouth of the crypts (arrow). H & E x 120.

In the control animals the above changes were much less marked. Accumulations of inflammatory cells were seen in the duodenal mucosa of Pig 25 and the villi were shortened in the upper part of the small intestine. Capillary dilatation was present in the laminal propria of the ileum of Pig 27, and mild inflammatory change was noted in the ileum of all 5 pigs.

Cryptosporidium was seen in the mucosal epithelium of Pig 28. Mild dilatation of the crypts were seen in the colonic mucosa of all pigs.

Bacteriological findings

Colonies of C. coli were isolated in large numbers from the mucosa of the ileum, caecum and colon in all the inoculated animals. Small numbers were isolated from the jejunum and from the duodenum in 3 of the 4 animals. Colonies of C. coli were isolated in small numbers from the mucosal surface of the colon in 3 of the control animals (Pigs 25, 27 and 29). Similar numbers of colonies were recovered from the ileal mucosa of 2 of these 3 pigs (Pigs 25 and 27).

C. coli was recovered from the mesenteric lymph nodes of 3 of the 5 infected animals but was not present in large numbers. The organism could not be recovered from the liver or gall bladder in any pig in this study. The identity of the isolates was confirmed by themethods described in Chapter 2. Table 25 shows the sites of isolation of C. coli at post mortem examination in the pigs of this experiment.

TABLE 25

Sites from which C. coli was isolated in experimental pigs killed 21 days following infection with pure cultures of the organism.

Site of isolation	Infected					Control				
	20	21	22	23	24	25	26	27	28	29
Stomach	-	-	-	-	-	-	-	-	-	-
Duodenum	-	-	+	+	+	-	-	-	-	-
Jejunum	+	+	+	+	+	-	-	-	-	-
Ileum	+++	+++	++	++	++	+	-	+	-	-
Caecum	++	++	++	++	++	-	-	-	-	-
Colon	++	++	+	++	+	+	-	+	-	+
Mesenteric Lymph Nodes	+	+	+	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-
Gall bladder	-	-	-	-	-	-	-	-	-	-
Lungs	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-	-

For key see Table 19.

Serological findings

Agglutinating antibody to the inocular strain of C. coli was present at titres of 1:320 (3/5) and 1:640 (2/5) in the sera of the infected animals at slaughter 21 days post-inoculation; but only at 1:10 and 1:20 in the controls. Serum antibody to the inocular strain was present in pre-inoculation sera at titres 1:10 and 1:20 in pigs of both the control and infected groups. The results are shown in detail in Table 26.

TABLE 26

Levels of agglutinating antibody to the inocular strain of C. coli in the sera of the conventional weaned pigs in Experiment 3.

Animal No.	Infected	Titre present	
		Day 0	Day 21
20	+	1:10	1:320
21	+	0	1:640
22	+	1:10	1:320
23	+	1:20	1:640
24	+	0	1:320
25	-	1:10	1:10
26	-	0	1:10
27	-	1:20	1:20
28	-	1:10	1:20
29	-	0	1:10

EXPERIMENT 4

OBJECTIVE: To determine the pathogenicity of C. coli for weaned hysterectomy-derived, colostrum-deprived pigs.

MATERIALS AND METHODS

A litter of hysterectomy-derived, colostrum-deprived piglets was produced by the methods described in Chapter 2 and for Experiment 1. Four piglets were reared to weaning by the methods described in Chapter 2. They were then fed on the ration used in the previous experiment from the 4th week of life onwards.

The 4 pigs were divided into 2 groups of 2 at 6 weeks of age and ear numbered 283, 284 (infected), 285 and 286 (controls); and housed in 2 separate pens bedded with sawdust. Water was made available freely at all times. A disinfectant footbath and separate clothing and equipment were used for each pen. The animals were monitored prior to infection and observed by the methods described in Chapter 2 and in this chapter for Experiments 1, 2 and 3. Clotted blood samples were taken from the animals prior to infection and the sera separated and stored as described in Chapter 2.

The inoculum was prepared by the same method and from the same isolate as that used in Experiments 1, 2 and 3. It contained 2.1×10^9 organisms. Inoculation was carried out as described in Chapter 2 and Experiment 3. The period of observation lasted for 18 days post-infection and the animals were killed on the 19th day by barbiturate injection and exsanguination, and examined post mortem by the methods

described in Chapter 2. Clotted blood samples were taken at euthanasia from all 4 pigs, and the sera stored and examined for the presence of antibody to C. coli by the methods described in Chapter 2.

Histological and bacteriological examinations were carried out using the methods described in Chapter 2.

RESULTS

Clinical findings

The faeces of all 4 pigs in this study were normal prior to infection. No Salmonella spp., B-haemolytic E. coli, C. perfringens Types A and C or campylobacters were isolated from the rectal swabs of the experimental animals prior to infection. No coccidial oocysts or nematode eggs were reported present.

Slight changes in faecal consistency were noticed in the infected pigs from the second day following inoculation. The faeces passed by the infected pigs were loose and very soft. The colour of the faeces varied from pale yellow to dark brown. Clear mucus was present on the faeces of the infected pigs as from the 3rd day following inoculation. Mucus was still present on the faeces even when it had become firm. The changes in the faecal consistency of pigs following inoculation are summarised in Table 27 below.

The faeces of the 2 control animals remained normal in appearance and consistency throughout the period of observation.

TABLE 27.. Faecal changes in HDCD weaned pigs following inoculation with pure cultures of C. coli and isolation of the organism from their faeces.

Pig's No.	Infected	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
283	+	N -	N -	S +	SM +	SM +	NM +	NM +	NM +	SM +	SM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	K
284	+	N -	N -	S +	SM +	SM +	SM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	NM +	N +	K
285	-	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	K
286	-	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	N -	K

N = Normal faeces
S = Soft faeces
M = Presence of mucus
B = Presence of blood
K = Killed

\+ = C. coli isolated
\- = No C. coli isolated

The infected pigs appeared depressed for 3 days from the 3rd day post inoculation. Even though the bodily condition of the inoculated pigs deteriorated throughout the period of observation when compared with the controls, they still remained active. The hair coat became rough in Pig 283 from the 5th day post inoculation and in 284 from the 6th day post inoculation. Pig 284 became thin and was seen to have hollow flanks towards the end of the experiment. The bodily condition of the uninoculated controls was normal in marked contrast to that of the infected animals (Figs. 49 and 50).

A rise in rectal temperature was noted as from the first day following inoculation in the 2 infected pigs (Pig 283 - 39.5°C, and Pig 284 - 40.1°C). The rise in rectal temperatures was maintained throughout the period of observation in Pig 284 but fluctuated as from the 9th day following inoculation in Pig 283. These changes in rectal temperatures are shown in Fig.51.

Faecal culture

Colonies of C. coli were isolated in large numbers from the faeces of the inoculated pigs from day 2 to the end of the experiment. C. coli was not isolated from the faeces of the controls throughout the period of observation.

Neither B-haemolytic E. coli, nor B-haemolytic Cl.perfringens was isolated from any of the experimental animals. The bacteria isolated from the faeces consisted largely of non-haemolytic E. coli and faecal streptococci.

FIG. 49: HDCD weaned pigs, 19 days post inoculation with C. coli, Experiment 4.
Note the poor bodily condition.

FIG. 50: Uninfected control HDCD weaned pigs, Experiment 4.

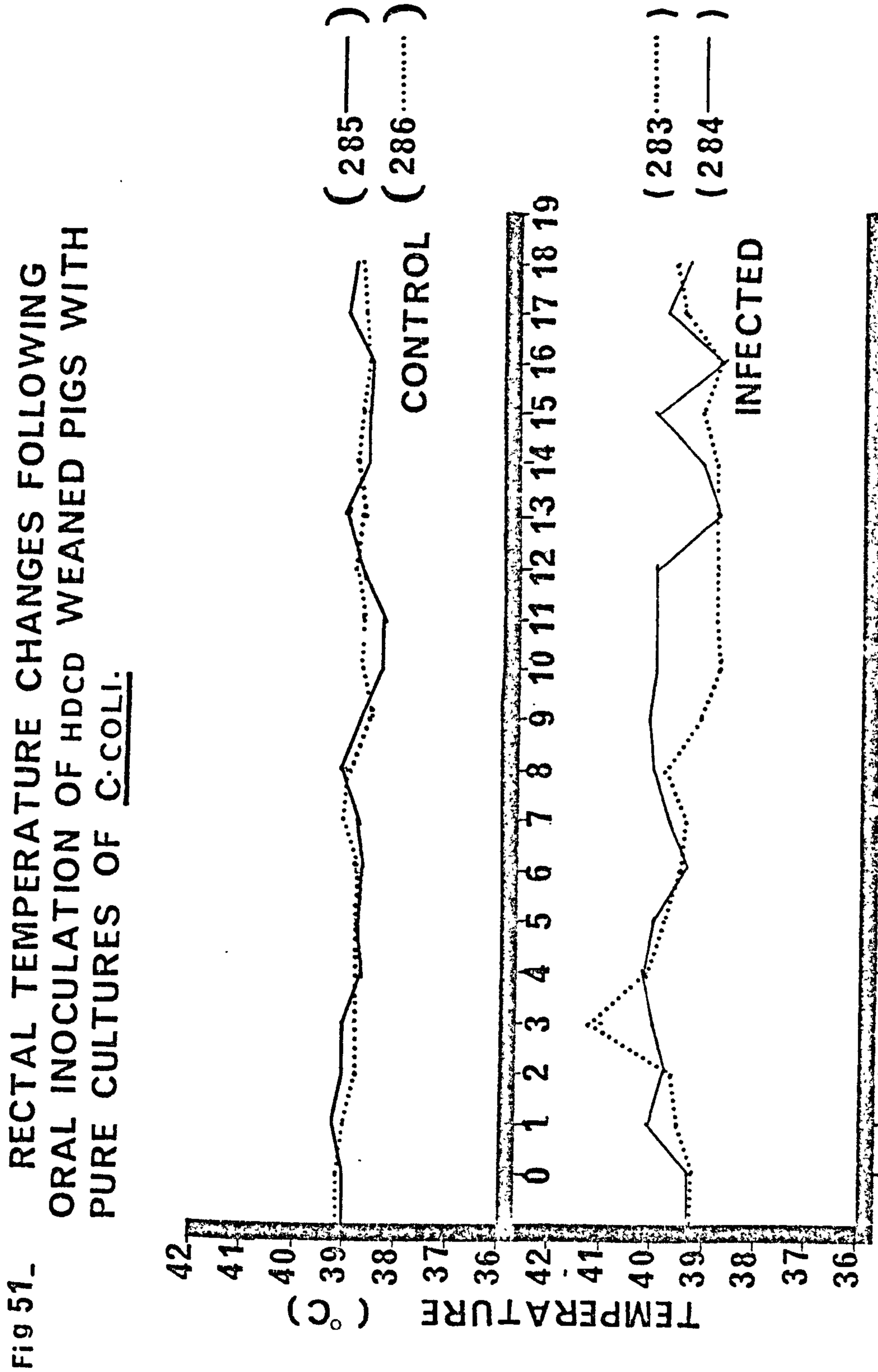


Fig 51_ RECTAL TEMPERATURE CHANGES FOLLOWING ORAL INOCULATION OF HDCD WEANED PIGS WITH PURE CULTURES OF C. COLI.

The identity of bacterial isolates was confirmed by the methods described in Chapter 2.

No nematode eggs or coccidial oocysts were reported to be present in their faeces.

Pathological findings

At post-mortem examination on day 19 post inoculation, the infected pigs were in relatively poor bodily condition when compared with the uninoculated controls. The infected animals had faecal staining or soiling of the perineum and had sunken eyes, hollowed flanks and hairy coats. The thoracic cavity and its organs were grossly normal. The spleen was slightly enlarged in Pig 284. The livers of the 2 infected pigs were slightly pale. The mesenteric lymph nodes were enlarged in both infected pigs, pale in Pig 283, and slightly yellowish and oedematous in Pig 284.

The stomach, its contents and mucosa were normal in both animals as was the duodenum. The jejunal wall appeared normal but the ileal serosa appeared pale and thickened especially in the distal portion.

The contents of both jejunum and ileum in both pigs were fluid, slimy with clear obvious mucus. The mucosal surface of the jejunum was mildly inflamed in places; while the ileal mucosa was hyperaemic particularly in the terminal portion. The wall of the ileum was thickened and fleshy. The height of the villi of the ileum was slightly reduced when compared with those of the controls under the dissecting microscope. The contents of caecum in both pigs were pasty

and contained some obvious mucus. Pinpoint haemorrhages were present on the mucosal surfaces. The colonic contents were firm in Pig 283 but pasty in Pig 284. Tags of clear mucus were present in both. The colonic mucosa was grossly normal.

The two control animals, Pigs 285 and 286 were in good bodily condition. The thoracic and abdominal cavities and their organs were grossly normal and the gastrointestinal tract was normal with normal contents and mucosa throughout its length. The mesenteric lymph nodes were markedly smaller than those of the infected group.

Histological findings

The most obvious changes were noted in the gastro-intestinal tract. The gastric mucosa appeared normal but there was slight lowering of the villi, dilatation of the crypts and the presence of excess mononuclear cells in the lamina propria of the duodenal mucosa of both pigs. In Pig 283, some inflammatory cells and red cells were present on the mucosal surface. The jejunal mucosa of both animals resembled the duodenal mucosa of 283 with lowered villi, and some dilated capillaries but without the cellular exudate.

Marked changes were seen in the ileal mucosa. In both animals there was stunting of the villi, an increase in the numbers of mononuclear cells in the lamina propria and very prominent submucosal lymphoid tissue which was reactive (Figs.52 and 53). There was some congestion and dilatation of the submucosal blood vessels. In addition to these

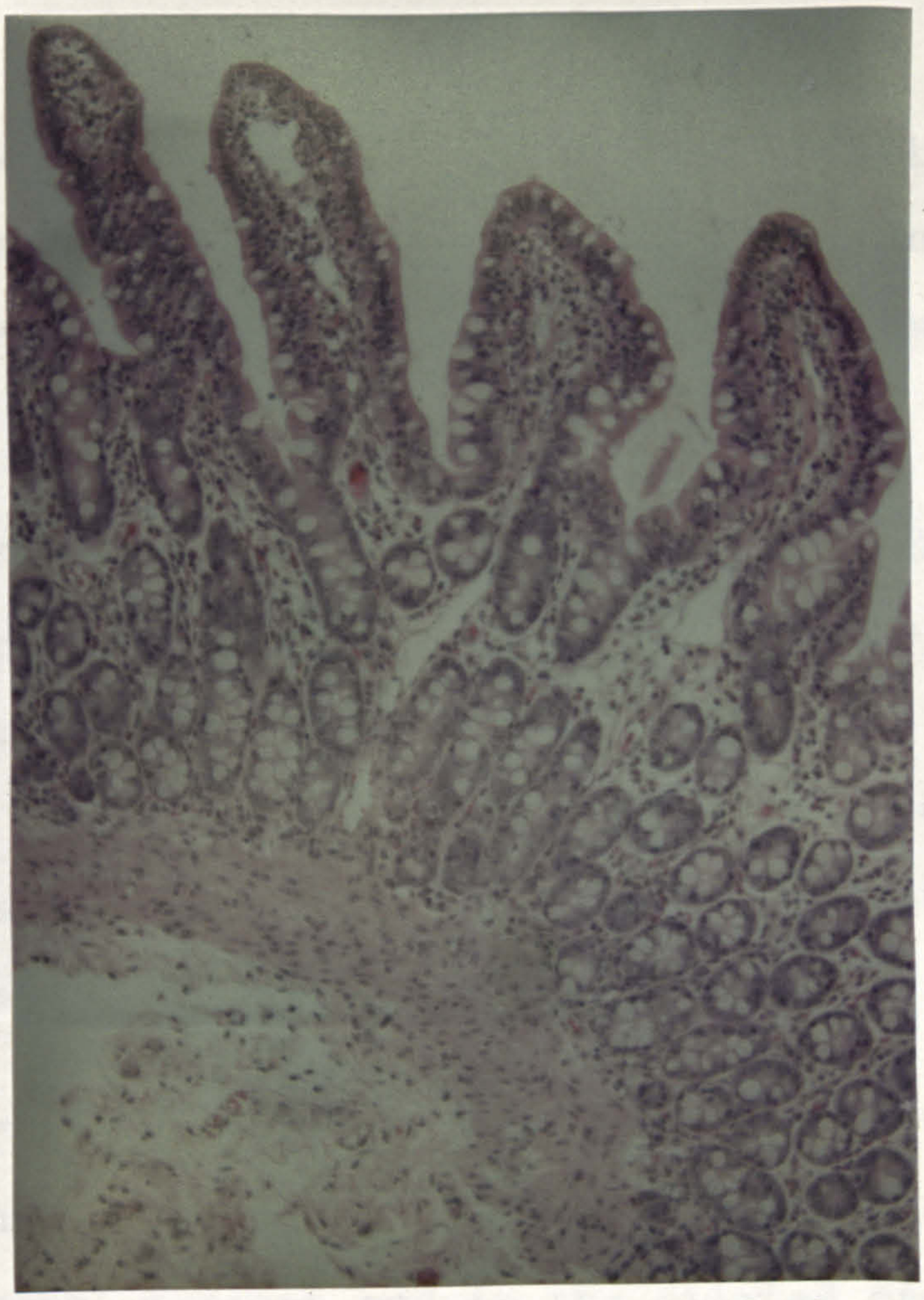


FIG. 52: Histological section of the ileal mucosa of Pig 285 - uninfected control HDCD weaned pig. Note the normal appearance of the villi and the lamina propria as compared with Figure 53.
H & E x 120

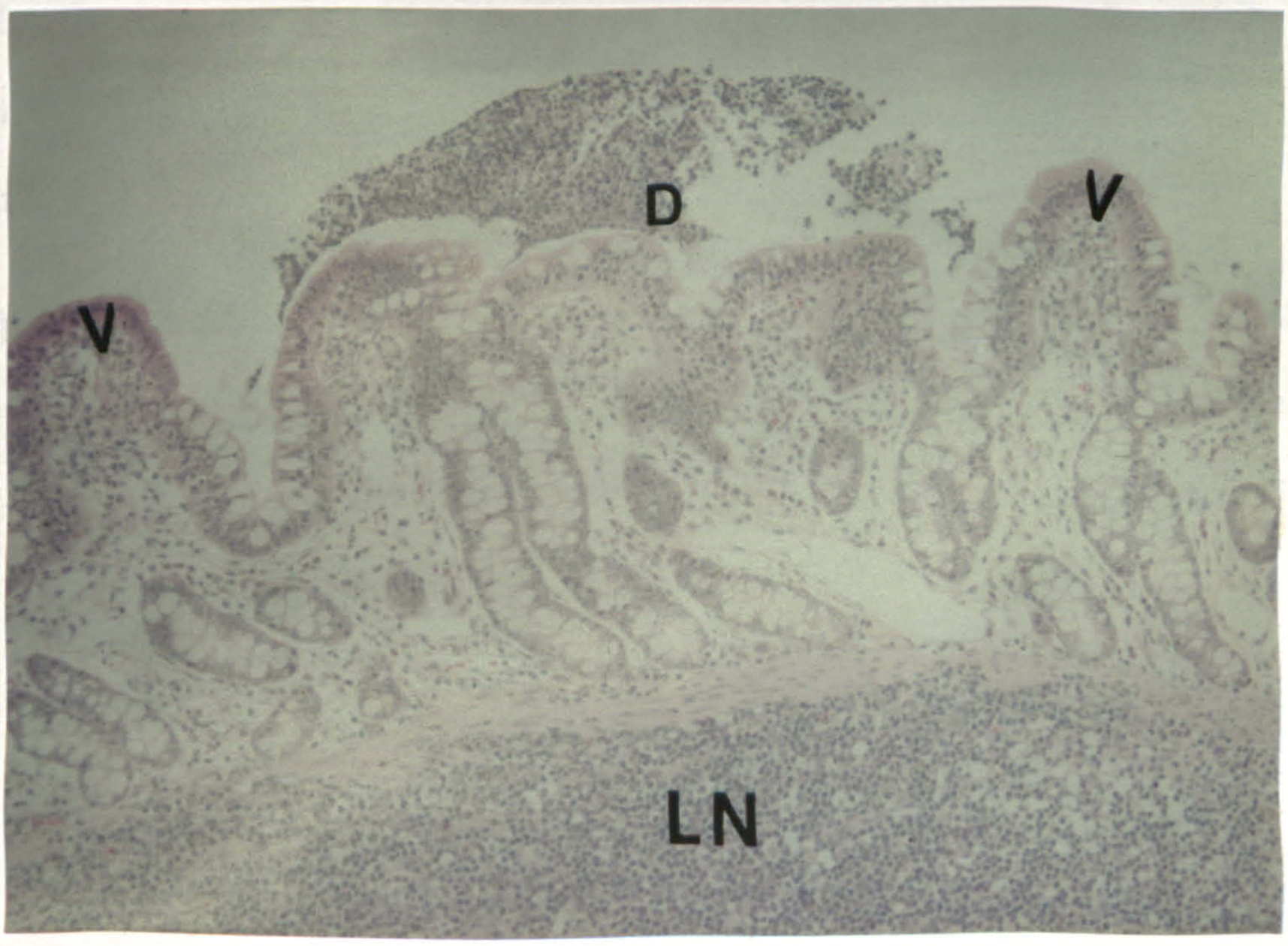


FIG. 53: Histological section of the ileal mucosa of HDCD weaned Pig 283, 19 days post inoculation with C. coli. Note the stunted villi (V), cellular debris adjacent to the lumenal surface (D) and submucosa lymphoid hyperplasia (LN).
H & E x 110

changes there was cellular debris which contained inflammatory cells and, apparently, some lymphocytes on the lumenal epithelium of Pig 283 and some oedema of the submucosal layer in Pig 284.

The large intestinal mucosa was similar in both animals. Varying amounts of cellular debris was present on the lumenal surface but disruption of the mucosal epithelium was not seen. The crypts were dilated and some contained cellular debris. There was local capillary dilatation in the lamina propria of Pig 283 (Figs.54 and 55).

Silver-stained bacteria were observed between the villi and in the crypts of the ileal mucosa. They were also seen on the lumenal surface and in the dilated crypts of the colonic mucosa but were not observed in the deeper tissues of the section (Fig.56).

The mesenteric lymph nodes were reactive in both animals and oedema and the presence of neutrophils was particularly prominent in those of Pig 283.

The gastrointestinal mucosa of the control animals was normal throughout although submucosal lymphoid tissue in the ileum was present but not reactive as in the infected animals. No silver-stained bacteria were observed in either the ileal or colonic sections from the controls.

Bacteriological findings

C. coli colonies were isolated in large numbers from the mucosa of the ileum, caecum and colon in both inoculated

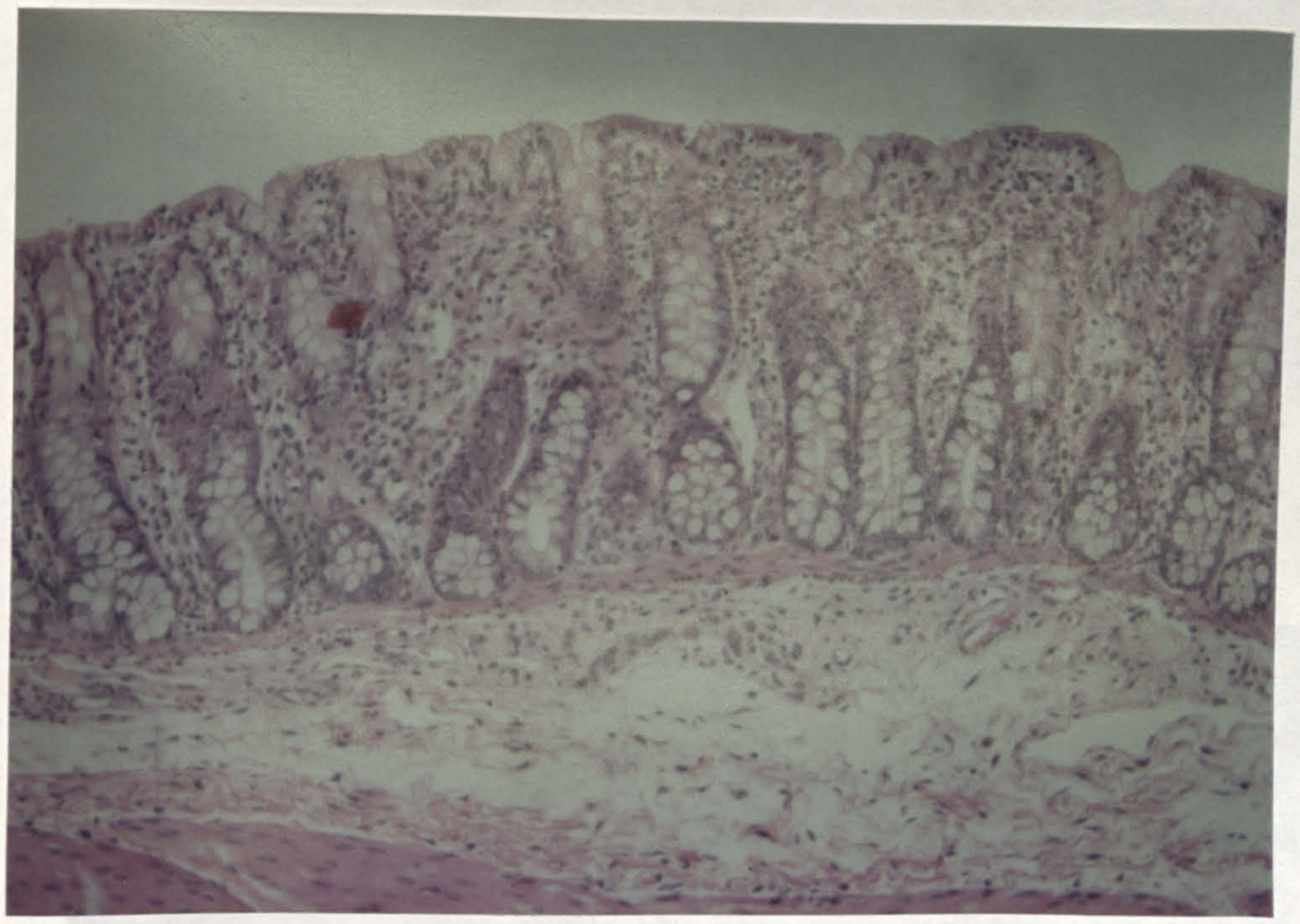


FIG. 54: Histological section of the colonic mucosa of Pig 285, uninfected control HDCD weaned pig.
Note the normal crypts.
H & E x 120

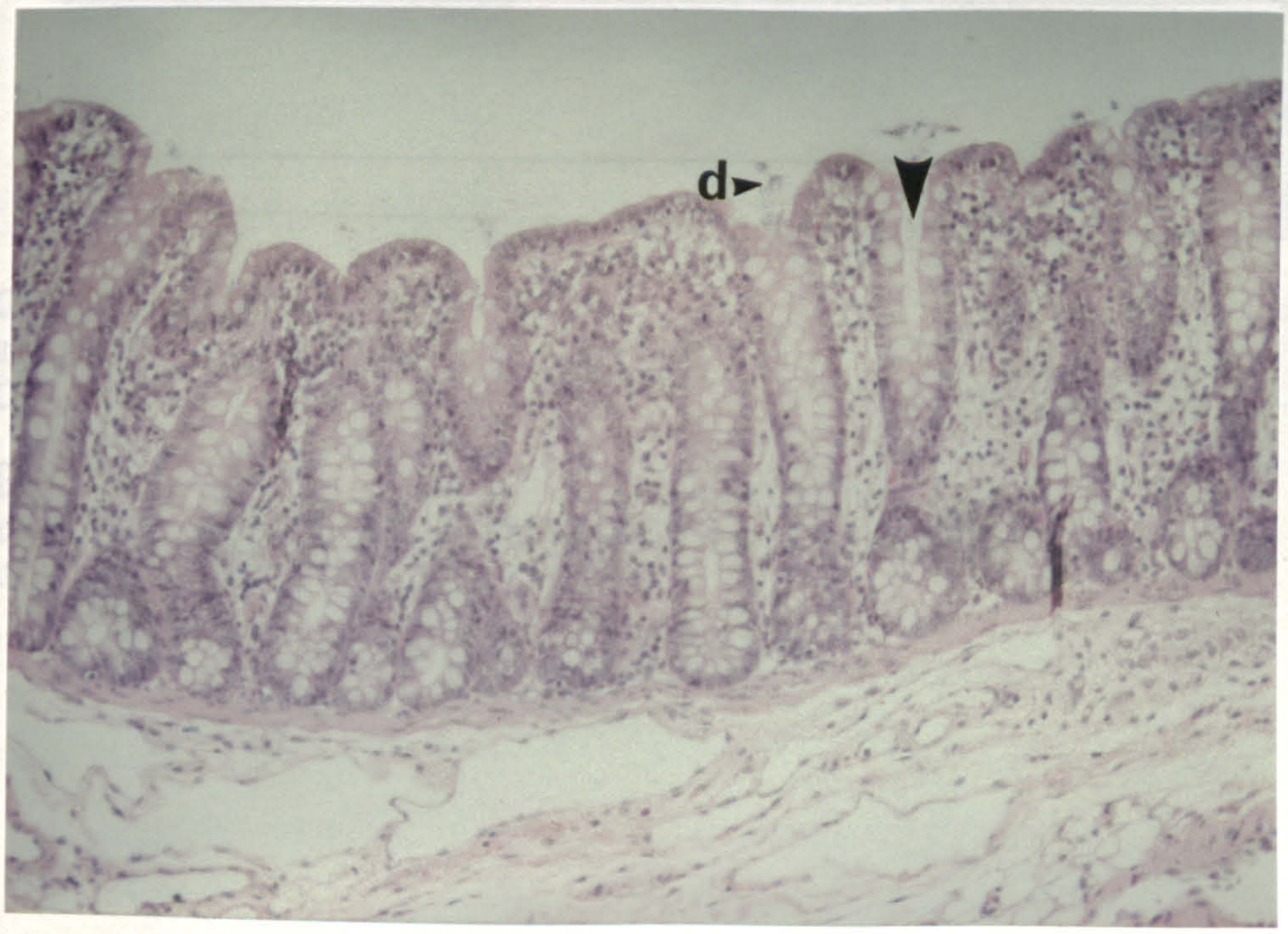


FIG. 55: Histological section of the colonic mucosa of HDCD weaned Pig 283, 19 days post inoculation with C.coli.
Note the dilated crypts (arrow) and the cell debris (D).
H & E x 120

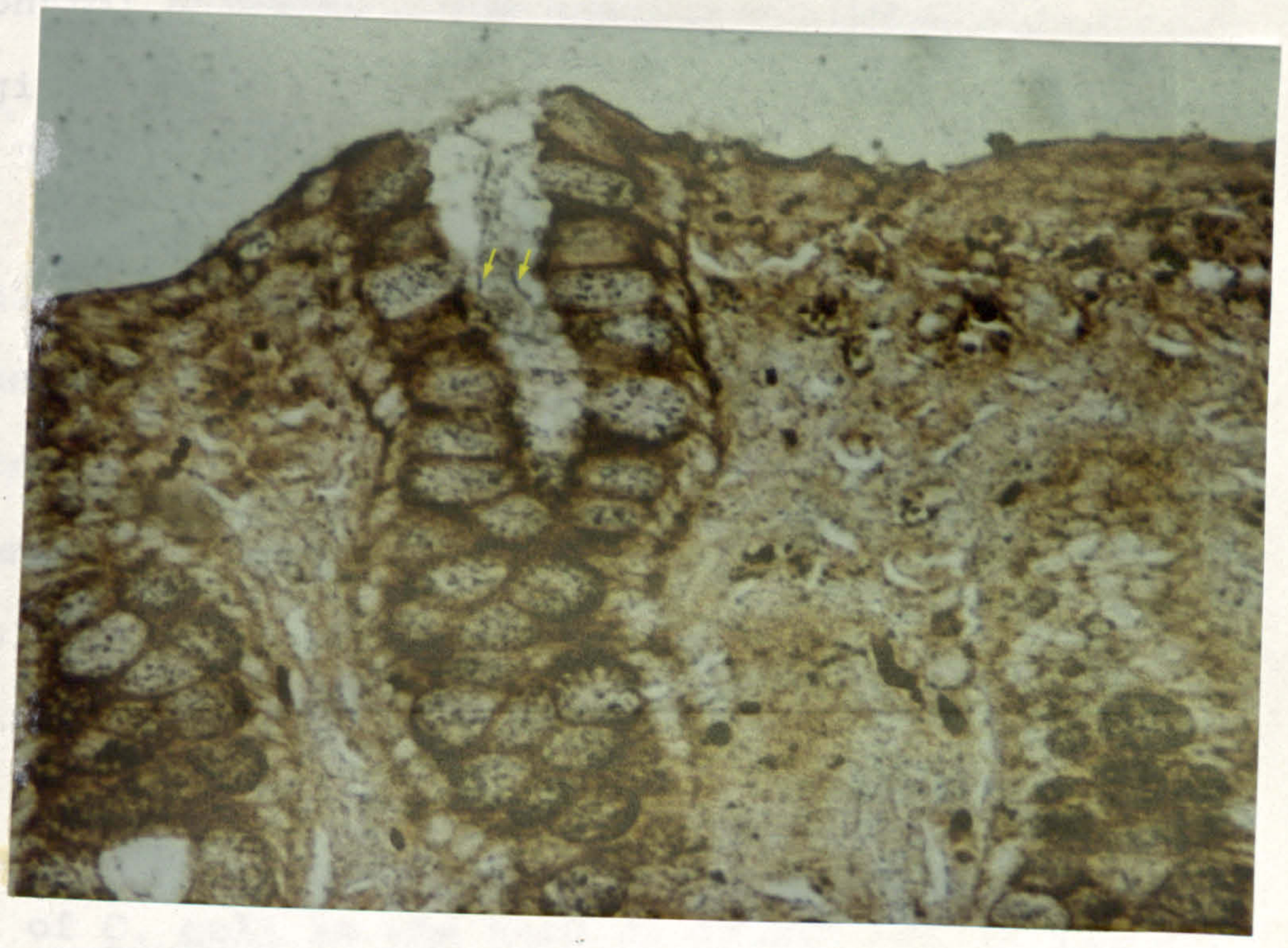


FIG. 56: Silver-stained histological section of the lumenal border and crypts of the colonic mucosa of HDCD weaned Pig 283, 19 days post inoculation with C. coli.
Note the silver-stained material which contained curved rods in the mouth of the crypt.
Young's x 1200

pigs. Smaller numbers of colonies were isolated from the jejunal mucosa of both animals. A few colonies of the organism were isolated from the mesenteric lymph nodes of the 2 inoculated animals. Two colonies were isolated from the liver of Pig 283. The organism was not isolated from any organs other than those listed above, nor was it isolated from the control animals. The results of the bacteriological examination for C. coli are summarised in Table 28.

Antibody to the inocular strain of C. coli was not found in the serum samples from the controls or from the infected group at the beginning of the experiment. At slaughter on the 19th day following infection, agglutinating antibody to the inocular strain was present in the sera of infected animals at titres of 1:640.

SECTION 2

Studies of C. coli in the herd of origin and in vitro

INTRODUCTION

The material reported in this section provides background information useful in assessing the infected status of the experimental animals (Studies 1 and 2) and the relationship of the organism to the host (Study 3).

The pigs used in these experimental studies were obtained from the Animal Husbandry Department Pig Herd (Chapter 2) and were found to be carriers of C. coli (Experiment 3). In order to obtain more information about them and about the carriage of C. coli, the faeces of pigs

TABLE 28

Sites from which C. coli was isolated from HDCD weaned pigs killed 19 days following inoculation with pure culture of the organism.

Site of inoculation	Infected		Uninfected controls	
	283	284	285	286
Stomach	-	-	-	-
Duodenum	-	-	-	-
Jejunum	+	+	-	-
Ileum	+++	++	-	-
Caecum	+++	++	-	-
Colon	++	++	-	-
Mesenteric Lymph Nodes	+	+	-	-
Liver	+	-	-	-
Gallbladder	-	-	-	-
Spleen	-	-	-	-
Kidney	-	-	-	-
Lungs	-	-	-	-

For key see Table 19.

in the herd were sampled and examined for the organism and other possible pathogens. This examination was carried out in 2 parts, the first as an in herd control to Experiment 2 monitoring a single litter on several occasions and the second as a general herd survey to provide information about infection especially in the animals from the weaned and fattening age groups used for studies such as Experiment 3.

STUDY 1. FAECAL MONITORING OF A LITTER OF PIGS CONTEMPORARY TO THE LITTER USED IN EXPERIMENT 2.

Materials and Methods

The conventional litter used was comprised of 10 piglets suckled by a single sow but containing 6 of her own and 4 fostered piglets from a contemporary farrowing. They were kept in the farrowing house under the management conditions described in Chapter 2 and were fed solely by the sow during the 12 days of observation. Iron injections were given on the 1st day of life. Three of these piglets were treated orally with antibiotic (Penbritin, Beecham) on the 3rd day of life as part of normal farm practice.

The appearance of the piglets, the consistency of their faeces and their rectal temperatures were recorded on days 2, 4, 7, 9 and 12 after birth. Rectal swabs were examined for the presence of C. coli and other bacteria by the methods described in Chapter 2. Whole rectal faeces from some of the piglets were negatively stained and examined for the presence of viral particles by electron microscopy.

Three piglets, 4217, 4218 and 4219 were killed on days 4, 9 and 12 of life by the methods described in Chapter 2.

Clotted blood samples were taken at slaughter from these piglets. The sera were stored and examined for the presence of agglutinating antibody to C. coli by the methods described in Chapter 2.

Post-mortem examination was carried out as described in Chapter 2. Histological and bacteriological examinations were also carried out by the methods described for the infected conventional piglets. The identity of the bacterial isolates was confirmed by the methods described in Chapter 2.

Results

Day 2. Watery diarrhoea was present in 2 of the 10 piglets on the 2nd day after birth. One of the piglets had splayleg. Rotavirus particles were demonstrated in the faeces of 1 of 3 faecal samples taken on that occasion. No B-haemolytic E. coli, haemolytic clostridia, salmonellae or campylobacters were isolated and no coccidial oocysts or nematode eggs were reported to be present. The results are summarised in Table 29.

Day 4. Three piglets had diarrhoea on this visit. One, with splayleg had severe mucoid diarrhoea and this animal was sacrificed and examined post mortem. Blood was seen on the firm faeces of one of the remaining animals. Two of those with normal faeces had been given antimicrobial therapy. One of these had a markedly raised rectal temperature (41°C)

TABLE 29

Summary of clinical and microbiological findings in the 10 conventional sucking piglets contemporary to the inoculated group in Experiment 2.

Day	Rectal temperatures in excess of 39°C	Number with diarrhoea	C.coli isolated	Viral particles demonstrated
2	N.D.	2/10	0/10	1/3 tested
4	1/10*	3/10	0/10	-
7	0/8	3/8	0/8	-
9	1/8	1/8	4/8	N.D.
12	0/7	3/7	5/7	N.D.

N.D. = Not Done

* = $\frac{\text{Number affected}}{\text{Number present}}$

No campylobacters were isolated from any of the samples and B-haemolytic E. coli, haemolytic C. perfringens and salmonellae were not isolated. Non haemolytic E. coli, non haemolytic clostridia and a weakly haemolytic streptococcus were isolated. No nematode eggs or coccidial oocysts were reported as present.

Day 7. One piglet died between the 4th and 7th day of life but was not made available for post mortem examination. Three of the remaining piglets had diarrhoea but none had raised rectal temperatures. C. coli was not isolated from any of the rectal faeces samples and no parasites were reported present.

The other bacteria present were the same as those isolated on Day 4.

Day 9. One animal had a rectal temperature of 40.8°C and was killed for examination. One of the remaining animals had pasty faeces with blood but the remainder had normal faeces. No nematode eggs or coccidial oocysts were reported as present in any of the faecal samples. C. coli was isolated from the faeces of 4 animals including the sacrificed animal with the raised rectal temperature and the one with pasty faeces. The other organisms isolated resembled those isolated on previous occasions.

Day 12. Three of the 8 remaining piglets had mucoid diarrhoea and 1 had normal faeces with a coating of excess mucus. One of the diarrhoeic animals which had an arthritic joint was killed and examined post mortem. C. coli was

isolated from the faeces of 5 animals including 3 with faecal changes. The other bacteria isolated resembled those identified on Day 4.

Pathological findings

The animal killed on day 4 (4217) had splayleg and enteritis. Villi were absent from the jejunum and those in the ileum were stunted. Pasty contents were adherent to the large intestinal mucosa. Rotaviral particles were demonstrated in the intestinal content when examined with electron microscope. Histological lesions of the small and large intestine consistent with those of rotavirus infection were seen. No campylobacters were isolated from the gastrointestinal tract and no B-haemolytic E. coli were isolated from the tract. A few colonies of a non haemolytic E. coli were isolated from all levels of the tract and a few colonies of a faecal streptococcus were also present in the large intestinal mucosa.

The animal killed on day 9 (4218) appeared grossly normal but on histological examination was found to have large numbers of coccidial bodies in the epithelium of the ileum. A few colonies of C. coli were isolated from the ileum and the colon of this pig and the other findings resembled those reported for pig 4217.

The animal killed on day 12 was found to have pallor of the jejunal and ileal serosa. The jejunal contents were fluid. The ileal contents were mucoid and adherent to the mucosa as were those of the caecum. Intestinal villi were

reduced in height. Small intestinal changes resembling those of late rotavirus or coccidial infection were seen histologically. Dilated crypts were present in the colon. A few colonies of C. coli were isolated from the ileum, caecum and colon of Pig 4219.

The other bacteria isolated were those described above under Pig 4217. The post mortem findings are summarised in Table 30.

Antibody to the strain of C. coli used in the transmission experiments was present at titres of 1:10 in the serum of the piglet killed on day 12.

STUDY 2. FAECAL MONITORING FOR C. COLI IN DIFFERENT AGE GROUPS OF PIGS IN THE HERD FROM WHICH ALL EXPERIMENTAL ANIMALS WERE OBTAINED (COCHNO FARM)

Materials and Methods

The numbers and ages of pigs sampled are shown in Table 31. The creep feed (Suckler care 402, B.O.C.M.) contained 50 ppm olaquindox (Fedan I.C.I.) at the time of sampling and was fed to animals from 10 days to 6 weeks of age. No other non nutrient additives were being fed. Rectal swabs were taken, placed in Amies transport medium (Vipak, Exogen Ltd.) and kept on ice pending examination. The samples were streaked onto selective medium plates containing Campylobacter selective medium and incubated under microaerophilic conditions as described in Chapter 2. Samples from 4 diarrhoeic pigs were streaked onto sheep blood agar and MacConkey plates which were incubated

TABLE 30

Sites from which C. coli was isolated in three conventional piglets killed at similar intervals to those used in Experiment 2.

Site of isolation	4th day	9th day	12th day
Stomach	-	-	-
Duodenum	-	-	-
Jejunum	-	-	-
Ileum	-	+	+
Caecum	-	-	+
Colon	-	+	+
Liver	-	-	-
Gallbladder	-	-	-
Mesenteric Lymph Nodes	-	-	-
Spleen	-	-	-
Lung	-	-	-
Kidney	-	-	-

For key see Table 19.

aerobically, and on to spectinomycin blood agar which was incubated anaerobically.

Plates were examined after 24 hours, 48 hours and 72 hours incubation. Plates were reincubated for longer periods as necessary. The bacteria isolated were presumptively identified by their colonial morphology and their identity was confirmed by the methods described in Chapter 2.

Results

Four weaned pigs were observed to have clinical diarrhoea at the time of observation. Three of these pigs were in the 6-8 weeks age group, and were receiving the creep feed medicated with olaquindox at 50 ppm.

The faeces passed by these pigs was fluid and dark brown in colour. Necrotic debris were present in the faeces. The animals themselves had hollow flanks and were hairy but not more so than unaffected pen mates.

The remaining animal was 15 weeks of age and had similar diarrhoea but without any obvious loss of condition. At a distance, the faeces presented a shiny appearance, even though clear mucus was not noted to be present.

All the remaining pigs examined in this study did not have clinical diarrhoea at the time of examination (Table 31).

TABLE 31. Results of Study 2, the distribution of faecal shedding of C. coli in the Cochno Farm herd. (Farm 3).

	Age	Total number examined	No.with clinical diarrhoea	No.from which C.coli was isolated
Sucking piglets with no creep feed	8 days	7	0/7	0/7
Sucking piglets with creep feed	21 days	5	0/5	1/5
Sucking piglets with creep	27 days	6	0/6	1/6
Weaners (in flat deck) with creep feed	4-6 weeks	10	3/10	4/10 *
Weaners (in flat deck) without creep	6-8 weeks	10	0/10	0/10
Growers	8-10 weeks	10	0/10	3/10
Fatteners	15 weeks	10	1/10	6/10 *
Farrowing sows		6	0/6	0/6
Sows in Dry House		20	0/20	3/20

* pigs with diarrhoea yielded profuse cultures of C. coli and non T. hyodysenteriae spirochaetes.

Small numbers of colonies were isolated from the pigs shown in Table 31. The organism was only isolated in profuse culture from the 4 pigs with clinical diarrhoea.

A weakly B-haemolytic spirochaete differing from T. hyodysenteriae was isolated from all the 4 diarrhoeic samples. A weakly haemolytic faecal streptococcus was also isolated from all 4 pigs. B-haemolytic E. coli was not isolated from any of the 4 diarrhoeic faeces and even E. coli was rare in the samples from animals receiving olaquindox.

STUDY 3. ADHESION STUDIES WITH BACTERIAL CULTURES AND ISOLATED INTESTINAL BRUSH BORDERS

In this study an attempt was made to investigate the interaction between the host mucosal surface and C. coli using E. coli and C. perfringens Type A as controls. C. coli cultures were studied for their ability to adhere to isolated brush borders.

Materials and Methods

The isolates of C. coli and of C. perfringens Type A used in this study were those obtained from enteric lesions in the survey of enteric bacteria as described in Chapter 3 and were the same as those used in the transmission studies described in Chapters 4 and 5.

B-haemolytic E. coli isolated from a piglet in the survey with diarrhoea was used as a positive control for this study.

The bacteria under test were maintained as freeze-dried isolates at the passage numbers described in the appropriate chapters, but the E. coli was stored on a nutrient agar slope. Cultures were prepared for this test within one subculture of the stored state.

Brush borders were prepared from small intestines obtained from weaned pigs slaughtered in the course of experiments at the Veterinary School. These pigs had no enteric lesions and were negative on culture for C. coli, B-haemolytic E. coli and C. perfringens Type A.

The method used was that of Sellwood et al (1975) with minor differences in the concentrations of brush borders and organisms used. These are described below:

.Horse blood agar plate cultures were inoculated overnight or for 48 hours at 37°C, under appropriate atmospheres.

The growth was suspended in Krebs-Henseleit buffer and washed twice with the same solution and finally resuspended in the buffer. These suspensions were standardised at tube 8 using Wellcome Opacity Tubes (Wellcome Reagents Ltd.).

Brush borders were resuspended in the same buffer and also standardised at tube 8.

Results

Campylobacters: Bacteria were seen to adhere loosely and in relatively small numbers to the isolated brush borders. They did not obscure the brush border entirely.

C. perfringens Type A: This acted as a negative control and showed no adherence to the brush borders. Bacteria were seen lying adjacent to the brush borders but were not attached to or adherent to them.

E. coli: These provided a positive control and adhered to the brush borders in the numbers described by Sellwood et al. (1975).

DISCUSSION

The results of Experiment 1 suggest that the isolate of C. coli used was capable of initiating clinical signs in HDCD piglets. The faeces of the inoculated pigs were bulky, soft, pale in colour and contained mucus and on one occasion, fresh blood. The changes were not dramatic and did not cause death. The incubation period appeared to be 2-3 days. The rise in the rectal temperatures of the inoculated group occurred at a time when low environmental temperatures were present and were corrected and can best be seen by comparison of the rectal temperatures of both groups (Fig.29). In this study, the condition of the infected pigs was markedly reduced when compared with that of the controls. The inoculated piglets remained depressed from the 3rd day of infection until the end of the experiment with hollow flanks, hairy coats, sunken eyes and general deterioration.

The control piglets remained in good condition until killed and passed hard dark coloured faeces, providing a marked contrast with the inoculated animals. These findings also applied to Pig 4 in spite of its destruction on humane grounds.

The clinical signs in conventional suckling piglets were less pronounced than those seen in HDCD piglets. However, mild fever, softening of the faeces with obvious mucus and reduced bodily condition were observed in the infected piglets in Experiment 2. Soft faeces was passed by 2 piglets on the 3rd day following infection. Faecal changes were noted in all infected piglets within 6 days of inoculation as shown in Table 21. None of these changes were observed in the piglets killed prior to infection and 4 hours after infection. Infection, as in Experiment 1, did not result in death of any of the piglets.

The litter of pigs used in this study did not appear to develop the clinical signs seen in contemporary animals in the litter monitored in the herd of origin (Study 1) although it is possible that some of the agents identified there were present. That litter with its mixture of pigs from 2 sources may have been especially susceptible to disease and was also located in the farm farrowing house while the litter used in Experiment 2 was in separate cleaned accommodation.

In Experiment 3, changes observed in conventional weaned pigs were mild. These were limited to slight fever, slight softening of the faeces with some excess mucus

(Table 24). The slight nature of the changes was underlined by the similarity of feed conversion ratio and liveweight gain in the 2 groups.

The changes in faecal consistency lasted for 6 days but obvious mucus was still present in firm faeces. Elevation of rectal temperature occurred within 1 and 4 days of infection and reached a maximum of 41°C in Pig 20 (Fig.45). The raised rectal temperatures persisted for a variable length of time and were often very inconsistent.

In experiment 4, the clinical changes noted in infected HDCD weaned pigs were more pronounced than those seen in infected conventional weaned pigs. Changes in faecal consistency were noticed as from the 2nd to the 3rd day following inoculation. The faeces were loose and obvious mucus could be seen as shown in Table 27. Fever was noted as from the 1st day following inoculation. The body condition was depressed in the infected group as compared with the uninfected controls (Figs. 49 and 50). The clinical changes seen in the infected animals were no longer evident from day 10 onwards. None of these changes were observed in the uninoculated controls.

These results appear to indicate that inoculation of pigs with pure cultures of C. coli is followed by a specific syndrome in which a rise in rectal temperature to 40-41°C occurs within days and faecal changes develop within 2-3 days and last for at least 12 days. These faecal changes include softening of the faeces or diarrhoea and the presence of mucus which on occasions may contain blood. The

changes are most obvious in young non-immune pigs (Experiment 1) and young conventional pigs suckling a mother from a herd in which infection is present in sows (Experiment 2, and Study 2). In older weaned pigs the changes are slight but are again more pronounced in non immune pigs (Experiment 4) than in immune pigs (Experiment 3). The changes are associated with loss in condition although this was not quantified in the younger and non-immune pigs.

In all 4 experiments, C. coli was recovered from the faeces of the infected pigs within 2 days of inoculation and there was no doubt that it had become established. In Experiment 3, small numbers of colonies of C. coli were isolated from the faeces of conventional weaned pigs prior to infection and from the control group in the experiment. No attempt was made to distinguish between faecal C. coli organisms of the inocular and resident strains in the infected animals. The absence of C. coli from the faeces of the control animals in Experiments 1 and 4 emphasises their causal role in the syndrome noted. In experiments 2 and 3 it is probable that immunity of some type was already present as C. coli was found in piglets aged 9 days or more in the contemporary litter control in Study 1 and to be present in every age group of pigs except for the youngest in Study 2 on the farm of origin. This supposition was confirmed by the presence of agglutinating antibody in the sera of the conventional animals used in Experiment 3 prior to infection even though it was only present at a low level. Inoculation with C. coli was, however, followed by a rise in agglutinating antibody to the inocular strain in all 4

experiments and this, with its recovery in profuse culture from the faeces of the inoculated pigs provides evidence that C. coli initiated the syndrome.

Gross pathological changes attributable to C. coli infection were seen in infected animals in all 4 experiments. The changes seen were observed 10 or more days post infection in Experiments 1, 3, 4 and in 4 pigs in Experiment 2. They were, therefore, late or chronic lesions and in this study, earlier lesions were only examined in Experiment 2 in which piglets were killed 4 hours, 4, 5 and 7 days post infection. The changes seen in all 4 experiments were most striking in the non immune piglets of Experiment 1 and non-immune weaned pigs of Experiment 4. The ileum was pale and fleshy in appearance and the contents were fluid with some excess clear mucus. The cut surface of the ileal mucosa was thickened and there was patchy erythema of its lumenal surface. The mesenteric lymph nodes were enlarged and pale. These findings resembled those seen in field cases of pigs from which C. coli was isolated. In some cases, particularly in Experiment 3 some degree of ileal change was seen in the controls but in all cases comparison of infected and control intestines and mesenteric lymph nodes emphasised the changes described above. The changes noted in the large intestines of the pigs in this series were more difficult to attribute to C. coli particularly in Experiment 1.

The histological changes were slight in most cases. In all cases lymphoid hyperplasia and cellular infiltration of the lamina propria of the ileum was a feature of the infected animals. In some of the cases, particularly in

Experiments 1 and 4, this type of change was not observed in the controls but in the conventional weaned pigs of Experiment 3 it was present to some degree. Other changes of campylobacter infection such as stunted villi, accumulation of lymphocytic and inflammatory cells in the crypts, capillary dilatation and polymorphonuclear leucocyte infiltration of the lamina propria were also seen in varying degrees in the conventional weaned control animals in Experiment 3. In all cases these changes were not obvious in the inoculated animals.

The large intestinal changes were either present to some extent in both control and infected pigs (Experiment 1) or were marked (Experiment 2). In Experiments 3 and 4, dilated crypts filled with bacteria were seen in the infected animals and were less obvious in the controls.

Silver-stained bacteria were observed in the lumenal surface of the small intestine, and in between the villi (Fig. 43). Organisms were not present in the apical cytoplasm. In the large intestine, few crypts were colonised. The organisms were present in the crypts but not in the epithelial cells or in the lamina propria. The findings here are in contrast with the situation with C. sputorum ss. mucosalis, where bacteria are demonstrated intracellularly in the apical cytoplasm of altered mucosal epithelial cells.

Other changes seen in the pigs of Experiment 2 may have been due to subclinical infection with the agents identified in Study 1 (rotavirus and coccidia) although the latter were not identified in that experiment. The presence

of cryptosporidium in the pigs of Experiment 3 is also of unknown significance.

C. coli was recovered from the mucosa of the ileum and large intestine in large numbers, less frequently from the duodenum, jejunum and mesenteric lymph nodes and on one occasion (Experiment 4, Pig 283) from the liver (Tables 19, 22, 25 and 28). Few colonies were isolated from the infected mucosal sites in the control conventional pigs (Experiment 3 and Study 1), markedly fewer than the numbers isolated from similar sites in infected animals although counts would be needed to provide objective information on this point.

C. coli was isolated from the sites at which the most obvious changes described above were seen, i.e. the ileum and, to a lesser extent the colon providing further evidence for the involvement of the organism in the lesions described. The only discrepancy was the difficulty in determining their role in the large intestinal mucosa from which they were isolated in profuse culture but in which few specific gross or microscopic changes were identified in infected animals when compared with the controls. This was particularly relevant in Experiment 1.

In the silver-stained sections, bacteria were seen lying closely to the brush borders of the epithelial mucosa but not closely attached to them. This finding was confirmed in Study 3 in which the organisms were seen to adhere loosely to the isolated brush borders in contrast to the close attachment seen with E. coli.

The results discussed above suggest that C. coli can initiate clinical signs and pathological changes when fed orally to HDCD piglets and weaned pigs; and can also initiate some features of the syndrome observed in them when given both to conventional piglets and to conventional weaned pigs. The changes seen in the conventional pigs were less marked and more difficult to interpret than those in the HDCD pigs because of the presence of other infectious agents and some degree of immunity in the herd of origin and in control litters.

CHAPTER 5

Experimental infection with Clostridium perfringens Type A

INTRODUCTION

C. perfringens Type A was isolated from inflammatory lesions in the intestinal tracts of 23 pigs in the survey described in Chapter 3. C. perfringens Type A is known to cause food poisoning in man (Hobbs, 1965), necrotic enteritis in chickens (Al Sheikhly and Truscott, 1977a,b) and has been considered to cause diarrhoea in lambs (Hauschild et al., 1967) and calves (Niilo and Dorward, 1971). Although Type A has been isolated from pig faeces and intestinal contents on some occasions (Amtsberg et al., 1976), it is still uncertain whether or not it plays any significant part in enteric diseases of pigs.

In order to assess the importance of C. perfringens Type A as a pathogen of pigs, a series of controlled experiments was carried out in HDCD piglets (Experiment 5) and in conventional weaned pigs (Experiments 6 and 7). The organism used in these studies was an isolate of C. perfringens Type A obtained from enteric lesions in the small intestine of a 3-day old diarrhoeic piglet (70, Appendix 1). It had been cloned twice and then freeze-dried using the method described in Chapter 2.

THE INOCULATION OF PIGS WITH PURE CULTURES OF C. PERFRINGENS TYPE A

EXPERIMENT 5

OBJECTIVE: To determine the pathogenicity of an isolate of C. perfringens Type A for hysterectomy-derived, colostrum deprived (HDCD) piglets.

MATERIALS AND METHODS

The HDCD piglets were produced and reared as described in Chapter 2 and were of the same group as those used in Experiment 1 (Chapter 4). They were housed individually in isolators in a temperature-controlled room. Infected and control piglets were maintained in separate blocks of cages. The 3 infected animals were P7, P8 and P9, while P4, P5 and P6 formed the control group and have already been described in Experiment 1 (Chapter 4).

Faecal samples from each piglet were examined daily for 4 days prior to inoculation for the presence of B-haemolytic E. coli: C. perfringens, campylobacters, salmonellae and other bacteria, nematode eggs and coccidia by the methods described in Chapter 2.

The inoculum was prepared from the isolate of C. perfringens Type A described above by the methods outlined in Chapter 2. 10ml of inoculum containing approximately 2.0×10^9 organisms was given to each piglet on day 4 of life after it had been fasted for 24 hours.

All piglets were examined daily and their appearance, appetite, rectal temperature and the consistency of their faeces were noted and recorded.

Rectal faeces samples were taken from animals with diarrhoea and rectal swabs from all other animals on each occasion that clinical observations were made. These samples were examined daily for the presence of C. perfringens Type A and other bacteria by the methods described in Chapter 2. In this experiment reinforced clostridial medium was not yet in use and horseblood agar plates were used for anaerobic culture. The colonies of C. perfringens Type A were identified by the methods described in Chapter 2.

Negatively-stained preparations were made from faeces of piglet P8 on day 2 when diarrhoea was observed, and these were examined by electron-microscopy for the presence of virus particles.

Clotted blood samples were taken at post mortem from all piglets, stored according to the methods described in Chapter 2, and examined for the presence of antibody to C. perfringens Type A of the inocular strain by the methods described in Chapter 2.

The period of observation lasted for 12 days. Piglet P8 that died in the acute stage of the disease was examined at post-mortem. The second piglet (P9) was killed and examined at post-mortem on the 3rd day following inoculation, while P7 was killed on the 12th day following inoculation. The control animals were killed on the days

described in Experiment 1 and shown in Table 18. Post mortem examination, histological and bacterial examinations were carried out on all the 6 piglets by the methods described in Chapter 2.

RESULTS

Changes in faecal consistency were noted in all 3 inoculated piglets within 48 hours of inoculation. Softening of the faeces was first observed in Pig P7 on day 1 and in piglets P8 and P9 on day 2. The diarrhoea was profuse, creamy in colour and soiled the hindquarters of all 3 piglets. The faecal changes persisted in all the 3 piglets until death or until the end of the observation period. Flecks of blood were noted in the soft faeces of Pig P7 from the 4th day following inoculation (Fig.57). The faecal changes are summarised in Table 32.

Piglets P8 and P9 were severely affected on the 2nd day following infection. They were weak, in poor bodily condition, dull and lying down showing no inclination to feed. Pig P7 was able to stagger around. Piglet P8 was found dead in the early morning of the 3rd day following infection, and piglet P9 was moribund. It was killed later on day 3. Piglet P7 remained weak with signs of illness for the rest of the time of observation after which it was killed with the surviving controls on day 12. None of these clinical signs were observed in the controls (see Experiment 1, Chapter 4).

TABLE 32

Changes in faecal consistency following the inoculation of HDCD piglets with pure cultures of C. perfringens Type A, and isolation of the organism.

Piglet No.	Infected	Day of experiment 0	1	2	3	4	5	6	7	8	9	10	11	12
P7	+	N −	S +	D +	D +	DB +	SB +	SB +	SB +	SSM +	SM +	SB +	SB +	K
P8	+	N −	N +	D +	Died									
P9	+	N −	N +	D +	D_K									
P4	−	N −	N −	N −	N −	N_K								
P5	−	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K
P6	−	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K

N = Normal firm faeces
S = Soft faeces
D = Diarrhoea
B = Presence of blood in faeces
M = Presence of mucus in faeces
\+ = C. perfringens Type A isolated
− = No C. perfringens Type A isolated
K = Killed

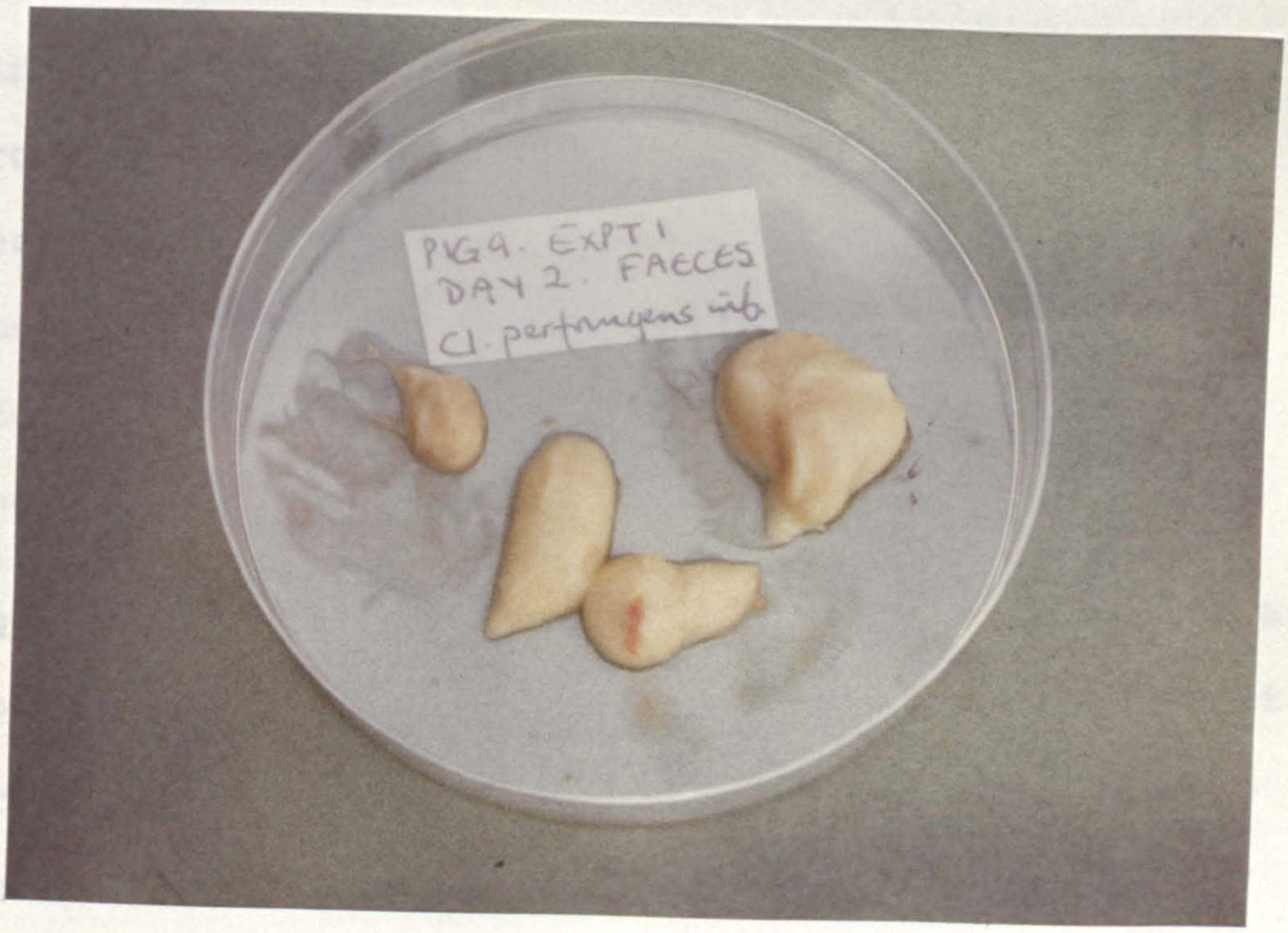


FIG. 57: Faeces passed by HDCD Piglet 9, 2 days post inoculation with C. perfringens Type A. Note the presence of blood on the soft faeces.

A transient rise in rectal temperature to 40°C was noted in piglet P9 on the 2nd day following inoculation and then dropped to 34.4°C on day 3 (Fig.58).

Faecal culture

C. perfringens Type A was isolated from the rectal swabs and faeces of all the inoculated animals from the lst day after inoculation. The organism was consistently isolated from the faeces of infected piglet P7 which lived until the end of the experiment (Table 32). The organism could not be isolated from the faeces of the inoculated animals prior to infection and was never isolated from the rectal swabs of the control animals.

No virus particles were demonstrated in the faeces of piglet P8. Nematode eggs and coccidial oocysts were not reported present. B-haemolytic E. coli and campylobacters were not isolated. Non haemolytic E. coli and non haemolytic clostridia were isolated from faeces as described in Experiment 1 (Chapter 4).

Pathological findings

The post-mortem findings in the 3 control piglets have been described in Experiment 1, Chapter 4.

A number of changes were noted in the inoculated animals.

P8, which died in the early hours of the 3rd day following inoculation had an acute diarrhoea. The general bodily condition was poor. Signs of dehydration were

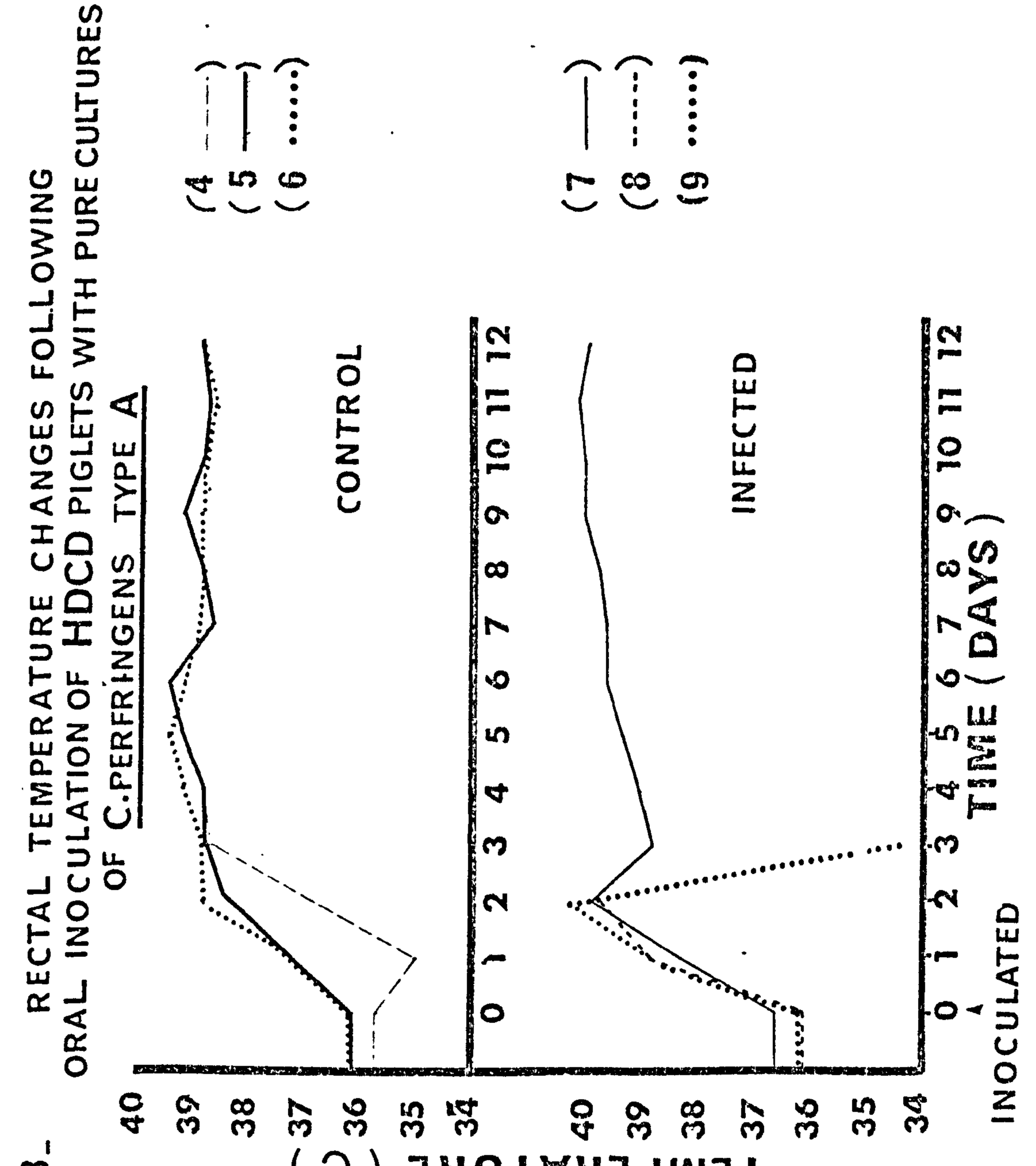

RECTAL TEMPERATURE CHANGES FOLLOWING
ORAL INOCULATION OF HDCD PIGLETS WITH PURE CULTURES
OF C.PERFRINGENS TYPE A
CONTROL
INFECTED
(4 —·—)
(5 ——)
(6 ·····)
(7 ——)
(8 ----)
(9 ·····)
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME (DAYS)
INOCULATED
TEMPERATURE (°C)
40
39
38
37
36
35
34

Fig 58_

evident. The skin was hairy and over the abdomen was dark and discoloured. The eyes were sunken and the flanks hollow.

The hind quarters were soiled with creamy faeces (Fig.59). The thoracic cavity and viscera were normal, except for the accumulation of pericardial fluid.

The abdominal cavity was filled with clear straw-like fluid and there was some fibrinous peritonitis. The liver was dark in colour and enlarged (Fig.60). The spleen was also dark in colour but was normal in size and the kidneys were congested and dark in colour but otherwise appeared normal. The musculature of the abdominal wall was dark in colour and so was that of the hindlimbs adjacent to the abdominal cavity. The stomach was distended with milk and some gas. The gastric mucosa was slightly congested. The small intestine was distended with gas. The lower portion of the duodenum and the whole of jejunum appeared reddened from the serosal surface, while the ileum, though distended, had a pale looking serosal surface.

The duodenum contained fluid with gas but its mucosal surface was only mildly inflamed. The jejunal contents were frothy and contained necrotic debris and some flecks of blood and were brownish in colour. Small areas of necrosis and haemorrhage were seen on the heavily congested mucosal surface (Fig.61). Examination using a dissecting microscope showed the presence of necrotic debris and stunted villi.

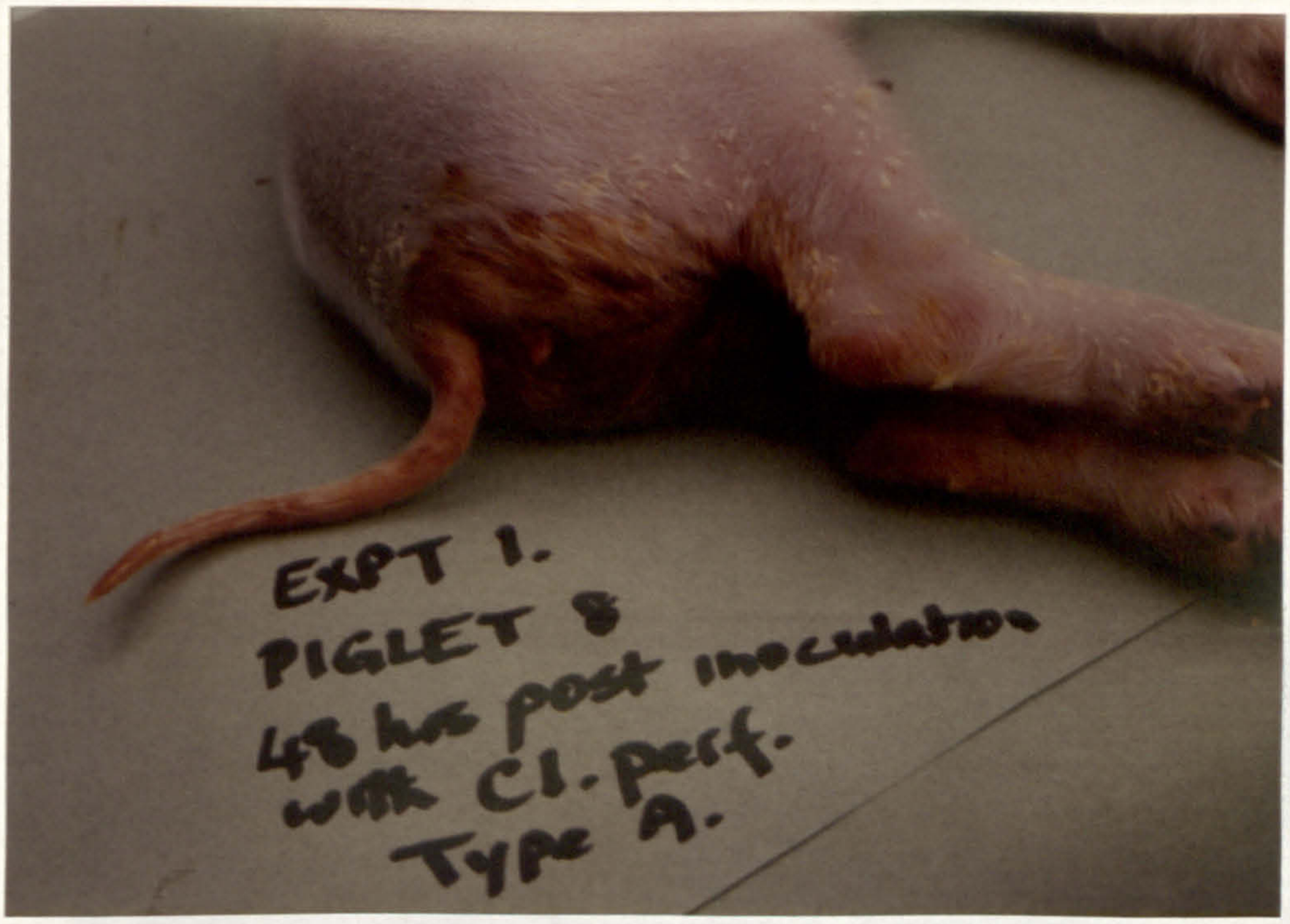


FIG. 59: Perineal region of HDCD piglet, P8, 2 days post inoculation with C. perfringens Type A.
Note the soiling of the hindquarters with fluid faeces.

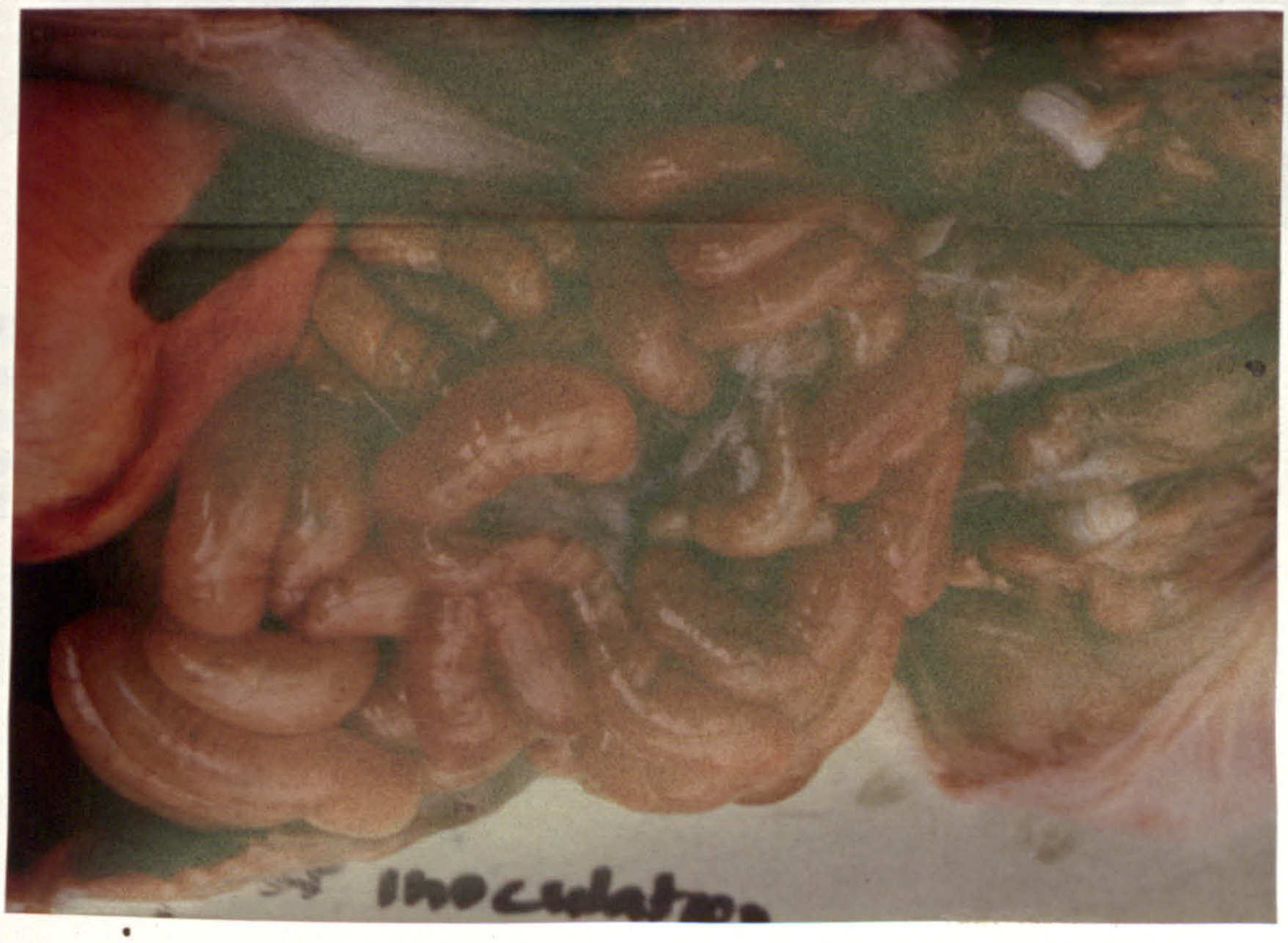


FIG. 60: Gross appearance of the abdominal viscera of HDCD piglet, P8, 2 days post inoculation with C. perfringens Type A.
Note distension and congestion of the small intestine.

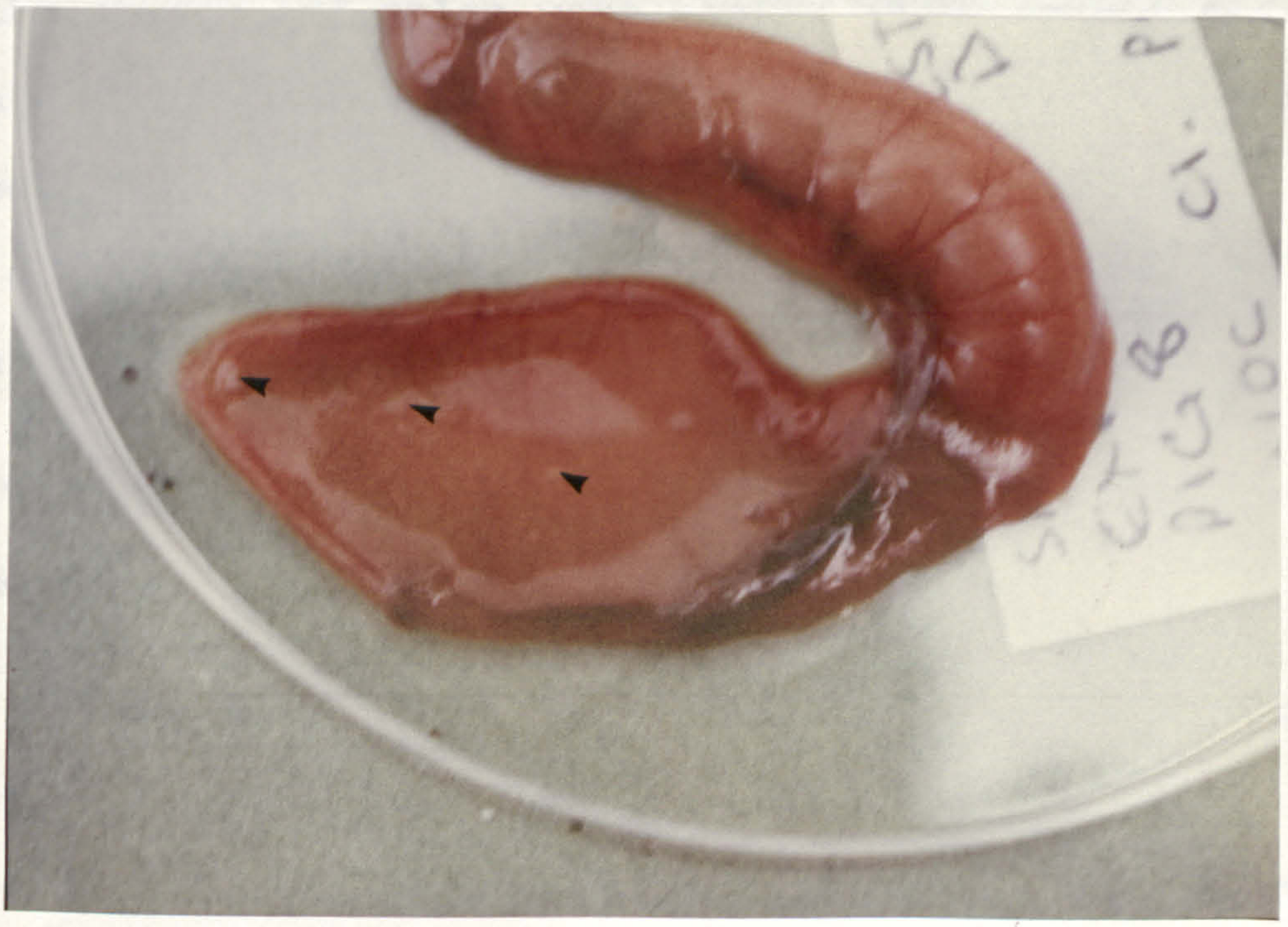


FIG. 61: Macroscopic appearance of the jejunal mucosa of the HDCD piglet, P8, 2 days post inoculation with C. perfringens Type A.
Note the congestion of the mucosa and the presence of flakes of necrotic material (arrow).

The ileal contents were fluid with some flecks of blood, small amounts of whitish necrotic debris and very small mucoid tags. Pinpoint haemorrhages were present in some areas of the mucosal surface, but this was not generalised. The intestinal wall was very thin.

The serosal surface of the large intestine was pale and the caecum and colon were nearly empty except for scanty pasty contents which adhered to the mucosal surfaces. Scanty flecks of blood could be seen on the adherent contents, and the mucosal surfaces of the caecum and colon were normal except for some local pinpoint haemorrhages.

The mesenteric lymph nodes were enlarged and mildly congested.

Piglet P9 was critically ill when it was killed on the 3rd day after inoculation. There was marked soiling of the hindquarters by the creamy fluid faeces. The general bodily condition was very poor at post-mortem examination. The eyes were sunken and the flanks were hollowed. The skin was hairy and sticky. Excess fluid was present in the thoracic cavity, the lungs were normal. The heart was, however, slightly pale in appearance. No fluid was present in the abdominal cavity, the liver was pale and fragile and the spleen and kidneys appeared to be normal. The stomach was distended with gas. Only traces of milk were present on the normal mucosa surface.

The small intestine was flaccid and heavily distended with gas and fluid. The middle part of the small

intestine had a reddened appearance of the serosal surface (Fig.62).

The contents of the duodenum were fluid in consistency. The mucosal surface was pale except for pinpoint haemorrhages noticed on the mucosa surface at the proximal end.

The jejunal contents were frothy with a mixture of gas and fluid. Necrotic debris could be seen in both the content and on the mucosal surface. Flecks of blood were present on the inflamed mucosal surface. The mucosa of the lower jejunum was particularly congested.

The ileal contents were fluid and contained white specks of necrotic debris along with some flecks of blood. The mucosal surface was covered with the contents and there were areas of pinpoint haemorrhages. The contents of both the caecum and colon were scanty and pasty. The mucosal surface was pale and appeared to be normal. The mesenteric lymph nodes were slightly congested and enlarged.

The 3rd infected piglet (P7) was killed on day 12 after inoculation.

The general bodily condition was poor. The eyes were sunken. The flanks were hollowed. The perineal region was matted with dry crusts of faeces.

The thoracic organs were normal, with no excess fluid unlike in the other 2 infected piglets that died or were killed in the acute stages of the disease. No fluid

FIG. 62: Gross appearance of the abdominal viscera of HDCD piglet, P9, 3 days post inoculation with C. perfringens Type A.
Note the distended, pale small intestine.

was present in the abdominal cavity and the liver was pale but the spleen and kidneys were normal. The stomach was only half filled with milk. The gastric mucosa was normal in appearance. The small intestine was distended and flaccid with some darkening of the serosal surface of the mid portion (Fig.63).

The duodenal contents were fluid. The mucosa was pale except for some old petechiation which was restricted to the proximal end.

The jejunal contents were fluid and off-white in colour. White necrotic debris could be seen on the mucosa which was slightly inflamed. The intestinal wall was thin at this point. The ileal contents were fluid with some necrotic material. Some mucoid or necrotic tags were seen on the mucosa which was congested and inflamed.

The serosal surface of the large intestine was normal in appearance but both the caecum and colon had scanty greyish pasty contents, with a few flecks of blood. The mucosal surface of the large intestine was normal.

The mesenteric lymph nodes were moderately enlarged and oedematous.

The pathological findings in the control animals have been described in Experiment 1, Chapter 4. No changes were seen in the wall of the intestine, in its mucosa or contents.

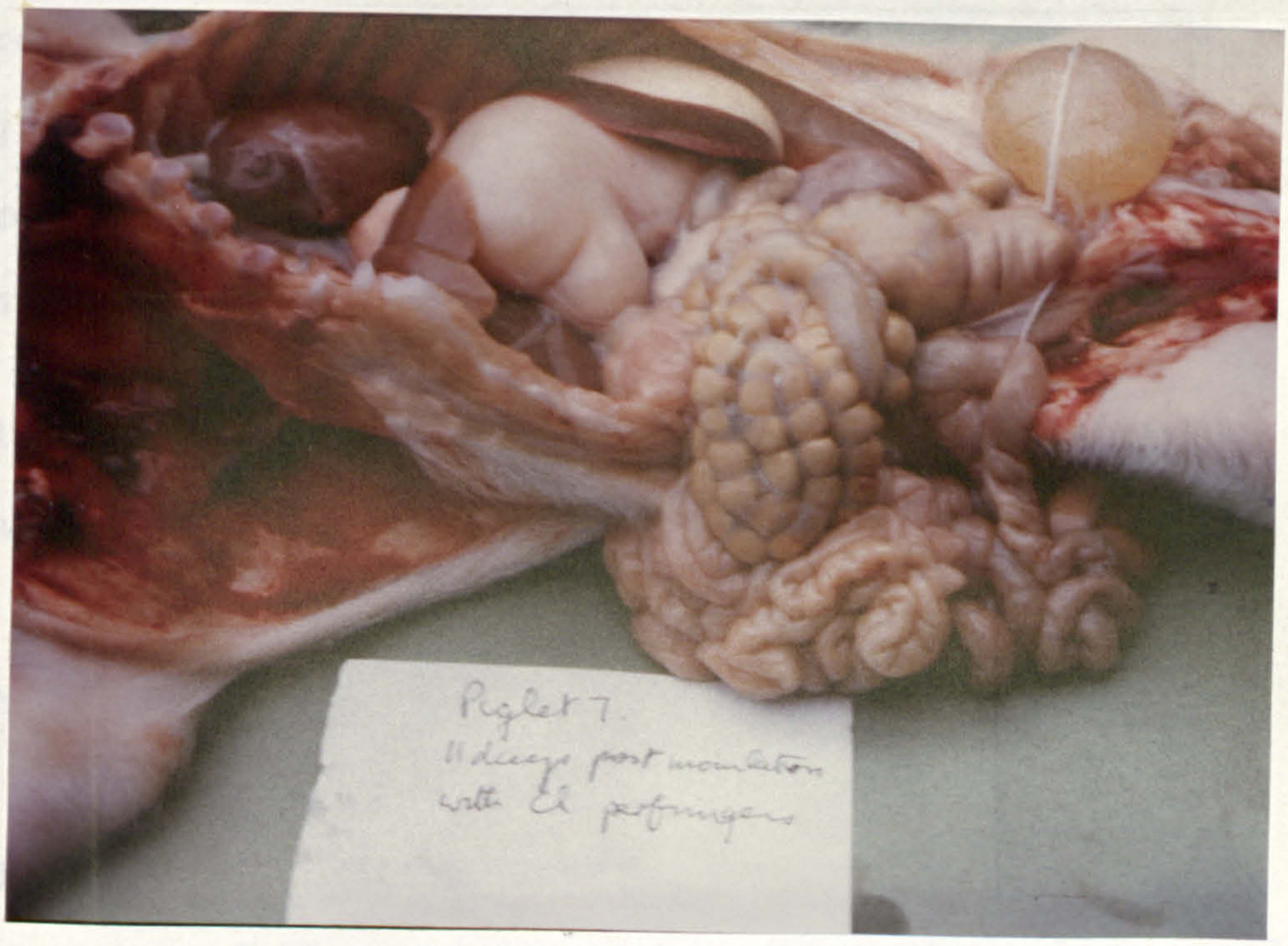


FIG. 63: Gross appearance of the abdominal viscera of HDCD piglet, P7, 12 days post inoculation with C. perfringens Type A.
Note the pale, small intestine which was flaccid.

Histological findings

Marked changes were noted in the intestinal mucosa of all inoculated animals. They were most severe in Pigs P8 and P9 and were least obvious in Pig P7 killed at the end of the experiment. As the changes seen in Pig P8 were affected by post mortem change, the description of the findings in these 2 pigs is of the findings in Pig P9 with occasional reference to the findings in P8. The changes found in Pig P7 are reviewed later. Few changes were noted in the controls, Pigs P4, P5 and P6 and these have been described in detail in Experiment 1, Chapter 4.

Histological changes in Pigs P8 and P9 were most prominent in the small intestine and consisted of massive destruction of the villous architecture, necrosis and inflammation of the mucosa and the surviving villi and the presence of large quantities of cell debris in the lumen (Figs.64 and 65). In the duodenum there was oedema of the submucosal layers and the presence of a few mononuclear cells. The mucosal blood vessels were grossly dilated and there was patchy destruction and distortion of the villi with surface necrosis and loss of epithelium. Cell debris which contained necrotic epithelial cells and inflammatory cells was seen to lie adjacent to the lumenal epithelium or the surface of the denuded villi and within the lumens of the surviving crypts.

Similar changes were seen in the jejunum which, in Pig P8 was completely devoid of villi. In both animals massive numbers of desquamated cells were present on the

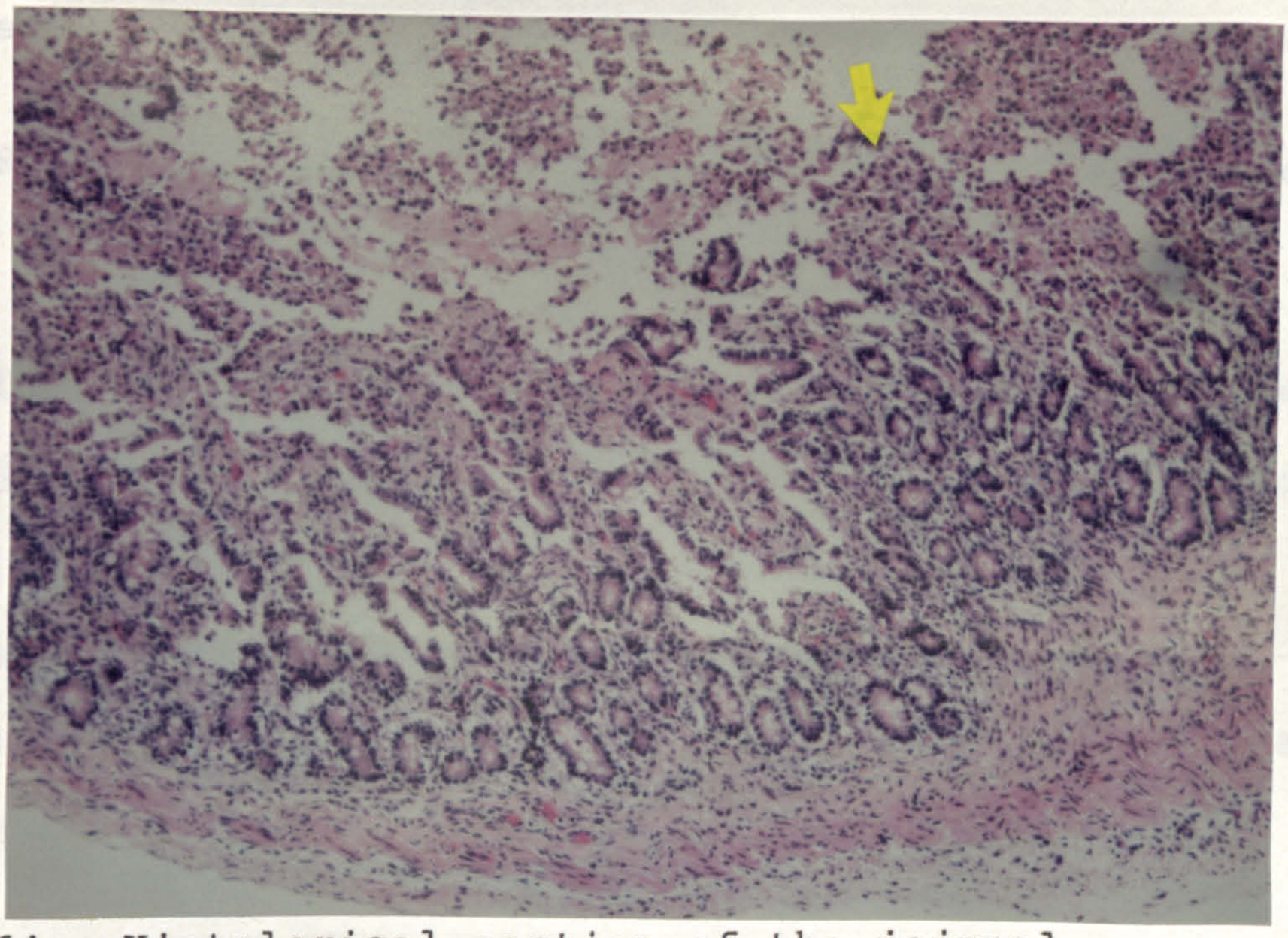


FIG. 64: Histological section of the jejunal mucosa of HDCD piglet, P9, 3 days post inoculation with C. perfringens Type A.
Note the massive destruction of the villous architecture (arrow).
H & E x 110

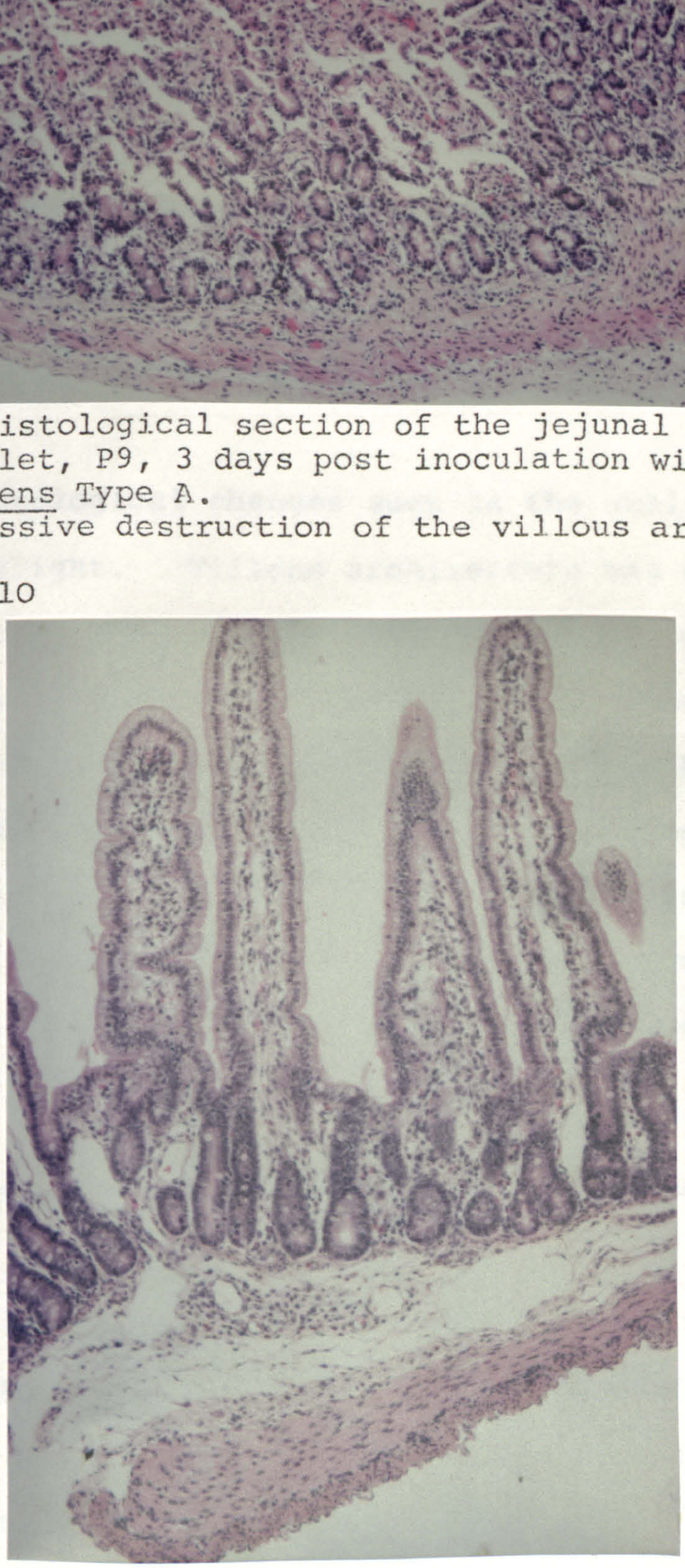


FIG. 65: Histological section of the jejunal mucosa of an uninfected control, HDCD piglet, P4.
Note the normal villi.
H & E x 110

lumenal surface.

Similar necrotic changes could be seen in the ileum and they were milder than in the jejunum.

The mucosal layers of both the caecum and colon were intact but were covered with debris. The epithelial cells of the crypts were vacuolated and small capillaries were prominent in the lamina propria. The crypts themselves were dilated (Figs.66 and 67) and some contained bacteria. The submucosal layer was particularly oedematous in the colon.

The histological changes seen in the small intestine of Pig 7 were slight. Villous architecture was essentially normal although slight local stunting was seen. Occasional strands of eosinophilic material, some of it containing bacteria and cells, were seen on the lumenal epithelium and in crypts. The lamina propria between the crypts and in the cores of the villi contained mononuclear cells and some eosinophils. There was some capillary dilatation. In the ileum the cells of the villous epithelium were all vacuolated and pale staining.

In the caecum, the mucosa was normal but with dilated crypts. Inflammatory cells were seen in small numbers in the lamina propria. The surface epithelium was uneven and covered with a thick layer of basophilic bacterial cells. In the colon a few bacteria could be seen in this position and a few inflammatory cells were also present. There was local cytoplasmic irregularity of the lumenal epithelium.

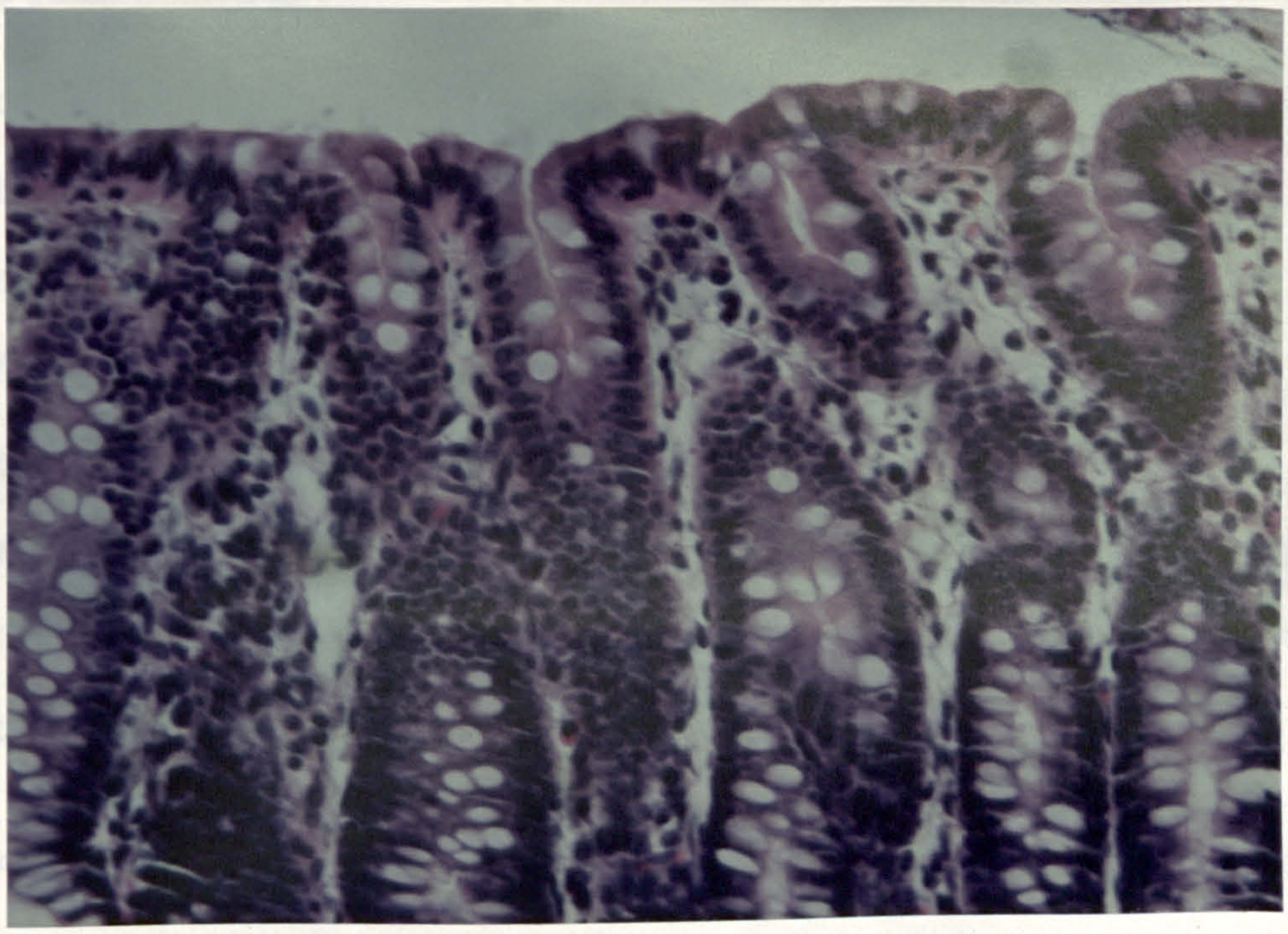


FIG. 66: Histological section of the colonic mucosa of uninfected control HDCD piglet, P4.
Note the normal crypts.
H & E x 110

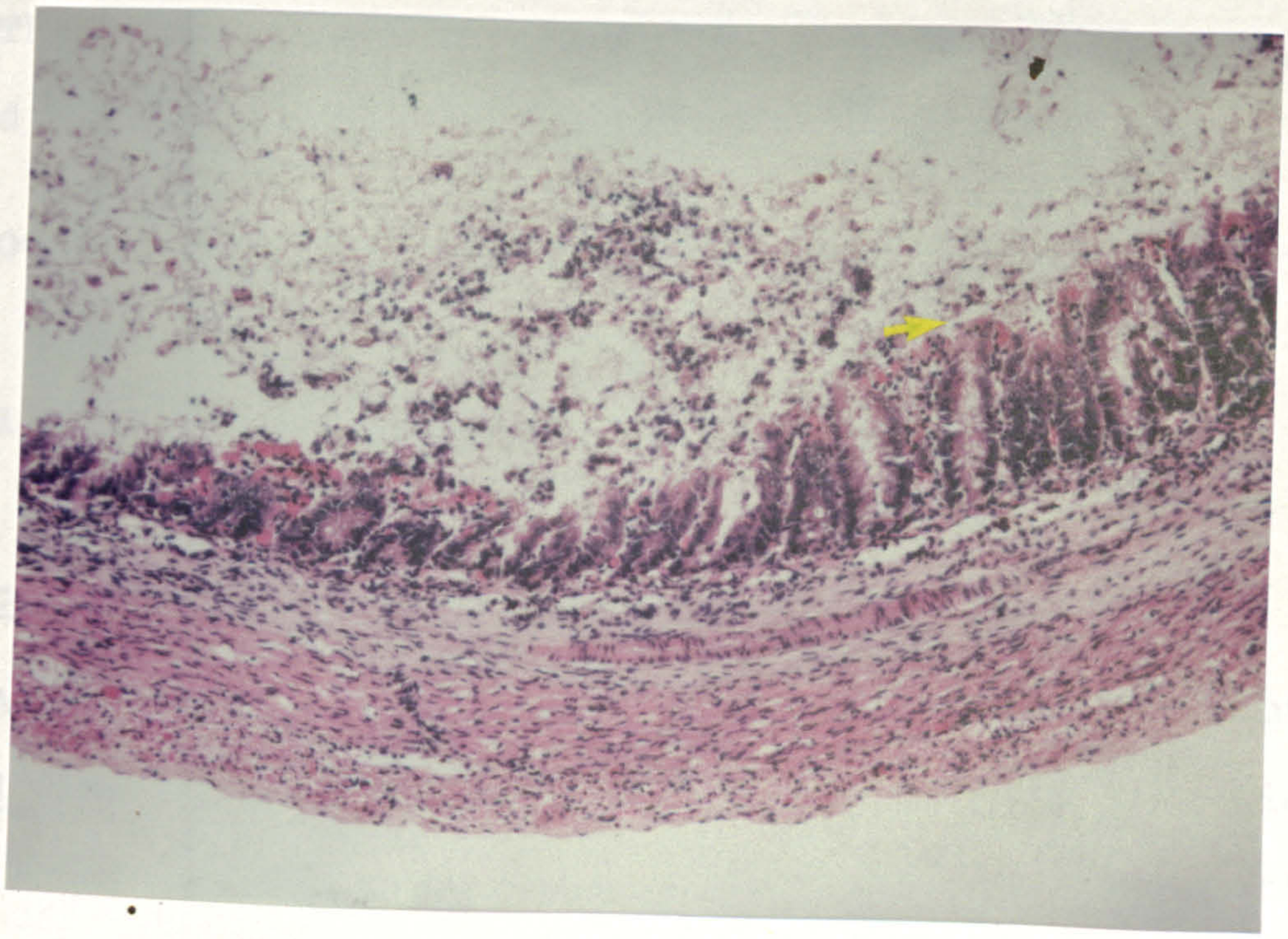


FIG. 67: Histological section of the colonic mucosa of HDCD piglet, P9, 3 days post inoculation with C. perfringens Type A.
Note the debris on the lumenal surface (arrow) and the destruction of the lamina propria.
H & E x 110

Bacteriological findings

Colonies of C. perfringens Type A were isolated in large numbers from the mucosa of the jejunum and ileum of all 3 inoculated animals; while the isolations of the organism from the duodenum, caecum and colon were inconsistent and generally fewer in number. No colonies with the morphology and haemolysis pattern of the inocular strain of C. perfringens could be isolated from any of these sites in the control animals (Table 33).

The Gram-stained smears prepared from the mucosa of these organs contained gram-positive rods with no demonstrable spores, with the morphology similar to C. perfringens Type A (Fig.68). They were absent from similarly stained mucosal smears from the controls.

The identity of the organisms isolated were confirmed by the methods described in Chapter 2.

Other bacteria were also isolated. They were the same non haemolytic clostridium and the non haemolytic E. coli isolated from the faeces.

Serological findings

Agglutinating antibodies to the inocular strain of C. perfringens Type A were present in the sera of both pigs and were highest in Pig P7 which survived to the end of the experiment. The end point was blurred in the serum from Pig P9 but could be read at 1:160. The full results are shown in Table 34.

TABLE 33

Sites from which C. perfringens Type A was isolated from HDCD piglets examined at post-mortem following inoculation with pure cultures of the organism.

Site of isolation	INFECTED ANIMALS			UNINFECTED CONTROLS		
	P7	P8	P9	P4	P 5	P 6
Stomach	-	+	+	-	-	-
Duodenum	+	++	+	-	-	-
Jejunum	++	+++	+++	-	-	-
Ileum	+	+++	+++	-	-	-
Caecum	+	+	+	-	-	-
Colon	+	+	+	-	-	-
Liver	-	-	-	-	-	-
Gallbladder	-	-	-	-	-	-
Mesenteric Lymph Nodes	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Kidney	-	-	-	-	-	-

\+ = Less than 8 colonies of C. perfringens Type A isolated

++ = Between 8 and 10 colonies of C. perfringens Type A isolated

+++ = Profuse culture of C. perfringens Type A isolated

\- = No isolation of C. perfringens Type A

TABLE 34

Levels of agglutinating antibody to the inocular strain of C. perfringens Type A and presence of antilecithinase activity in the sera of the animals of Experiment 5.

Infected Animal	Agglutination titre	Antilecithinase activity
P7	1:640	+
P8	N.D.	N.D.
P9	1:320	+
Uninfected control		
P4	-	-
P5	-	-
P6	-	-

N.D. = Not Done

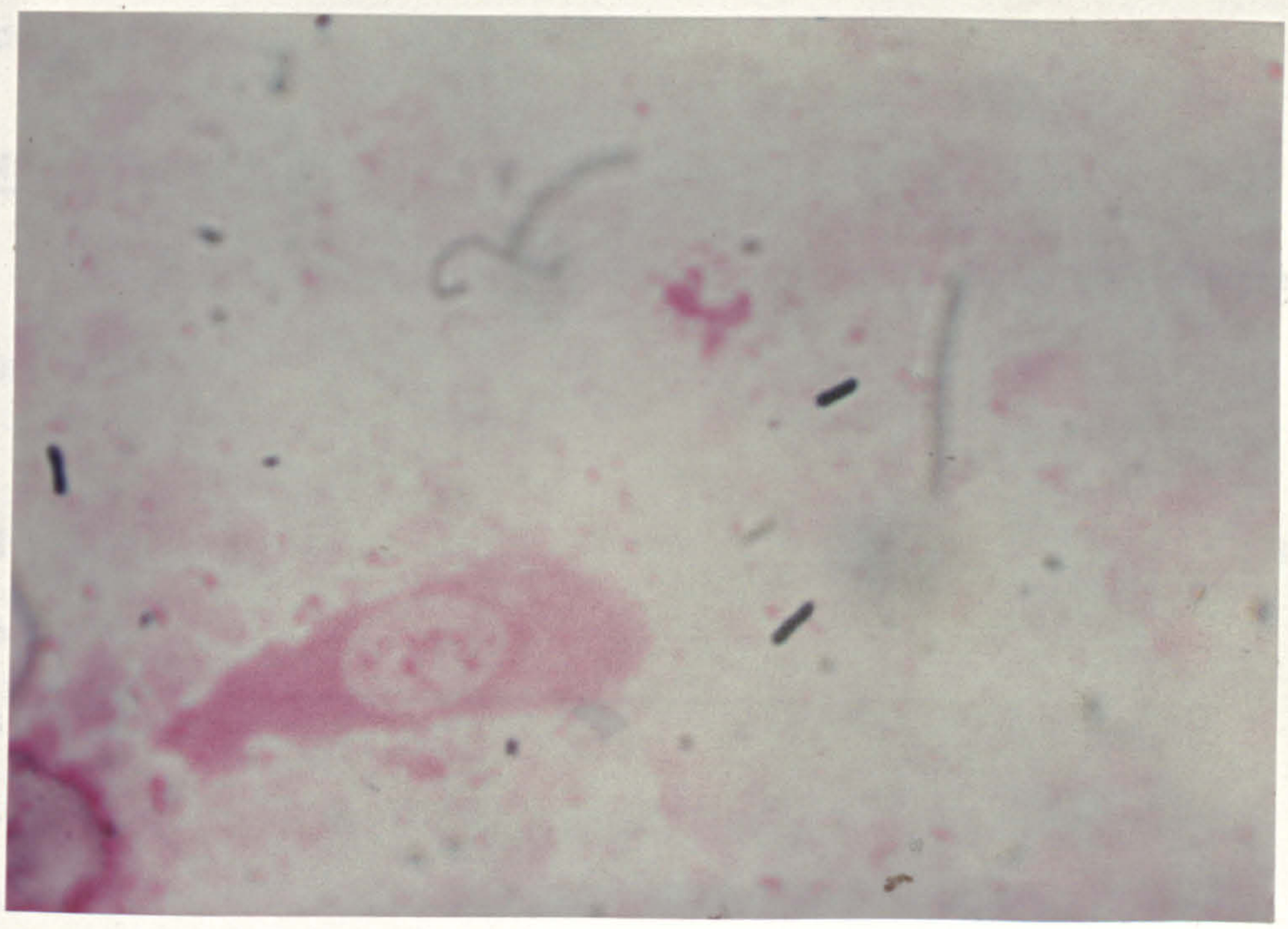


FIG. 68: Smear of the jejunal mucosa of HDCD piglet, P7, 12 days post inoculation with C. perfringens Type A. Gram positive rods with the morphology of C. perfringens were present. Grams' x 1200

In addition, antibody to the lecithinase activity of C. perfringens Type A was demonstrated using the Nagler reaction as described in Chapter 2. It was demonstrated only in the inoculated pigs and was best developed in Pig P7.

EXPERIMENT 6

OBJECTIVE: To determine the pathogenicity of C. perfringens Type A for conventional weaned pigs.

MATERIALS AND METHODS

Ten conventional weaned pigs of 8 weeks of age were obtained from the University of Glasgow Animal Husbandry Department. They were housed in the conditions described in Chapter 2 and divided into 2 groups of 5 in separate pens. They were observed and monitored for 7 days prior to infection as described in Chapter 2. Animals numbered P2006 - P2010 were infected while animals numbered P2011 to P2015 were maintained as uninfected controls.

The inoculum was prepared by the same method as in Experiment 5, and 3.5×10^{10} organisms were used for each pig.

Animals were examined daily for the parameters described in Chapter 2. Daily weight gains were measured in all 10 pigs and feed consumption was measured on a pen basis.

Rectal faeces samples and rectal swabs were examined daily for the presence of C. perfringens Type A using horseblood agar plates as in Experiment 5.

The period of observation lasted for 21 days post-infection and animals were killed on the 22nd day post-infection and were examined by the methods described in Chapter 2.

RESULTS

No B-haemolytic E. coli, salmonellae, campylobacters or C. perfringens Type A were isolated from the rectal swabs of the pigs prior to infection. T. hyodysenteriae was not isolated.

Changes in the consistency of the faeces were limited to the appearance of mucus in semi-solid faeces which started from the 4th day following inoculation. The rectal contents of Pig 2008 were completely mucoid for about 4 days starting from the 8th day following infection. AT no time during the course of the experiment was the faecal consistency of the pigs fluid. None of these changes were noticed in the control groups (Table 35).

Changes in the clinical condition of the infected animals were noted from the 2nd day following inoculation. These changes included dullness and reduction of activity in the infected pigs. The infected pigs showed some nervous signs, particularly Pig 2008 which staggered, was incoordinated and had a twisted neck. The infected pigs were depressed for about 10 days. These signs were not observed in the control group. The bodily condition of the infected pigs was noticeably worse than that of the controls from the 5th day after infection.

TABLE 35. Changes in faecal consistency of conventional weaned pigs following infection with pure cultures of Cl. perfringens Type A and the isolation of the organism.

Pig No.	Inf-ected	DAY OF EXPERIMENT 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
2006	+	N −	N +	N +	SB +	SMB +	NM +	NM +	NM +	NM +	N +	F +	M +	NM +	N +	N −	NM −	N −	N +	NM +	N −	N −	N +	K
2007	+	N −	N −	N +	S +	SMB +	SM +	SM +	SM +	NM +	NM +	N +	+	NM +	N +	N −	N −	N −	N +	N +	N −	N −	N +	K
2008	+	N −	N −	N +	N +	SMB +	SM +	SM +	SM +	NM +	NM +	NM +	NM +	NM +	N +	N +	NM +	N −	N −	NM +	N +	N −	N −	K
2009	+	N −	N +	N +	N +	SB +	SM +	SM +	NM +	NM +	NM +	N +	N +	NM +	N +	N −	N −	N +	N −	N −	N −	N +	N −	K
2010	+	N −	N −	N +	SB +	SM +	NM +	NM +	NM +	NM +	N +	NM +	N +	N +	N +	N +	N −	N +	N −	N −	N −	N −	N −	K
2011	−	N −	N −	N −	N −	N −	N −	N +	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K
2012	−	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K
2013	−	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K
2014	−	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K
2015	−	N −	N −	N −	N −	N −	N −	N −	N −	N −	N +	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	N −	K

For Key see Table 32.

The rectal temperature of the inoculated group rose to 39.2°C on the second day following infection and reached 40.5°C in one case (Pig 2009) on the 11th day following infection.

The feed conversion ratio of the infected pigs was calculated at the end of the experiment to be 3.5 as compared with 2.9 for the controls. The daily liveweight gain was 332g/day (infected) and 489g/day (control).

Faecal culture

No B-haemolytic E. coli, salmonellae or campylobacters were isolated from the rectal swabs or faeces of the animals used in this study.

C. perfringens Type A was isolated from the rectal swabs of the inoculated animals on the day following inoculation (P2006 and P2009) and from all the rest of the inoculated animals as from the 2nd day following inoculation. The organism was isolated from all infected pigs until the 13th day after inoculation; after this isolation from these animals was inconsistent (Table 35).

Pathological findings

At post-mortem examination the infected group of animals were found to be in fair condition only. The changes noted.were common to all 5 animals and are given separately where they differed from those in the remainder of the group. The thoracic cavity and its organs were all grossly normal as were the abdominal cavities and the spleen

and kidneys. In some animals there was scarring of the surface of the liver. The changes which were noted were slight and were restricted to the gastrointestinal tract and its associated lymph nodes. The stomach and its contents were normal but the small intestines were distended with fluid ingesta and were flaccid. The jejunal contents were fluid and sometimes dark in colour with a mucoid appearance (Fig.69). Moderate inflammation of the mucosa was present throughout the jejunum and when viewed using the dissecting microscope, local areas of villous atrophy and adherent flakes of necrotic material were seen. The ileal wall was flaccid, the contents were mucoid and frothy and the ileal mucosa was mildly hyperaemic especially in animals P2007, 2008 and 2009.

The contents of the caecum in all inoculated animals were fluid and dark in colour. Some necrotic debris was seen in the contents; while the colonic contents were pasty and contained variable amounts of mucus. There was patchy hyperaemia of the mucosa of the large intestines of animal P2008 and P2010.

The mesenteric lymph nodes were enlarged in all inoculated animals with varying degrees of congestion and hyperaemia.

The control animals were found to be grossly normal, with the exception of a mild congestion of the duodenal mucosa and enlargement of the spleen of P2014.

FIG. 69: Gross appearance of an opened portion of the small intestine of Pig 2006, 22 days post inoculation with C. perfringens Type A.
Note the mucoid contents (arrow).

Histological findings

Histological changes were present in the small and large intestines of the inoculated animals and were similar in all cases.

Small amounts of cellular debris were present on the lumenal surface of the jejunal mucosa. Some cell debris could be seen in the crypts. Destruction of the tips of villi was present in some places but this was not general. Jejunal villi were stunted with dilated lacteals. There was local oedema of the lamina propria and submucosal layers.

Lymphoid tissues were very prominent in the ileum and limited areas of necrosis and cell debris were seen on the mucosal surface (Fig.70). There appeared to be some cell metaplasia on the villi and in the crypts. There was congestion of the small blood vessels in both the mucosal and submucosal layers.

Cellular debris (Fig.71) and bacteria were present on the lumenal surface of the mucosa of the caecum in all the inoculated animals. There was some oedema and inflammation of the lamina propria. Similar changes were seen in the colon. There was no disruption of the lumenal epithelium of the mucosa. The changes seen in the controls were minimal.

Bacteriological findings

Colonies of C. perfringens Type A were isolated from the jejunum and ileum of all inoculated animals and from the caecal mucosa of some of the animals (Table 36). Other

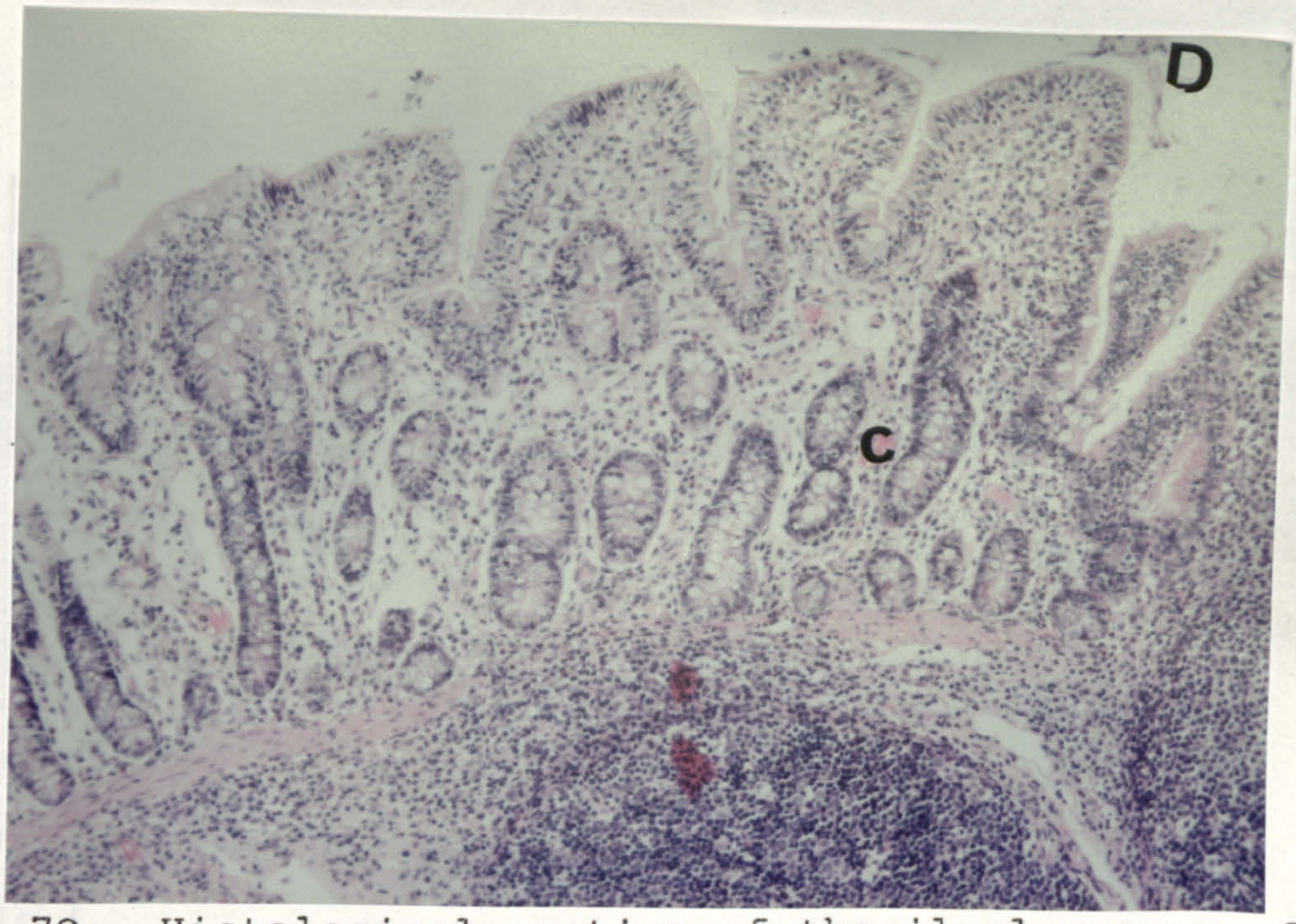


FIG. 70: Histological section of the ileal mucosa of Pig 2006, 22 days post inoculation with C. perfringens Type A.
Note the cell debris in the lumen (D) and the dilated mucosal capillaries (C).
H & E x 110

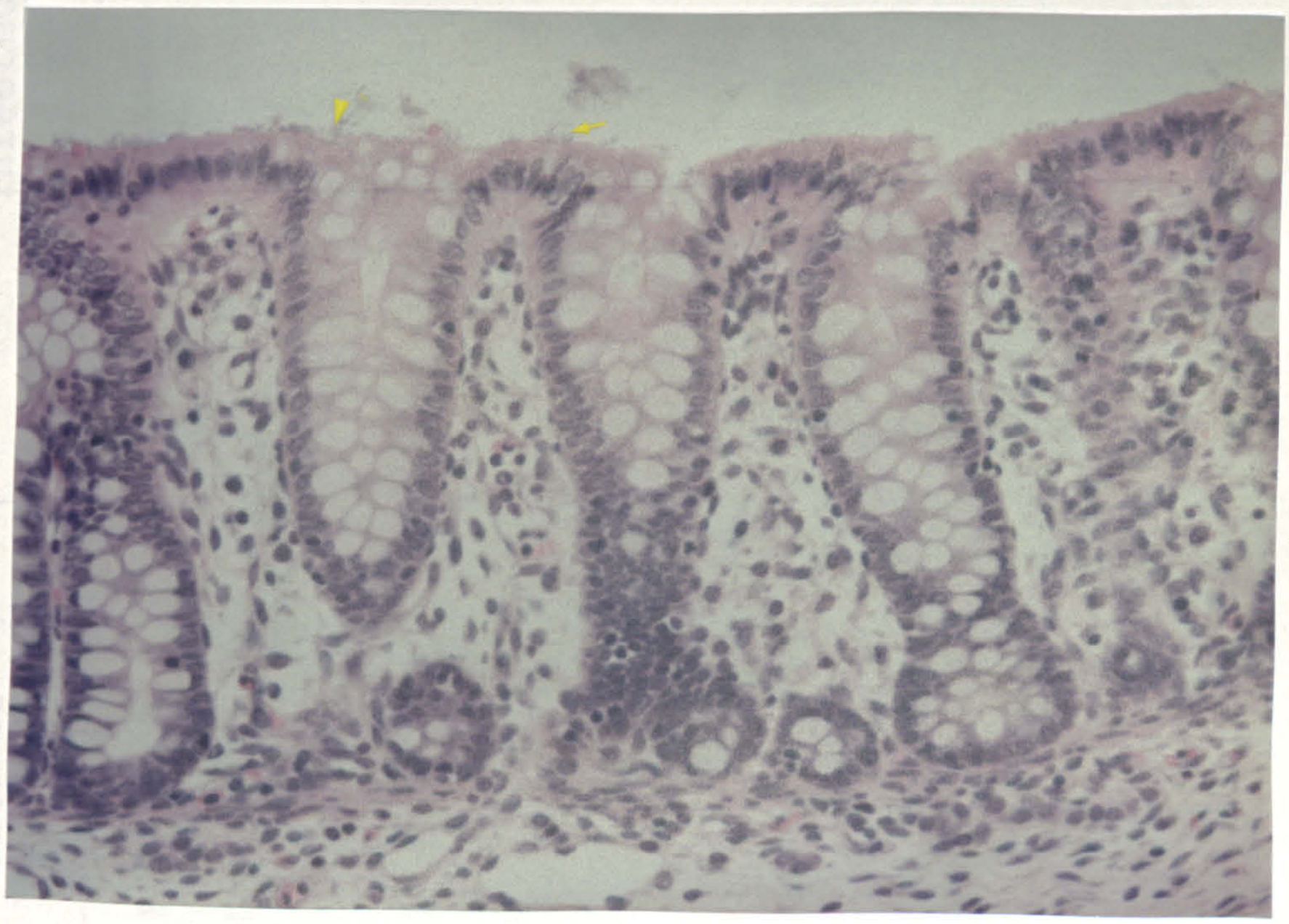


FIG. 71: Histological section of the colonic mucosa of Pig 2006, 22 days post inoculation with C. perfringens Type A.
Note the bacteria on the lumena surface (arrow).
H & E x 250

TABLE 36 Sites from which C. perfringens Type A was isolated from conventional weaned pigs killed 22 days following inoculation with pure culture of the organism.

Site of Isolation	Infected					Uninfected control				
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Stomach	-	-	-	-	-	-	-	-	-	-
Duodenum	+	-	+	-	+	-	-	-	-	-
Jejunum	++	+	+	+	+	-	-	-	-	-
Ileum	+	+	+	+	+	-	-	-	-	-
Caecum	+	+	+	+	+	-	-	-	-	-
Colon	+	+	-	-	+	-	-	-	-	-
Mesenteric Lymph Nodes	-	-	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-
Gallbladder	-	-	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	-	-	-

For key see Table 33

bacteria isolated from the gastro-intestinal tract included non-haemolytic E. coli, Bacillus spp., non-haemolytic clostridia sp., faecal streptococci and staphylococci on some occasions. C. coli was present in small numbers in the small and large intestinal mucosa of animals in both the control and infected groups.

Agglutinating antibody to the inocular strain of C. perfringens Type A was present in the sera of the animals of this experiment at titres shown in Table 37. The end points in the agglutination test were found difficult to read and incomplete agglutination occurred frequently. No antilecithinase activity was demonstrated.

EXPERIMENT 7

OBJECTIVE: To confirm the results of Experiment 6 in which the pathogenicity of the C. perfringens Type A isolate was examined in conventional weaned pigs.

MATERIALS AND METHODS

Ten conventional weaned pigs of 8 weeks of age were obtained from the same Animal Husbandry Department farm as in Experiment 6. They were housed and monitored using the accommodation and methods described in Experiment 6. They were divided into 2 groups of 5 in separate pens. They were observed and monitored for 7 days prior to infection as in Experiment 6. Animals numbered P81, P82, P83, P84 and P85 were infected, while animals numbered P86, P87, P88, P89 and P90 were maintained as uninfected controls. The inoculum was prepared from the isolate used in Experiments 5 and 6

TABLE 37

Levels of agglutinating antibody to the inocular strain of C. perfringens Type A and presence of antilecithinase activity in the sera of animals of Experiment 6.

Pig No.	Infected	Agglutination titre		Antilecithinase Activity	
		Day 0	Day 22	Day 0	Day 22
2006	+	-	1:320	-	-
2007	+	-	1:320	-	-
2008	+	-	1:320	-	-
2009	+	-	1:320	-	-
2010	+	-	1:320	-	-
2011	-	1:10	1:10	-	-
2012	-	1:0	1:10	-	-
2013	-	-	-	-	-
2014	-	-	-	-	-
2015	-	-	-	-	-

using the methods described in those experiments and in Chapter 2. 3.3×10^{10} organisms were given to each pig. Animals were orally inoculated after 24 hours' starvation.

Animals were examined in detail on days 2, 4, 8, 14 and 20 following inoculation for the parameters described in Chapter 2 and Experiment 6. Daily liveweight gains were calculated in all 10 pigs and the feed consumption was measured on a pen basis.

Rectal faeces samples and rectal swabs were examined for the presence of C. perfringens Type A using both horse blood agar plates and reinforced clostridial medium on the days on which the pigs were examined in detail. These samples were also examined for the presence of other bacteria by the methods described in Chapter 2.

The period of observation lasted for 21 days but the animals could not be killed until 29 days following infection when they were examined post mortem by the methods described in Chapter 2 and for Experiment 6.

Clotted blood samples were obtained from all animals prior to infection and at slaughter and the sera tested for the presence of agglutinating antibody to the inocular strain of C. perfringens Type A and to antibody to lecithinase by the methods described in Chapter 2.

RESULTS

Clinical findings

All the animals were clinically normal prior to inoculation. Changes in the consistency of the faeces were noted from the 4th day post infection. The faeces of P82, P83 and P85 were soft on this particular day. On day 8 following infection, only P81 had soft faeces. Mucus was seen in the faeces of P81, P82 and P83. When the animals were examined on day 14, the faeces of all the animals were normal in consistency but mucus was present on the faeces of animals P82 and P83. The presence of mucus on the firm faeces of P82 was the only faecal change noticed on day 21. At no time during the period of examination was the faecal consistency completely fluid. None of these changes were observed in the control group (Table 38).

The bodily condition of the infected animals was poor on day 4. The animals were weak and generally depressed. The depressed state of the infected animals noticeably improved as from day 14, and the animals were as active as the controls on day 21. At no time during the period of observation was the bodily condition of the control group depressed.

Rectal temperatures were found to be slightly raised in the infected group on days 2 and 4, when compared with those of the controls (Table 39).

The feed conversion ratio of the infected pigs was calculated at the end of the experiment to be 1.7 as compared with 2.6 for the controls. The daily liveweight

TABLE 38

Changes in faecal consistency following oral inoculation of conventional weaned pigs with pure cultures of C.perfringens Type A and isolation of the organism.

Pig No.	Infected	DAY OF EXPERIMENT						
		0	2	4	8	14	20	29
81	+	N −	N +	N +	SM +	N −	N −	K
82	+	N −	N +	S +	NM +	NM +	NM +	K
83	+	N −	N +	S +	NM +	NM +	N −	K
84	+	N −	N +	N	N +	N −	N +	K
85	+	N −	N +	S +	N +	N −	N +	K
86	−	N −	N −	N −	N −	N −	N −	K
87	−	N −	N −	N −	N −	N −	N −	K
88	−	N −	N −	N −	N −	N −	N −	K
89	−	N −	N −	N −	N −	N −	N −	K
90	−	N −	N −	N −	N −	N −	N −	K

For Key see Table 32

TABLE 39

Rectal temperature changes following oral inoculation of weaned conventional pigs with pure cultures of Cl. perfringens Type A.

Pig No.	Inf-ected	DAY OF EXPERIMENT 0	2	4	8	14	20	
81	+	39.3	40.0	39.3	39.6	39.4	39.4	K
82	+	39.4	39.7	39.4	39.4	39.4	39.4	K
83	+	39.1	39.7	39.4	39.3	39.4	39.5	K
84	+	38.9	40.1	39.3	39.5	39.6	39.6	K
85	+	38.9	40.2	39.5	39.2	40.0	40.0	K
86	-	38.9	39.1	38.9	39.3	39.3	38.7	K
87	-	39.0	38.9	39.4	39.0	39.2	39.4	K
88	-	38.7	38.9	39.0	39.4	39.2	39.3	K
89	-	38.9	39.4	39.0	39.1	39.5	39.0	K
90	-	38.9	39.0	38.7	38.9	39.1	39.0	K

gains were 293g/day (infected) and 195g/day (control).

Faecal culture

No B-haemolytic clostridia or E. coli were isolated from the rectal swabs of the animals prior to infection. C. perfringens Type A was isolated from the rectal swabs of all the inoculated animals on days 2 and 8 and was isolated from some from day 14 onwards. The organism was not isolated from the rectal swabs of any of the animals in the control group (Table 38).

Pathological findings

At post mortem examination on the 29th day following infection, all the animals were in fair to poor bodily condition. Those in the control group were if anything in poorer bodily condition than the infected animals.

The changes noted were slight and are common to all members of the infected group. The thoracic cavity and organs were grossly normal as was the abdominal cavity. The liver was pale in pigs P82, 83 and 84. The spleen and kidneys were normal in all the infected animals.

The small intestine was distended with fluid contents and gas in all the 5 infected pigs. The contents of the jejunum in particular were foamy with bubbles or dark and mucoid (Fig.72). Necrotic material was observed in the jejunal contents of Pigs P81, P83 and P85. The necrotic material was adherent to the mucosal surface in places. The surface of the jejunal mucosa was only mildly congested

FIG. 72: Gross appearance of a portion of the jejunum of Pig 82, 29 days post inoculation with C. perfringens Type A.
Note the dark, mucoid fluid content.

in the infected animals. The ileal contents were fluid and interspersed with clear, firm, mucoid material. The mucosal surface of the ileum was normal in all the infected animals. The large intestinal contents were firm except in Pigs P84 and P85 in which the contents were pasty. The mucosal surface of the caecum and colon was normal in all cases. Mesenteric lymph nodes were enlarged in P81, and they were very small in P83.

Under the dissecting microscope, necrotic material was seen adhering to the surface of the jejunum of all the infected pigs. The villi were moderately reduced in height mostly in the jejunum but also in some areas in the ileum.

The control animals were normal in most cases. The liver was pale with small 1-2mm pinpoint white foci on P87 and P88. The gastrointestinal contents were normal except in P86 which had pasty colonic contents. The mesenteric lymph nodes were slightly enlarged in P87. No necrotic material was seen on the mucosal surfaces of the small intestine when examined under the dissecting microscope. The villi were normal in height in most cases.

Histological findings

Minimal histological changes were observed in the infected animals. Cellular debris containing bacteria was present on the lumenal surfaces of most of the segments of the gastrointestinal tract. The height of the villi of the duodenum was slightly reduced and their lacteals were slightly dilated. A limited number of eosinophils were present in the lamina propria. The small blood vessels of

the submucosal layer were slightly congested. The crypts were dilated and there were increased numbers of goblet cells around the crypts particularly in P82.

The height of the villi was also slightly reduced in the jejunum. Necrotic debris, bacteria and cells were seen on the tips of the villi in places. Inflammatory cells were observed in the lamina propria. Oedema between the mucosa and submucosa layer was seen particularly in P81. There was congestion of the small blood vessels in both these layers.

Cellular debris, containing bacteria and cells was seen on the slightly reduced villi of the ileum. Mononuclear cellular infiltration of the lamina propria was observed in all the 5 infected animals. There was congestion of the small blood vessels in the submucosal layers.

The epithelial layers of both the caecum and colon were intact in all the infected animals. Cellular debris containing bacteria was present on the lumenal surface. There was dilation of the crypts, some of which contained some cellular material.

None of the histological changes described above appeared to be present to such a marked degree in any of the uninfected controls; except for the slight reduction of the height of the villi in P90 and some degree of inflammation in the colon of P87.

Bacteriological findings

C. perfringens Type A was only isolated in very small numbers from the sites shown in Table 40. The organism was not isolated from sites outside the gastrointestinal tract. Other bacteria isolated from the gastrointestinal tract included faecal streptococci, non-haemolytic E. coli, non-haemolytic clostridium, Bacillus spp., and Fusobacterium spp. The last species was isolated from the colon of P85.

Serological findings

Agglutinating antibody to the inocular strain of C. perfringens Type A was present at titres shown in Table 41. Anti-lecithinase activity was not detected in any of the sera of both the infected and uninfected animals.

EXPERIMENT 8

OBJECTIVE: To study the pathogenesis of C. perfringens Type A infections in conventional weaned pigs.

MATERIALS AND METHODS

The conventional weaned pigs used in this experiment were obtained from the same source as those in Experiment 7 and formed part of the same group. They were housed in adjacent pens, were monitored and observed on the same occasions and infected with the same amount of inoculum on the same day. One animal, P75, was killed and examined post mortem on Day O prior to infection and the remainder were killed at daily intervals for 4 days - P79 (Day 1), P8O (Day 2), P76 (Day 3), P78 and P77 (Day 4). All were

TABLE 40

Sites from which C. perfringens Type A was isolated from conventional weaned pigs killed 29 days following inoculation with pure cultures of the organism.

Site of isolation	Infected					Uninfected controls				
	81	82	83	84	85	86	87	88	89	90
Stomach	-	-	-	-	-	-	-	-	-	-
Duodenum	-	-	-	-	-	-	-	-	-	-
Jejunum	+	+	+	+	+	-	-	-	-	-
Ileum	+	-	+	-	-	-	-	-	-	-
Caecum	-	-	+	-	-	-	-	-	-	-
Colon	+	+	-	+	-	-	-	-	-	-
Mesenteric Lymph Nodes	-	-	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-
Gallbladder	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-	-
Lungs	-	-	-	-	-	-	-	-	-	-

For Key see Table 33

TABLE 41 . Levels of agglutinating antibody to the inocular strain of C. perfringens Type A and presence of antilecithinase activity in the sera of the animals of Experiment 7.

Pig No.	Infected	AGGLUTINATION		ANTILECITHINASE ACTIVITY	
		Pre-inoculation Titre	Titre 29 days Post Inoculation	Pre-inoculation	29 days Post Inoculation
81	+	-	1:640	-	-
82	+	-	1:320	-	-
83	+	-	1:640	-	-
84	+	-	1:640	-	-
85	+	-	1:320	-	-
86	-	-	-	-	-
87	-	-	-	-	-
88	-	-	-	-	-
89	-	-	-	-	-
90	-	1:10	1:10	-	-

examined post mortem in the manner described in Chapter 2.

RESULTS

All the animals used in this experiment were clinically normal prior to inoculation. C. perfringens Type A was not recovered from their faeces during this period.

The faecal changes seen in this experiment are summarised in Table 42. The changes noted in the faecal consistency of the inoculated pigs were slight, ranging from pasty or slightly loose faeces to firm faeces covered with some mucoid tags. The loosening of the faeces was noticed in animal P87 on the 2nd day following infection and mucus and slight traces of blood were observed on the faeces of P77 and P78 on the 4th day following infection.

During the period of experiment 2 of the inoculated pigs appeared depressed as from the 2nd day following inoculation.

C. perfringens Type A was isolated from the faeces of all inoculated pigs on Days 2 and 4. These results are shown in Table 42. No B-haemolytic E. coli or campylobacters were isolated from their faeces.

Pathological findings

The post mortem findings in the inoculated animals are described below according to the day of death.

TABLE 42

Changes in faecal consistency in conventional weaned pigs infected with pure cultures of C. perfringens Type A and

Pig No.	Infected	Day following infection 0	1*	2	3*	4
76	+	N −	N.D.	N +	K	
77	+	N −	N.D.	N +	N.D.	NM + K
78	+	N −	N.D.	S +	N.D.	SM + K
79	+	N −	K			
80	+	N −	N.D.	S + K		

* = Animal killed but other animals were not monitored

N.D. = Not Done

For key see Table 32

Control animal P75 killed prior to inoculation on Day 0

No gross lesions were seen in the carcase of this animal. The stomach was filled with meal and the small intestine contents and mucosa were normal. The jejunal and ileal mucosal surfaces appeared normal when viewed using the dissecting microscope. The large intestinal contents were normal in colour and consistency and its mucosa was normal in appearance.

P79 Killed on Day 1

The gross lesions found in the gastrointestinal tract were minimal. The stomach was full. The small intestine was distended with fluid, which was foamy in the duodenum. There were bubbles on the mucosa of the duodenum. The contents of the lower jejunum were dark in colour, and the mucosa was bile stained. The mucosal surface appeared normal, except for mild inflammation at the distal end. The villi appeared to be normal in height when viewed with the dissecting microscope. There was debris adherent to the tips of the villi.

The serosal surface of the ileum was pale and the wall was flaccid. The ileal content was limited to traces of bile stained fluid on the mucosal surface. The mucosal surface of the ileum appeared normal except for some pinpoint haemorrhages at the proximal end. The height of the villi appeared normal when examined under the dissecting microscope.

The large intestine contained firm contents covered by bile stained fluid. The mucosal surface appeared to be normal.

The mesenteric lymph nodes were pale and slightly enlarged. The liver was pale.

Pig 80, Day 2

The stomach was filled with feed. The mucosa was bile stained with free mucus and fibrinous tags adhering to its surface. The small intestine was distended with fluid and gas. The serosal surface of the duodenum was pale, its contents were fluid with some foam and the mucosal surface was mildly inflamed. There was a slight reduction in the height of the villi when examined under the dissecting microscope. The jejunum had a dark appearance from the serosal surface, its contents were fluid, bile stained and foaming. The mucosal surface of the jejunum was inflamed. The villi appeared stunted and covered by necrotic debris when examined under the dissecting microscope.

The serosal surface of the ileum was pale and its contents were fluid mucoid, foaming and bile stained (Fig. 73). The mucosal surface was normal except for pinpoint haemorrhagic areas at the proximal portion. The villi were slightly stunted when examined under the dissecting microscope. Sparse necrotic debris was present on some of the villi.

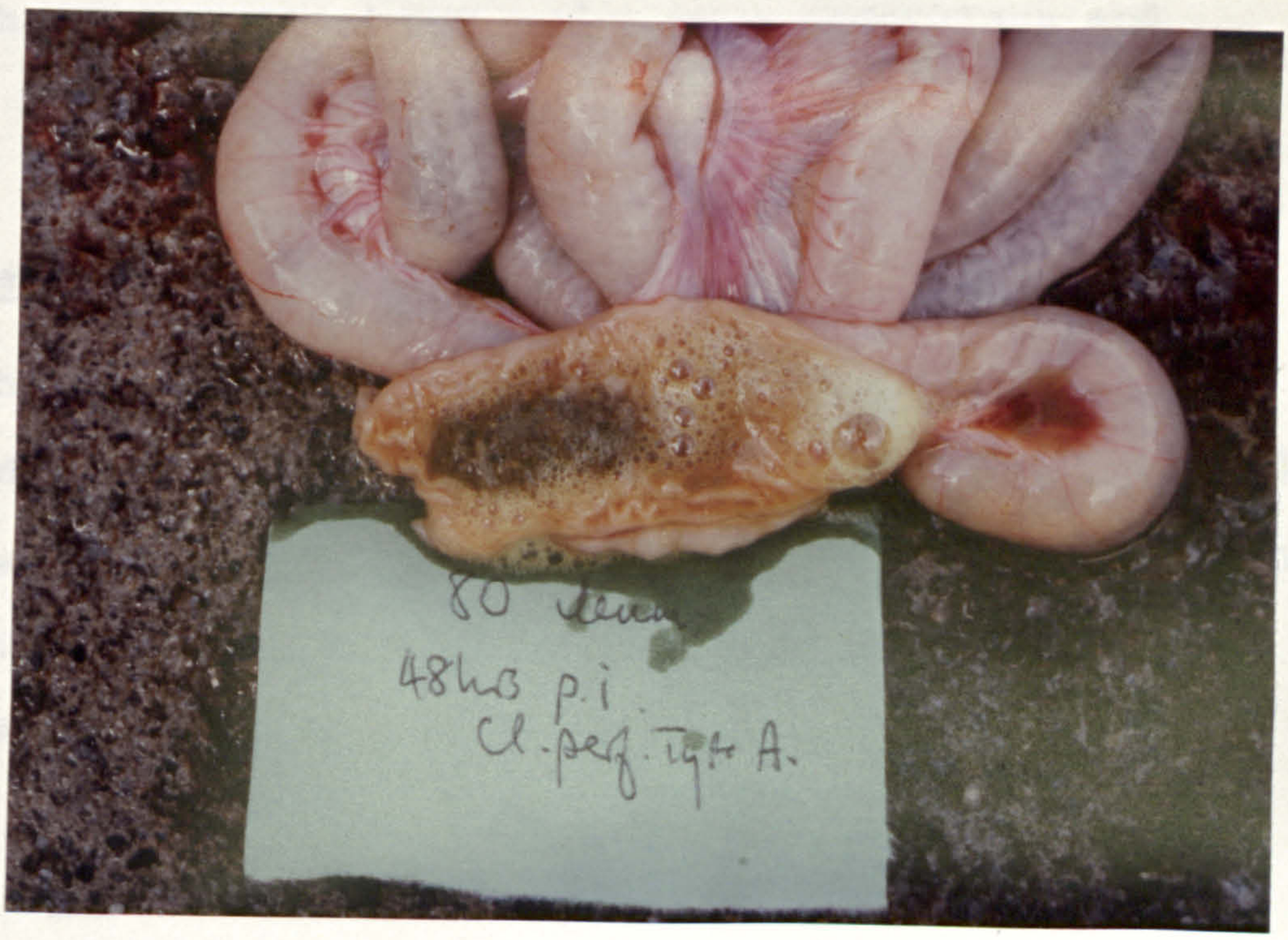


FIG. 73: Gross appearance of the ileum of Pig 80, 2 days post inoculation with C. perfringens Type A. Note the mucoid and foaming contents.

The serosal surface of the large intestine appeared grossly normal but the contents of the caecum were firm but covered with a layer of bile-stained fluid. The mucosa was grossly normal. The colonic contents were firm but some mucus was seen on the surface of the mucosa which was otherwise normal.

The mesenteric lymph nodes were oedematous and enlarged, but the liver was pale.

Pig 76, Day 3

The bodily condition was fair. The thoracic cavity and its organs were normal. The liver was pale and pinpoint white spots were seen on the surface. The spleen and the kidneys were normal. The stomach was filled with bile stained fluid, but the mucosal surface was grossly normal. The serosal surface of the duodenum was pale and flaccid. The contents were fluid and bile stained. The mucosal surface was bile stained and areas of mild inflammation were seen at the distal end. Under the dissecting microscope the villi appeared slightly stunted, and were covered in places by debris. The jejunum was distended with fluid. The serosal surface was dark in appearance where solid contents were present. The contents were fluid and foaming with gas bubbles (Fig.74). There were pinpoint haemorrhages on the mucosal surface and scattered hyperaemic areas were also present. The villi were stunted and covered with necrotic debris when examined under the dissecting microscope. The serosal surface of the ileum was pale. The contents were fluid and mucoid.

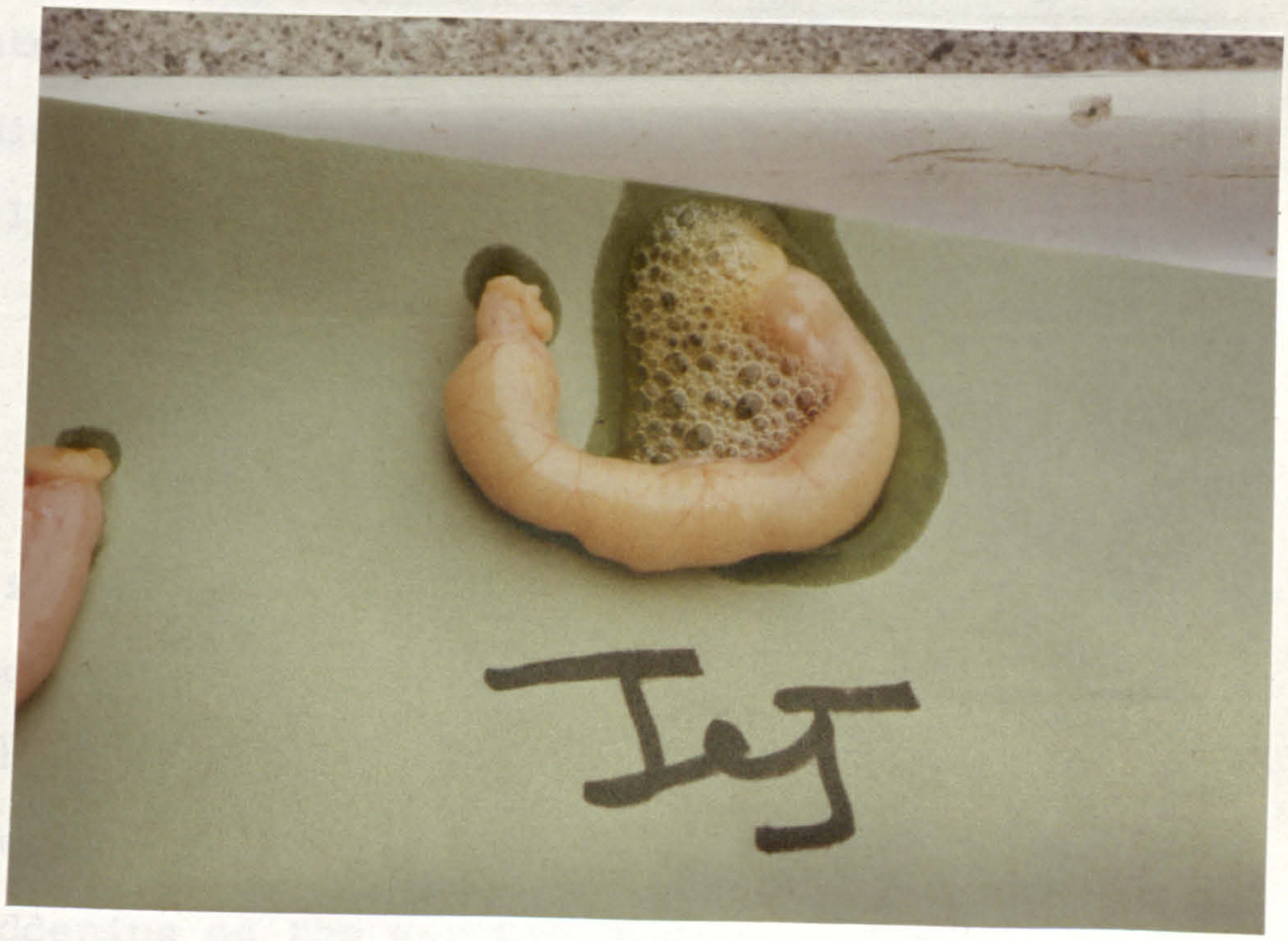


FIG. 74: Gross appearance of a portion of the jejunum of Pig 76, 3 days post inoculation with C. perfringens Type A.
Note the watery foaming contents.

Whitish necrotic debris was present in the mucoid content (Fig.75). The mucosal surface was mildly inflamed at the proximal end. Under the dissecting microscope, the villi appeared stunted and covered by traces of necrotic debris. The caecum appeared distended and its contents were bulky and pasty. Apart from the presence of clear mucus on the mucosal surface, the latter appeared grossly normal. The colonic contents were pasty and these were covered by bile stained fluid. The mucosal surface appeared normal. The mesenteric lymph nodes were enlarged and oedematous.

P77. Day 4

The bodily condition was fair to poor. The thoracic cavity and its contents were grossly normal. The liver was pale in appearance. The spleen and kidneys were grossly normal. The stomach was distended with bile-stained fluid. Free mucus was present on the mucosa; there were local areas of reddening on the gastric mucosa. The serosal surface of the duodenum was pale. The contents were fluid and bile stained. The mucosal surface had local areas of hyperaemia. The villi appeared slightly stunted when examined under the dissecting microscope.

The jejunum was distended and the wall was flaccid. The serosal surface was dark in appearance. The contents were fluid with bubbles and were bile stained (Fig.76) and contained whitish necrotic debris. Free mucus was present on the mucosal surface. The latter was hyperaemic particularly in the proximal half and contained pinpoint haemorrhages in the middle region. Under the dissecting

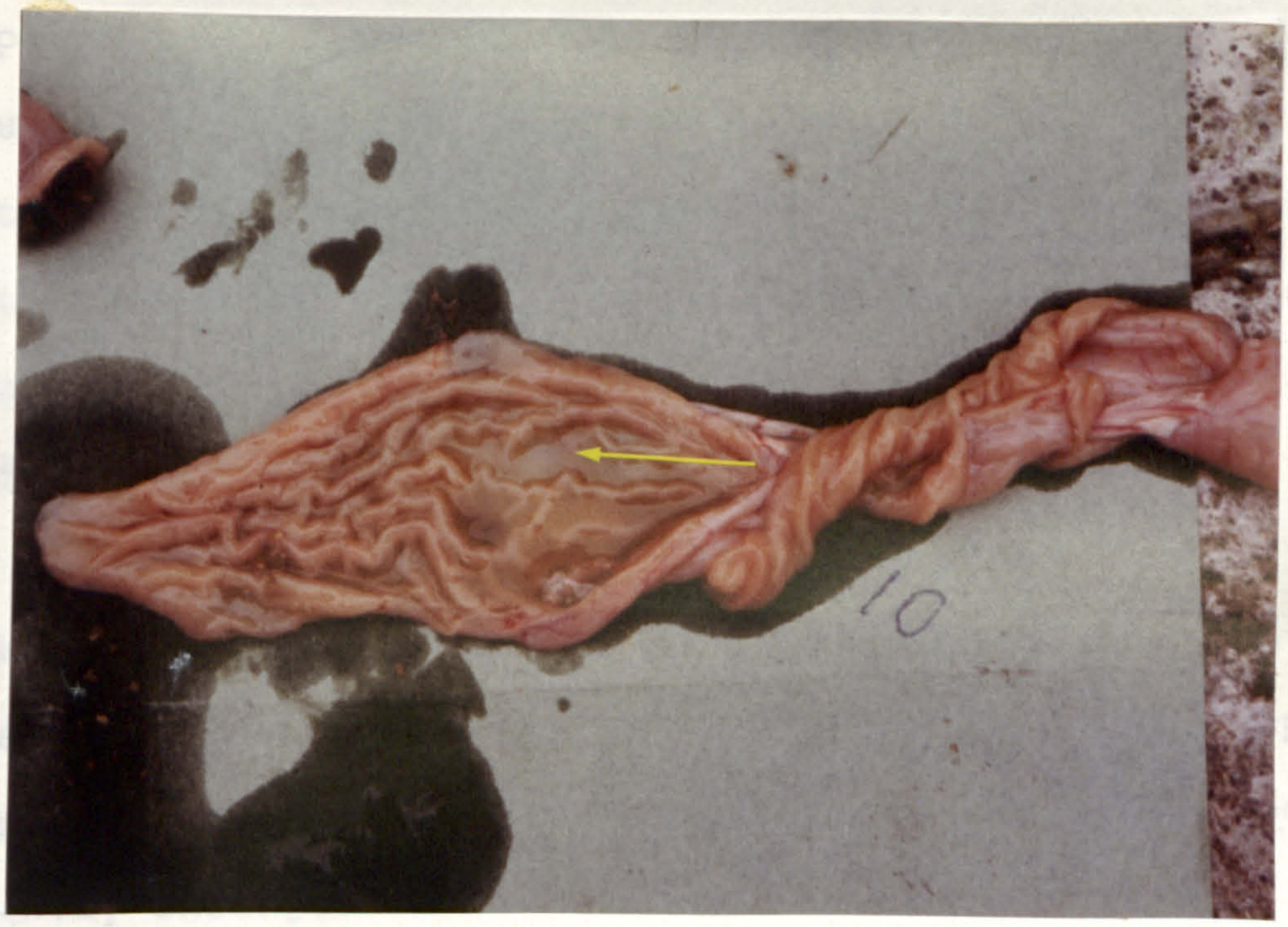


FIG. 75: Macroscopic appearance of the jejunal mucosa of Pig 76, 3 days post inoculation with C. perfringens Type A.
Note the pool of cloudy mucus (Arrow).

FIG. 76: Macroscopic appearance of the jejunal mucosa of Pig 77, 4 days post inoculation with C. perfringens Type A.
Note the foaming fluid contents with bubbles.

microscope, necrotic debris was seen adhering to the surface of the mucosa. The villi were stunted. The serosal surface of the ileum was less dark in colour. The contents were mucoid and bile-stained. There were localised areas of hyperaemia on the mucosal surface particularly that of the proximal portion. Under the dissecting microscope necrotic debris was seen to be present on stunted villi. The caecum and colon had pasty contents, which adhered to the mucosal surface. The latter was grossly normal except for the presence of free mucus. The mesenteric lymph nodes were slightly enlarged and oedematous.

Pig 78, Day 4

The bodily condition was fair. The stomach was filled with ingesta covered by a layer of free bile-stained fluid. The gastric mucosa was normal. The duodenal contents were mucoid and fluid in consistency. There were restricted areas of hyperaemia on the mucosal surface. The villi appeared stunted under the dissecting microscope. The serosal surface of the jejunum was dark in appearance. The contents were fluid and bile stained. Free whitish necrotic debris were present in the contents. The mucosal surface had adherent mucus and necrotic debris. There were areas of local hyperaemia on the mucosal surface. Under the dissecting microscope, the stunted villi were covered in places by the adherent necrotic debris. Pinpoint haemorrhages were evident in most areas. The serosal surface of the ileum was pale. The contents were fluid and bile stained. Clear mucus was present on the mucosal

surface. There were local areas of reddening of the mucosal surface. The height of the villi appeared slightly reduced when examined under the dissecting microscope. Traces of necrotic debris were present on the tips of the villi. The large intestinal contents were normal and the firm contents were covered with free bile stained fluid. The mucosal surface was normal except for some pinpoint haemorrhages present in the caecum. The mesenteric lymph nodes were enlarged and oedematous.

Histological findings

Day 1, Pig 79

The height of the villi in the duodenum remained unaltered. There was however scanty cellular debris containing bacterial cells on the lumenal surface. Some of the crypts contained some bacteria. The epithelial layer of the jejunum was intact. Bacteria were seen adjacent to the villi. The lamina propria was deeply stained. Some mononuclear cells were present in the lamina propria. The submucosal layer was oedematous. Cellular debris and some bacteria were present adjacent to ileal lumenal surface. There was shrinking of some villi and congestion of small blood vessels in both the mucosal and submucosal layers. The caecum and the colon were normal except for the presence of some bacteria adjacent to the lumenal surface.

Day 2, Pig 80

Stomach. Massive cellular debris was present on the intact epithelial layer. Mononuclear cells and bacteria were seen in some gastric glands.

There was distortion of the tips of some of the duodenal villi, and bacteria and cells could be seen near the epithelium. Mononuclear cells were present in the lamina propria. There was congestion of the small blood cells amd the capillaries in the lamina propria and submucosal layer.

In the jejunum there was a slight destruction of the tips of the villi in places, and they were reduced in height. Free bacteria were present adjacent to the lumenal surface. Mononuclear cells were present in the lamina propria. There was congestion of the small blood vessels in both the mucosal and submucosal layers. The height of the villi in the ileum was slightly reduced. Some of the villi were covered by a layer of cellular debris containing bacteria. The lymphoid tissue in the ileum was very prominent and some was reactive.

The epithelial layer of both the caecum and the colon was intact. Cellular debris was present in the lumenal surface. Some mononuclear cells were seen in the lamina propria. The crypts appeared dilated and some of them contained bacteria.

Day 3, Pig 76

. There was a reduction of the height of the villi of the duodenum. Cellular accumulation was present on the lumenal epithelium. Inflammatory cellular infiltration of the lamina propria was present. The submucosal layer was oedematous. There was lowering of the height of villi in the jejunum and some local fusion of villi. Inflammatory cells were present in the lamina propria. There was congestion of small blood vessels of both of the mucosa and the submucosa. There was a reduction in the height of the villi of the ileum. Layers of cellular debris were present on the lumenal surface. Inflammatory cellular infiltration of the lamina propria was noted. There was hyperplasia of lymphoid tissues, some of which were reactive. The submucosal layer was oedematous.

The caecum and colon had a thin layer of cellular debris on the lumenal surface. The epithelial layer was intact. The crypts were dilated and some contained cells. The histological findings in the 2 animals killed on the 4th day were the same as those observed in animals killed on day 3 (Figs.77 and 78).

None of the histological changes described above were observed in any of the uninfected controls. The histological findings in Pigs P86 and P90 had been recorded in Experiment 7. The mild changes observed in Pig P75 were slight staining of the lamina propria of the duodenum and ileum, and increased goblet cells in the epithelial layer of the ileum.

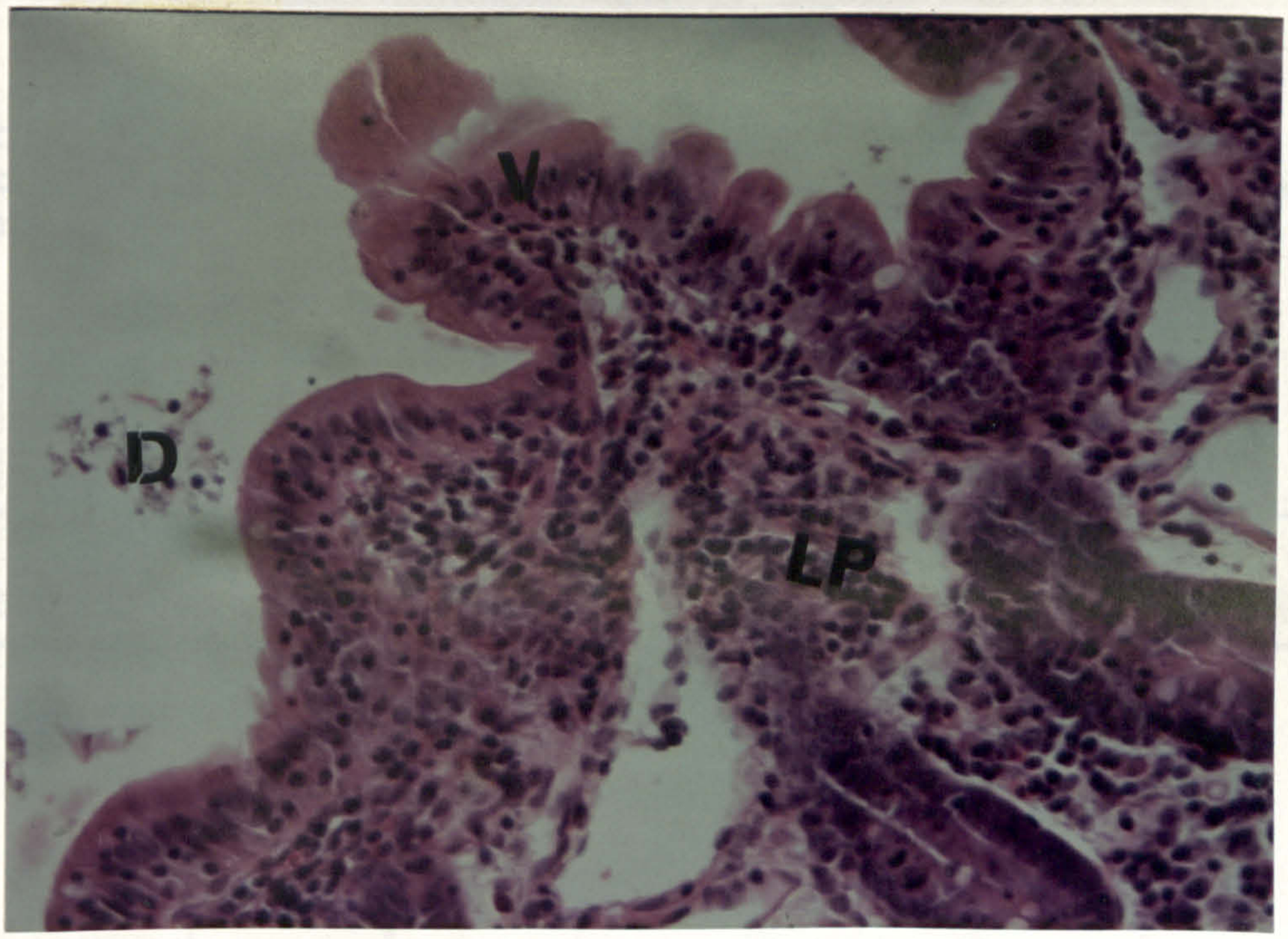


FIG. 77: Histological section of the jejunal mucosa of Pig 77, 4 days post inoculation with C. perfringens Type A.
Note the shortened villi (V), the marked cellularity of the lamina propria (LP) and the debris on the lumenal surface of the mucosa (D). H & E x 250

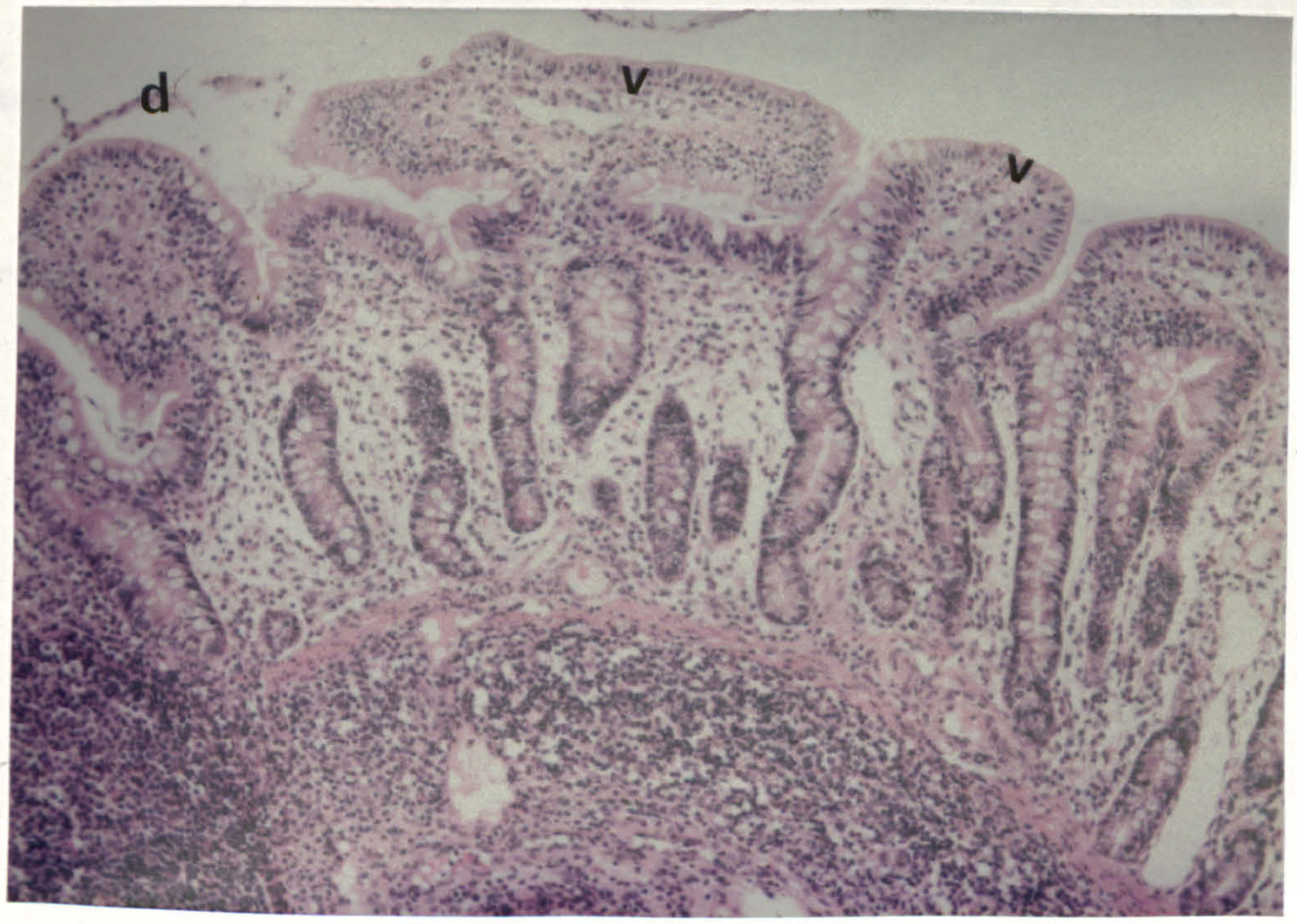


FIG. 78: Histological section of the ileal mucosa of Pig 77, 4 days post inoculation with C. perfringens Type A.
Note the distorted and stunted villi (V) and the cellular debris on the lumenal surface of the mucosa (D).
H & E x 110

TABLE 43

Sites from which C. perfringens Type A was isolated from conventional weaned pigs killed daily following inoculation with a pure culture of the organism.

Site of isolation	75* Day 0	79 Day 1	80 Day 2	76 Day 3	77 Day 4	78 Day 4
Stomach	-	+	+	-	-	-
Duodenum	-	++	++	+	+	+
Jejunum	-	++	++	++	++	++
Ileum	-	+	++	++	++	+
Caecum	-	-	+	+	+	+
Colon	-	-	+	+	+	+
Mesenteric Lymph Nodes	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Gallbladder	-	-	-	-	-	-
Lungs	-	-	-	-	-	-

For Key see Table 33

* = Uninfected control

Bacteriological findings

C. perfringens Type A was only isolated from the mucosal surface of the gastrointestinal tract of the infected pigs (Table 43). Other bacteria isolated included faecal streptococci, non-haemolytic E. coli, and non-haemolytic clostridia from the small intestine; and in addition to those organisms lactobacilli and Bacteroides vulgatus from the large intestine.

C. perfringens Type A was not isolated from the mucosal surface of the gastrointestinal tract of the control animals. Other bacteria isolated were the same as those isolated from the infected group.

Serological findings

Agglutinating antibody to the inocular strain of C. perfringens Type A was present in the sera of the pigs as shown in Table 44. No antibody to lecithinase was demonstrated in any of the sera.

TABLE 44

Levels of agglutinating antibody to the inocular strain of C. perfringens Type A and presence of antilecithinase activity in the sera of the animals of Experiment 8.

Pig No.	Infected	Day killed	Titre	Antilecithinase activity
75	-	6	1:10	-
79	+	1	1:10	-
80	+	2	1:80	-
76	+	3	1:80	-
77	+	4	1:160	-
78	+	4	1:160	-

DISCUSSION

The results of Experiment 5 suggest that the isolate of C. perfringens Type A used was capable of initiating clinical disease in hysterectomy derived colostrum deprived piglets. Elevation of rectal temperature was not a marked feature of the disease produced although a transient rise in rectal temperature to 40°C was recorded for Pig P9 on day 2 post infection (Fig.58). The failure to control the environmental temperature makes the early records of rectal temperature unreliable (see Discussion, Chapter 4). Profuse creamy diarrhoea containing flecks of blood was noted in the inoculated piglets from the 2nd day following infection (Fig.57). As the disease progressed, whitish necrotic material and small amounts of clear mucus were seen in the diarrhoeic faeces.

Infection with the isolate of C. perfringens Type A caused death in 2 of the 3 inoculated animals within 3 days of inoculation (Piglets P8 and P9). One piglet, P7 survived to the end of the period of observation but remained in poor condition with diarrhoea. The infected piglets were depressed as from the 2nd day following infection. The condition of Piglet P7 deteriorated as from the 3rd day but became slightly better on day 9 following infection. Even with this improvement, the piglet was still weak with hollowed flanks, sunken eyes, hairy coat and with soiled hindquarters. The control piglets remained in good condition until killed and passed normal faeces, providing a marked contrast with the inoculated animals. Piglet P4 was destroyed on humane grounds on day 4 of the

experiment as described in Chapter 4.

. The clinical disease produced in conventional weaned pigs was less marked than that seen in HDCD piglets. A transient and variable rise in rectal temperature (to 39.2°C) was observed in the inoculated pigs of Experiment 6. Loose faeces with varying amounts of mucus, some of which contained blood, was passed from days 3 to 9 post inoculation. Clinical signs noticed in the infected pigs included depression, dullness, and in one case (Pig 2008) transient incoordination. The disease produced in this study was not fatal. The poor bodily condition noticed in the infected pigs lasted for 6 to 9 days in most cases. During this period, they developed hairy coats and other signs of weakness. None of these signs were noticed in the uninfected controls. The feed conversion efficiency of the infected pigs was depressed in one study (Experiment 6). There was a 30 per cent decrease in daily live weight gain and a 20 per cent increase in feed conversion efficiency in the infected pigs as compared with uninfected controls. The failure to observe this finding in Experiment 7 may have been due to the small number of pigs used, to factors irrelevant to this study affecting a single group in Experiment 6 or to the presence of some degree of protective immunity in the animals used. Those used in Experiment 7 may have had low levels of agglutinating antibody to C. perfringens Type A prior to infection (Table 41).

These results appear to indicate that inoculation of pigs with pure cultures of C. perfringens Type A is followed by a specific syndrome in which a transient rise

of temperature to 40.0°C occurred within 4 days and faecal changes develop within 2-4 days and last for an average of 9 days. These changes include profuse creamy diarrhoea in HDCD piglets; loose faeces with variable amounts of mucus in weaned conventional pigs of Experiments 6 and 7; and of necrotic material in the jejunal content of Pig 77, Experiment 8. The effect of the disease appeared to be more marked in HDCD piglets than in the conventional weaned pigs. Effects on productivity were noted in some but not all of the studies. In all the experiments, C. perfringens Type A was recovered from the faeces of the infected pigs within 2-3 days of inoculation, and there was no doubt that it had become established. The organism was not isolated from the faeces of the uninfected controls, nor from the faeces of inoculated animals examined prior to infection. The absence of C. perfringens Type A from the faeces of the control animals in all the experiments emphasises the likelihood that it caused the disease or syndrome noted. In Experiments 6 and 7, where the syndrome noted was milder than that noted in HDCD piglets, it is probable that immunity of some type or the age of the animals was responsible (Tables 37 and 41). In these 2 experiments C. perfringens Type A could not be recovered on every occasion when sampling took place from Day 14 onwards (Tables 35 and 38). The other bacteria recovered were similar in both control and inoculated groups.

Gross pathological changes attributable to C. perfringens Type A infection were seen in infected animals in all 4 experiments. The early lesions were

studied in both HDCD piglets (Experiment 5) and in the conventional weaned pigs killed daily in Experiment 8. The chronic lesions were also observed in Piglet 7 on day 12 following infection, and as late as 21 to 29 days in Experiments 5 and 6. The changes seen in all experiments were most striking in the HDCD piglets of Experiment 5. Their bodily condition at post mortem examination was very poor. The liver was dark in colour in the piglets which died early in the experiment but the most marked changes were seen in the small and large intestines. The serosal surface was congested and the intestine was flaccid with fluid or pasty contents. In the small intestine the contents were fluid and contained specks of blood and small pieces of necrotic debris.

The mucosa of the small intestine, particularly that of the jejunum, was congested with pinpoint haemorrhages, and localised areas of necrosis. There was villous atrophy, or on one occasion, erosion, when examined under the dissecting microscope. The large intestinal contents were creamy, pasty and contained flecks of blood. Localised areas of inflammation were seen in the large intestinal mucosa of the infected piglets but were also present in the controls and are therefore of uncertain significance.

The changes described above were not seen in the controls but varying amounts of the fluid were present in the pericardial, thoracic and abdominal cavities of inoculated Piglet P8. Fluid and oedema of the large intestinal mesentery was also present in control Piglet P5.

This may have been connected with the isolation of E. coli from the pericardial fluid in Piglets P4 and P5, a probable indicator of E. coli septicaemia in the group.

Infected conventional weaned pigs killed on 21 and 29 days after inoculation changes were minimal and restricted to the small intestine which was flaccid with mucoid contents, and mildly congested and necrotic mucosa. The changes noted in the large intestine were minimal and inconsistent and at times resembled those seen in the uninfected controls.

When conventional weaned pigs were killed at daily intervals the most prominent changes were seen in the jejunum between days 2 and 4 post inoculation. The small intestine was flaccid, congested with bile stained foamy fluid contents. The mucosa was pale on day 1 but became progressively more congested. The reduction of the height of villi was progressive from day 2 onwards. These changes were not observed in the pig killed prior to infection, or in the control pigs of Experiment 7 which were their contemporaries.

The histological changes were most striking in HDCD piglets, and were less marked in the conventional weaned pigs. The small intestinal changes in the HDCD piglets included congestion, destruction of the villi in Piglets P8 and P9 and necrosis. In Piglet P8 autolysis had occurred but even so, similar findings were present in P9 which was killed. Epithelial cell shedding and the presence of inflammatory and red cells in the lumen was also a feature.

Similar but less marked changes were seen in Piglet P7.

In the large intestine, there was accumulation of cellular debris on the intact mucosal layer. In some places there was necrosis of the epithelial layer. The mucosal layer was heavily congested and inflamed. Similar changes were observed in the uninfected controls but they were much less pronounced.

In conventional weaned pigs, histological changes were slight and restricted to the small intestine, which was mildly congested and inflamed with some evidence of mild necrosis and the presence of inflammatory cells in the lumen which was localised in most cases.

In conventional weaned pigs killed at daily intervals, the histological changes were principally those of villous atrophy with apical villous damage and oedema of the mucosa. These changes were progressive from Day 2 to Day 4 following infection. The changes in the large intestine were non-specific, and included some evidence of inflammation and cellular debris accumulation on the intact epithelial layer.

C. perfringens Type A was recovered from the mucosa of the duodenum, jejunum and at times ileum in larger numbers, less frequently and in lesser amounts from the stomach, caecum and colon. The organism was not isolated at any time in this study from any other organs apart from the gastrointestinal tract. On no occasion was the organism isolated from the mesenteric lymph nodes (Tables 33, 36, 39 and 42).

C. perfringens Type A was isolated from the sites at which the most obvious changes described above were seen, i.e. the duodenum, jejunum and ileum and to a lesser extent the caecum and colon. These findings applied to the acute studies, Experiments 5 and 8 but C. perfringens Type A was only isolated from the mucosal surfaces of the small intestine in very small quantities in conventional pigs killed on Days 21 and 29 following infection (Experiments 6 and 7).

Gram positive rods with the morphology of C. perfringens Type A were seen in large numbers in smears of the mucosal surface in those areas from which it was isolated in greatest numbers. It was not isolated from the controls in Experiment 5.

These findings and the serological results in which infection was followed by a rise in or the development of agglutinating antibody to the inocular strain of C. perfringens Type A in the sera of the infected pigs (tables 34, 37, 41 and 44) reinforce the conclusion that C. perfringens Type A caused the changes seen. Agglutinating antibody was difficult to detect in the weaned conventional pigs because of the blurred end point in the test in contrast to the clarity of the results in Experiment 5. Antibody to lecithinase was only noted in the HDCD piglets and it may be that it developed and declined before the conventional pigs were killed in Experiments 6 and 7 or that local antibody was already present and the toxin was not able to penetrate beyond the mucosa. This suggestion is given weight by the presence of low levels of agglutinating

antibody (1:10) in some of the conventional weaned pigs in Experiments 7 and 8.

The results discussed above suggest that C. perfringens Type A is capable of initiating clinical signs and pathological changes when fed orally to hysterectomy derived colostrum deprived piglets, and conventional weaned pigs even though the changes were less dramatic in the latter. Mortality may occur in nonimmune piglets (HDCD) and it is possible that productivity may be affected in older pigs.

CHAPTER 6

GENERAL DISCUSSION

INTRODUCTION

In this chapter, the results of Chapters 3, 4 and 5 are discussed in the context of enteric bacteriology and enteric disease in general. The results of the individual controlled studies and their validity have been discussed in the appropriate chapters and, as the work falls naturally into 3 main parts, this discussion has been organised in a similar manner.

In the first section, the results of the survey are discussed with relation to the literature reviewed in Chapter 1 and material which is considered relevant in view of those findings. Questions posed by the results are also discussed.

In the second section, the role of C. coli in porcine enteric disease is discussed not only in the light of the experimental studies described in Chapters 3 and 4 but also in terms of the literature relating to it and to closely related species. In the third section C. perfringens Type A is treated in a similar manner.

The whole is put into context by brief concluding remarks.

Results of the survey described in Chapter 3 and their interpretation in terms of porcine enteric disease

(a) Technical factors affecting the results obtained

A large number of bacterial species were isolated from pigs examined in the survey in Chapter 3. The sites from which they were isolated varied from apparently normal mucosa to severely haemorrhagic or necrotic lesions. In some cases, mucosa which was grossly normal was abnormal when examined histologically. As many of the animals had died, tissues from relatively few of them were examined histologically. Had it been possible to carry out a more extensive histological examination on recently dead animals or on animals killed while clinically ill, it might have been possible to associate lesions or changes with more species of bacteria. The survey was also limited in that the pigs provided often lacked a detailed history. The descriptions of the units from which the pigs were obtained are given in Chapter 3 but details of the treatment given to individual pigs or the length of time for which they were ill were rarely available. The limited number of units from which the pigs came may also have reduced the incidence of certain conditions and may have made others such as C. perfringens Type A and C. coli infections more prominent.

The techniques used for the isolation may have been inadequate for the recovery of anaerobes such as Butyrovibrio, Eubacterium, Bifidobacteria and Veillonella unless they were present in large numbers. The use of prereduced media rather than blood agar and the use of

totally anaerobic systems such as those described in the Anaerobe Laboratory Manual might have resulted in the isolation of many more bacteria from the lesions. An example of a well-defined lesion which has been thoroughly defined in terms of strict anaerobes is that of swine dysentery. Robinson et al. (1982) listed 14 species of bacteria which could be isolated from the normal large intestinal mucosa of pigs and 12 species which could be isolated from lesions of swine dysentery. Although their study was comprehensive, their isolates did not include microaerophilic and aerotolerant organisms such as those described in this study. Many of the organisms they found were not isolated and probably could not have been isolated using the conditions used here. These bacteria were, therefore, probably present in the lesions examined here.

The information about other agents such as viruses and parasites was insufficient to say with certainty whether one or more of these types of agents was or had been present in the enteric tracts of the pigs concerned. For that reason in addition to the others discussed above, conclusions about the involvement of such agents in the lesions found at the sites sampled and the role of bacteria in them must of necessity be tentative.

It is also possible that infectious agents such as coccidia, cryptosporidia or viruses may have been present in the tissue a short time prior to death but could not be demonstrated in the material at the time of examination. They may also have been present in areas adjacent to those sampled and have been missed when sampling was carried out.

The tendency of parasitological reports only to include parasites thought to be present in significant numbers may have léd to under reporting of these agents.

(b) <u>The relationship between the mucosal and the lumenal flora</u>

In all these cases, a large number of bacterial species was isolated from the enteric mucosa. The bacteria isolated differ in some respects from those isolated from the lumenal flora of the porcine enteric tract by workers such as Smith and Jones (1963), Pesti (1962), Willingale and Briggs (1955), Horvath <u>et al</u>. (1958), Mansson and Olson (1961,1962) and Szynkiewicz <u>et al</u>. (1982). Organisms such as Veillonellae, Lactobacilli, <u>Bacteroides spp</u>., <u>Peptostreptococcus spp</u>. and <u>Fusobacterium spp</u>. were rarely recovered from the mucosa although they have been reported to occur in large numbers in the contents. Some of these bacteria may not have been isolated for the technical reasons described above and others may have been present only in small numbers. Members of many of the genera described from the lumen were isolated, maintained and identified using the methods described in Chapter 2 so that technical factors may not account fully for the differences observed.

On the other hand, there may be a different bacterial flora in the mucosa and its crypts from that present in the lumen particularly during disease.

It is possible that the bacteria isolated from the mucosa in this study reflect the population actually

present there. The evidence for this view is that the bacteria seen in the direct smears taken from the sites used as the inoculum for cultures resembled those actually isolated. This was particularly so with morphologically distinctive organisms such as campylobacters, streptococci, F. necrophorum, C. perfringens and spirochaetes.

On the other hand, large numbers of bacteria described in the literature as common in the gut lumen of normal animals were isolated from the mucosal surface of the intestines in some of the cases examined. The bacteria included non-haemolytic E. coli, Streptococcus spp., Staphylococcus sp. and Peptostreptococcus spp.. These bacteria may be incidental findings in the mucosa, or individual strains or serotypes may be associated with enteric disease.

(c) Bacterial species associated with particular lesions

The discussion in Chapter 3 has considered each bacterial species isolated in relation to the lesions present at the site of isolation. Lesions were associated with the presence of C. sputorum ss. mucosalis, T. hyodysenteriae, other intestinal spirochaetes; B-haemolytic E. coli, C. coli and C. perfringens Type A.

C. sputorum ss. mucosalis was associated with lesions of proliferative intestinal adenopathy, T. hyodysenteriae with lesions of both natural and experimental swine dysentery and non T. hyodysenteriae spirochaetes with lesions resembling those of spirochaetal diarrhoea. B-haemolytic E. coli was isolated from lesions

in animals with a clinical history of neonatal and post weaning diarrhoea and post-mortem findings strongly suggestive of enterotoxic E. coli enteritis. The lesions found were similar to those described in the literature, reviewed in Chapter 1 and discussed in Chapter 3.

The association of C. coli with lesions of the intestinal tract was one of the more important results of the survey. Its isolation in profuse culture from the small intestine of piglets is described in the results of Chapter 3 and discussed later in that chapter. The observations made appeared to agree with the scanty descriptions of similar findings made by Deas 1960, Roberts 1956b and Warner, 1965. In spite of the apparent association between C. coli and the lesions other bacteria or agents were present in all cases and it was never possible to attribute the lesions seen unequivocally to C. coli. The results of the experimental studies to determine the role of C. coli in intestinal disease are described in Chapter 4 and discussed there and below in the second section of this chapter.

C. perfringens Type A has been recorded as a pathogen in other species of animals such as chickens, lambs and cattle. The association of the organism with enteric lesions in piglets was raised speculatively by Moon and Dillman in 1972. The isolation of the organism from haemorrhagic enteritis and congested lesions (particularly in piglets) in the field cases gave rise to a strong suspicion that it might have a causal role. Its association with lesions has been discussed in Chapter 3

and in Chapter 5 which describes the experimental studies carried out to evaluate its role in enteric disease. It is further discussed in the third section of this chapter.

C. perfringens Type A was isolated from the 3 cases of bloody gut described in the survey. Its association with that condition seems much less clear than its association with enteritis in piglets but its presence in the lesions of the condition is of interest and would repay further study.

(d) Other bacteria and their causal relationship to the lesions in which they were found

A number of bacterial isolates could not be associated specifically with any particular lesion. Non-haemolytic E. coli was a case in point, as it was isolated from a wide variety of mucosal sites in many of the animals examined. As no serotyping or pathogenicity testing was carried out, it was not possible to relate non-haemolytic E. coli to any particular type of lesion.

B. licheniformis was relatively commonly found, sometimes in association with severe lesions and sometimes in mildly altered mucosa. Most Bacillus spp. are considered as non-pathogenic in the gut with the exception of B. anthracis. B. licheniformis was recovered from all regions of the gut, although the organism was most commonly isolated from the small intestinal mucosa. It is very difficult to assess the importance of this organism from this study. It has been isolated in similar circumstances from lesions in the enteric tracts of cattle by Al Mashat

and Taylor (1982) who also failed to draw any conclusions as to its significance.

The criteria discussed above for the involvement of B. licheniformis in lesions also apply to many of the other bacteria isolated, especially B. vulgatus, B. melaninogenicus, Peptostreptococci, Lactobacilli and F. necrophorum. Further field cases might provide more evidence for their involvement in enteric lesions. Their role might also be resolved by experimental studies. However, when experimental infections with B. vulgatus, B. melaninogenicus, Lactobacilli and F. necrophorum and other fusobacteria have been carried out in gnotobiotic pigs these organisms have apparently failed to cause lesions (Meyer et al., 1975).

The part that they may play in established lesions or in conventional weaned pigs is not yet known.

Many of the remaining bacteria were present in such large mixtures of species, in such indistinct lesions, in normal mucosa, or in such small numbers that it was difficult to relate them in any way to specific pathological conditions. However, the studies with C. coli and C. perfringens Type A reported in Chapters 4 and 5 suggest the possibility that some of them may eventually be identified as pathogens or shown to have a specific role in lesions of the gut.

(e) Lesions with more than one potential pathogen

Interpretation of the results of the survey was made more difficult by the fact that more than 1 pathogen was present in many of the enteric lesions seen.

A good example of this problem is that of the small intestinal lesions in pigs from which pure cultures of B-haemolytic E. coli were isolated and in which viral particles were also demonstrated from the intestinal contents. The presence of Torulopsis glabrata with C. coli in intestinal lesions in Pig 125 (Chapter 3 and Appendix 1), the presence of C. coli and C. perfringens Type A in several cases, the isolation of C. coli and C.s.ss. sputorum from Pigs 126 and 127 with proliferative intestinal adenopathy (Chapter 3 and Appendix 1) and T. hyodysenteriae and C. coli from Pigs 51, 80, 87 and 89 are all further examples of this problem. In other cases the bacteria were present in lesions in which parasites were also found (Pig 51, C. coli and Trichomonas) and this also made difficult the interpretation of their contribution to the lesion.

In some cases no specific cause for the lesion could be identified and its origin or cause remains uncertain.

(f) Bacteria commonly present in the respiratory tract were found in enteric lesions

A number of bacteria normally associated with disease in the respiratory tract were found in the mucosa of the enteric tract. In some cases these bacteria were present in enteric lesions. The bacteria included

P. multocida, P. haemolytica and C. pyogenes. It is, however, not clear whether these organisms were found in these sites because they had colonised existing lesions by bacteraemic spread, whether they were ingested after being coughed up into the mouth or whether they actually initiated or perpetuated the enteric lesions seen. The association between respiratory and enteric disease is, however, well known. It is of interest to note that respiratory lesions were found in all cases from which these organisms were recovered from the enteric mucosa. It may be that lowered oxygen tension in the blood may enhance the development of lesions at the intestinal mucosal epithelium where the flora:cell relationship is at its closest and where even slight changes may stimulate bacterial invasion.

The relationship or association between respiratory and enteric lesions and pathogens is worthy of further investigation.

(g) Enteric bacteria described as pathogens in the literature and not found in this study

A number of bacterial species which might, from the literature review in Chapter 1, be expected in enteric lesions were not isolated from, or demonstrated in, the enteric tracts studied.

Salmonella spp. was one of the major pathogens of pigs which was not isolated. This failure is unlikely to have been for technical reasons as cultures of small intestinal contents were prepared in tetrathionate broth

from each animal and the intestinal mucosa, lymph nodes, and in some cases gall bladders were cultured on non selective medium. If salmonellae had been present in appreciable numbers they would have been detected. Their absence is therefore likely to reflect the rarity of the organism in the pig herds from which animals in the study were obtained. The same cultural methods were in regular use in the laboratory in studies of other species during the period of the survey and their use resulted in the isolation of several salmonellae.

A number of other bacteria reviewed in Chapter 1 were not demonstrated in this study. These included C. perfringens Type C which is rarely reported in Britain so that its absence from the bacteria isolated is not surprising in view of the small number of herds from which the samples were drawn.

Y. enterocolitica and Y. pseudotuberculosis were not detected in any of the cases examined. This is in contrast with the opinion of Wooley et al. (1980) that domestic animals including pigs, are potential reservoirs of Yersinia spp.. A recent study shows that there is a low incidence of carriage of Yersinia spp. in pigs from the south of England (Walker and Coleman, 1979). There is no published report of its isolation from pigs in Scotland. The lack of isolationsmay reflect the rarity of the organism in Scotland or the difficulties in identifying this organism when selective media are not used.

Klebsiella spp. was not demonstrated in any of the cases examined. The organism has been reported to be frequently isolated from diarrhoeic pigs faeces in Mexico and it has been suggested to be capable of initiating enteric diseases by Alvarez and Gonzalez (1982) and Alvarez et al. (1982). However Klebsiella spp. are not thought to be important as initiators of enteric disease of pigs in Europe.

C. jejuni was not isolated from any of the cases examined. All available information shows that C. jejuni is a pathogen of humans and other species of animals (Skirrow and Benjamin, 1980; Al-Mashat and Taylor, 1980) and may occur in pigs.

Prescott and Bruin-Mosch (1981) reported the isolation of C. jejuni from 2 out of 208 faecal samples in Canada, and Sticht Groh (1982) reported its isolation from faeces and washed intestines. In the past, most reports failed to distinguish between C. coli and C. jejuni because of the similarity of their biochemical properties. With the description of rapid hippurate hydrolysis test (Harvey, 1980; Skirrow and Benjamin, 1982) it is now possible to differentiate between the two. The results in these studies may indicate that C. jejuni is not common in pigs.

A new catalase positive Campylobacter which was reported to be isolated from porcine intestinal adenomatosis, and named C. hyointestinalis by Gebhart and Ward (1982) was not found in this study. An organism with similar cultural characters was isolated but did not produce hydrogen sulphide on TSI slants. If the organism was

present in any of the cases examined, it should have been detected because of the very intensive biochemical testing carried out on all the campylobacter species isolated in this study.

Candida albicans was not seen in this series

Campylobacter coli in porcine enteric disease

The isolation of C. coli and other campylobacters from inflammatory lesions of the porcine enteric tract has been discussed in Chapter 3. The findings discussed in that chapter and the literature reviewed in Chapter 1 suggested that C. coli might be a cause of lesions in both the small and large intestine in both sucking and weaned pigs in certain circumstances. The association of the organism with the small intestinal lesions found was of particular interest. The presence of C. coli could not be correlated with the presence of diarrhoea or any other clinical syndrome in those studies for the reasons discussed in Section 1 and in a paper describing these studies (Taylor and Olubunmi, 1981, Appendix 2).

The experimental studies described in Chapter 4 and discussed in that chapter suggest that the C. coli isolate used could initiate clinical signs and pathological changes when fed orally to HDCD piglets and could initiate some features of the syndrome observed in them when given both to conventional piglets and to weaned pigs. These clinical and pathological changes differed only in their severity in the different groups possibly because of the age or immune status of the pigs used (Olubunmi and Taylor,

1982, Appendix 2).

These results are in disagreement with the widespread belief that C. coli is a normal inhabitant of the porcine intestine. This belief arose as a result of the failure of workers such as Andress and Barnum (1968) and Warner (1965) to reproduce swine dysentery by feeding pure cultures of C. coli to weaned pigs and partly due to the demonstration that a spirochaete initiated swine dysentery (Taylor and Alexander, 1971). The actual results of the experiments described by these authors do not disagree with those obtained in Chapter 4 of this study. The actual changes associated with C. coli infection in the literature were reviewed in Chapter 1 and the conclusions reached in that chapter agree closely with the results obtained here. The isolation of C. coli from the small intestine of pigs in these studies resembles the findings of Deas (1960) who demonstrated the organism in the terminal ileum of weaned pigs with diarrhoea. He also produced transient diarrhoea in weaned pigs by feeding pure cultures of his isolate in experimental studies. Lawson and Rowland (1974) recorded the presence of C. coli in the small intestines of pigs with intestinal adenomatosis but considered that they played no part in that syndrome. Birrel (1957) described a syndrome in which yellowish diarrhoea occurred in piglets of 3 days of age onwards. Mild catarrhal enteritis was noted in the small intestine and vibrios were noted in smears. The isolation of such large numbers of organisms from enteric lesions both in the survey and in the experimental studies does not appear to have been reported previously. The

presence of the organism in the caecum and colon is more familiar from the work of Doyle (1944) and Roberts (1956a and b). In these experimental studies, however, the association between C. coli and colonic lesions was less clear. C. coli had been seen in and isolated from the colonic mucosa in the survey Table 4, Chapter 3 and was isolated from the colonic mucosa of all pigs in Experiments 1, 2, 3 and 4 but few specific lesions were associated with its presence in that region. These findings differed from those of Prescott et al. (1982) who suggested that colonic lesions did occur in gnotobiotic pigs following infection with the closely related C. jejuni.

C. coli and C. jejuni are closely related by their biochemical properties. These 2 species of Campylobacters were often confused with each other by various workers until recently. The 2 species can now easily be differentiated by the rapid hippurate hydrolysis test (Harvey, 1980; Skirrow and Benjamin, 1982), and growth at the temperature of 30.5°C (Skirrow and Benjamin, 1980). There is no doubt, however, that the isolates used in these studies were of C. coli (Chapter 3).

C. jejuni is of less importance in pigs as a pathogen but has been reported as a primary pathogen of man (Skirrow, 1977) and cattle (Al-Mashat and Taylor, 1980). There are few reports of its isolation from pigs, and these isolations are from faeces and washed intestines (Sticht Groh, 1982). The only experimental infection of pigs with C. jejuni was reported by Prescott et al. (1981) who used a human isolate. Their infection did not produce any

clinical changes but did produce colitis and they therefore considered that C. jejuni caused large intestinal changes. In the studies described in Chapter 4 this association was not clear, possibly because C. coli was used rather than C. jejuni and possibly because gnotobiotic pigs were not used.

The changes seen in experimental studies in Chapter 4 appear to be distinct from those of proliferative intestinal adenopathy which is said to be initiated by another species of campylobacter, C. sputorum ss. mucosalis. C. coli differs biochemically and serologically from C. sputorum ss. mucosalis. The lesions of proliferative intestinal adenopathy are constantly associated with the presence of intracellular vibrios, which have been identified as C. sputorum ss. mucosalis (Lawson and Rowland, 1975) but the studies reported in Chapter 4 failed to show either the presence of intracellular campylobacters or the proliferative change in the mucosa. The thickening seen in the terminal ileum in the experimental studies described in Chapter 4 was due to an increase in lymphoid tissue rather than to an increase in the thickness of the lamina propria as in proliferative intestinal adenopathy. While both C. coli and C. sputorum ss. mucosalis can be regarded as different pathogens of pigs causing different types of enteric lesions, there is evidence from the findings in Chapter 3 and from the accounts by Lawson and Rowland, 1974, that C. coli may complicate the lesions seen in C. sputorum ss. mucosalis infection.

The changes seen in C. coli infection might have been confused with those seen in coccidial or cryptosporidial infection, but coccidia were not seen in any of the histological sections in the experiments and cryptosporidia were only seen in 1 control pig (Pig 28) in Experiment 3. Coccidia were present in the herd and were demonstrated in Pig 4218, Study 1. Neither agent was demonstrated in the faeces of the experimental pigs and it seems unlikely that either was involved in the changes seen.

The studies described in Chapter 4 provide some information about the pathogenicity of C. coli in pigs of different ages and of different immune status. The information from that chapter is discussed here as a coherent account of the infection, its distribution in a herd and its significance.

C. coli is present in the faeces of both diarrhoeic and normal piglets (Studies 1 and 2). On Farm 3 it was found in the faeces of pigs aged 9 days and more but in Farm 1 it was clearly present in younger animals (Chapter 3 and Appendix 1). Since oral infection with C. coli (Chapter 4) produced a syndrome similar to that seen in pigs from the survey it appears likely that faeces from infected pigs containing C. coli must be ingested whole or as a contaminant of feed and water in order to initiate infection. Following infection, a transient fever (to 40.6°C) develops which may be maintained for 2-3 days. A watery or creamy diarrhoea containing mucus with occasional streaks of blood is present from the 3rd day following

infection. There is shedding of the bacteria in the faeces from the 3rd day following infection, and this lasts for 12 or more days. In weaned pigs, C. coli infection may be associated with chronic mucoid diarrhoea in which no blood is seen. In both sucking and weaned pigs, loss of condition appears to occur but mortality is not a feature.

In the studies described in Chapter 4, evidence was found for the presence of C. coli outside the gut and its associated lymph nodes only in 1 animal, Pig 283 in Experiment 4 in which it was isolated from the liver. In all 4 of the experiments described in Chapter 4 the organism was found in the mesenteric lymph nodes of some animals. It appears that C. coli does not regularly colonise organs other than the gut. It is not possible to say whether a bacteraemia occurs early in the disease as most pigs were killed more than 4 days post inoculation. Some evidence that C. coli may penetrate the basement membrane of the intestinal epithelium early in the infection is provided by the presence of circulating agglutinating antibody within 96 hours of inoculation (Table 23).

The organism appears to colonise the whole gastrointestinal tract and may be found in the stomach and duodenum within the first 4 hours of infection (P4208, Chapter 4, Experiment 2), but is not found in the stomach and only infrequently occurs in the duodenum in chronic cases. The greatest concentrations appear to occur in the ileum, caecum and colon although counts were not carried out (Tables 19, 22, 25 and 28).

Within an infected portion of the gut the organism is present in the mucosa as it may be isolated from the washed mucosa and demonstrated in smears made from the surface (Fig.5). It is probable that it is also present in the contents but counts were not performed on contents and mucosa in order to determine its distribution within the gut. From the histological studies in Chapter 4, the organism appeared to be within the crypts and at the mouth of the crypts, but was not intracellular. There is no evidence of cellular invasion by C. coli in the studies described in Chapter 4.

Organisms with the morphology of C. coli were adjacent to, but not closely adherent to the microvilli of the lumenal epithelial and crypt cells in the early stages of the disease (Figs. 36 and 43). This loose association was similar to that noted in the adhesion studies carried out in vitro and described in Study 3 of Chapter 4.

The failure to observe any close association may have been a reflection of the real relationship of C. coli with the tissues but may have been due to the time at which the pigs were killed after infection. In the conventional pigs, immunity, either passive (Experiment 2) or active (Experiment 3) may have been present as C. coli infection was widely distributed in both sows and weaned pigs in the herd from which the experimental pigs came (Study 2).

The effects noted in the mucosa and described and discussed in Chapter 4 were probably not due to invasion of the epithelium but may have been due to a toxic product of

the organism. Very little information is available in the literature about the type of the toxins produced by C. coli. It has been suggested that C. jejuni which is closely related to C. coli, produces an enterotoxin (Gubina et al., 1981). Similar observations were made by Butzler and Skirrow (1979), who suggested that it is not a heat labile enterotoxin. Fumarola et al. (1982) suggested that an endotoxin was responsible and the biochemical studies of Naess and Hofstad (1982) suggested that the endotoxin may be Lipid A. The cellular infiltration of neutrophils and lymphocytes into the lamina propria and crypts which occurs following infection of pigs with C. coli, is similar to the reaction described in man and other animal species with C. jejuni (Butzler and Skirrow, 1979; Al-Mashat and Taylor, 1980). This suggests that the mechanism of both disease and lesion production might be similar in infections with both organisms.

C. coli is a microaerophilic organism and its requirement for conditions of this type may govern both its location in the gut and the numbers present. The presence of the organism in the ileum, caecum and colon in infected pigs may reflect their microaerophilic requirements and their absence from the crypts may reflect a local increase in oxygen tension following inflammation. In the large intestine, where conditions are more anaerobic, the optimum site for growth may be the crypts of the mucosa and in particular the region at the mouth of the crypt. This requirement for microaerophilic conditions as well as the development of immunity may be responsible for the changes in the distribution of C. coli noted with time in Experiment

2 (Table 22). C. coli was originally found in the stomach, duodenum, jejunum and ileum but in animals killed later was restricted largely to the ileum caecum and colon. This finding may explain the colitis noted in the gnotobiotic studies of Prescott et al. (1982) with C. jejuni.

In conventional animals and in the survey, C. coli was present with other agents or in lesions initiated by them. It may be that the tissue debris, serum and blood produced by C. coli or other agents may enhance their ability to tolerate the anaerobic conditions and account for their distribution within the gut, especially in the large intestine.

It seems that C. coli persists in the mucosa of infected animals long after it could be recovered reliably from faecal samples (Experiment 3, Chapter 4) and all experimental animals were positive upon mucosal culture even when it had been difficult to isolate the organism from their faeces. This finding suggests that the detection of carriers by faecal sampling may be difficult, and the organism may be involved in enteritis of pigs caused by other agents which allow the proliferation of C. coli already present in low numbers in previously exposed animals which have remained as carriers.

This carrier state and the presence of low levels of serum agglutinating antibody does not appear to prevent colonisation by another isolate (Experiment 3, Chapter 4) or to prevent the development of some of the changes found following infection.

It is thus clear from the above discussion that C. coli is capable of initiating an enteric syndrome which can be detected by clinical signs and pathological changes particularly in non-immune piglets. In all animals, infection with this organism may contribute to the features described above - transient fever, mucoid diarrhoea which may contain blood particularly in piglets, thickening of the terminal ileum, gross and microscopic inflammatory lesions in the jejunum, ileum, caecum and colon and lymphoid hyperplasia, including enlargement of the mesenteric lymph nodes. In many cases C. coli is probably present in the inoculum which initiates the disease, but in others it may be present locally in the mucosa of the carrier pigs and multiply when conditions favour its growth. The studies described here did not suggest that C. coli caused death but its presence may contribute blood, mucus and some diarrhoea to enteric syndromes and some inflammatory changes in the jejunal, ileal, caecal and colonic mucosa to the pathology of some enteric diseases. The effects of infection of C. coli on productivity still need to be studied in more detail.

Clostridium perfringens Type A in porcine enteric disease

C. perfringens Type A was isolated from inflammatory lesions in the intestinal tract of piglets dying from a number of disease conditions as described in the survey in Chapter 3. Profuse cultures were isolated from the small intestinal mucosa (Table 5) but the organism was less common in the large intestinal mucosa. It was most commonly isolated in profuse culture from sucking pigs (Table 5 and

Appendix 1) and was present in the intestines of weaned pigs with 'bloody gut'.

As it was present in mixed culture in all lesions, an isolate considered to be typical of those found was identified biochemically and by means of C. perfringens Type A antiserum in the Nagler reaction as C. perfringens Type A (Chapters 2 and 3). It was used to infect HDCD piglets and conventional weaned pigs (Chapter 5). In these experimental infections transient fever, creamy faeces flecked with blood and mucus was passed by non-immune piglets and small amounts of blood and mucus were found in the faeces of weaners. The infection appeared to be fatal in non-immune piglets (Experiment 5), but the clinical signs were less severe in the older pigs. There may have been an effect on productivity but the evidence for this (Experiments 6 and 7) was equivocal.

The main site of the infection appears to be the small intestine and inflammatory and necrotic changes appeared to result from infection in piglets. In the older pigs, particularly in those killed during the early stages of the disease in Experiment 8, fluid production in the small intestine appeared to be the most consistent finding although there were inflammatory changes including cell shedding and congestion. The lesions seen in natural cases described in the survey resembled those seen in the experimental infections particularly those in the HDCD piglets which resembled closely those found in sucking piglets. The small intestinal changes and clinical signs

produced in the weaned pigs were much less marked than those seen in bloody gut but shared with them the fluid secretion and the cell shedding. The organism was also present in the small intestines in large numbers at the sites of the lesions. These studies appear to represent the first account of the isolation of C. perfringens Type A from piglet intestine and its relation to specific lesions in the porcine intestinal tract by experimental reproduction. The earlier reports by Moon and Dillman (1972) and Amstsberg et al. (1976) were mainly speculative or descriptive.

The experimental studies described in Chapter 5 show that C. perfringens Type A infection may cause an acute and fatal disease in non-immune piglets and it is possible that productivity may be affected in older pigs. These findings are similar to those reported in other species such as in man by Hobbs (1965); chickens by Al Sheikhly and Truscott (1977); lambs by Hauschild et al. (1967) and calves by Niilo and Dorward (1971).

The observations reported here in both Chapters 3 and 5 and those in the literature reviewed in Chapter 1 provide the basis for the construction of a hypothesis for the pathogenicity of C. perfringens Type A infections in pigs. Clostridial enteritis in other species results from oral infection and this was the case with the studies described here in Chapter 5. The organism is restricted to the gut even in animals killed in extremis (Experiment 5, Table 33) and early in the disease (Experiment 8, Table 42). It multiplies throughout the intestine but appears

to be most common in the small intestine during the initial stages of infection (Experiment 8). In recovered animals it is largely restricted to the ileum and large intestine (Experiments 6 and 7). This distribution may result from the development of immunity or to changes in oxygen tension (it is an oxygen tolerant anaerobe). The reasons for its initial colonisation of the anterior small intestine are not clear. Within the intestine it appears not to be closely associated with epithelial cells, and invasion was not a feature of C. perfringens Type A infections in the earliest cases examined (Experiment 8). No information about the early disease in HDCD piglets was available in this study and invasion or adhesion may occur in that class of animal. This is in contrast to infection with C. perfringens Type C which is known to invade the cells of the villous epithelium (Arbuckle, 1972). It appears that a toxin may be responsible for the disease produced by infection with C. perfringens Type A. This supposition is in agreement with information available in literature about the organism in other species. Necrotic enteritis was produced in 4 week old chickens with bacteria-free crude toxins of C. perfringens Type A (Al-Sheikly and Truscott, 1977b) and similar findings have been reported with experimental infections in lambs (Hauschild et al., 1971). The role of enterotoxin in C. perfringens Type C is, however, not well understood. Typical necrotic lesions were not reproduced in pig ligated intestinal loops injected with bacterial free toxin, whereas focal areas of bacterial invasion and necrosis were seen in loops injected with whole-broth cultures of C. perfringens Type A by Bergeland, 1972.

Evidence for the production of toxins by C. perfringens Type A in these studies is largely indirect. C. perfringens Type A was not seen to adhere to isolated brush borders when used as a control to C. coli in Chapter 4, Study 3 and was apparently present in the lumen of crypts in the earliest stages of the disease (Experiment 8). It also comes from the types of changes seen. In the older pigs the fluid contents of the small intestine and the slight changes seen in the mucosa suggest that an enterotoxin may be involved (Experiment 8). C. perfringens Type A is capable of producing an enterotoxin particularly in association with sporulation (Hauschild et al., 1970). Sporulation is encouraged by the presence of starch which would have been present in the diet of weaned pigs but not in that of milk fed HDCD piglets. It may be that this factor led to the difference in the effects of infection noted in the weaned animals when compared with the HDCD piglets.

Another factor suggesting that a separate mechanism is involved is that in the HDCD piglets serum antibody to alpha toxin (lecithinase) was shown to develop after infection. None was demonstrated in the serum of recovered weaned pigs (Experiments 6, 7 and 8). It may be that the toxin was not elaborated in the weaned pigs or that local immunity prevented its entry. In any case it was clearly involved in the immune response to infection in the HDCD piglet which survived the initial infection.

The development of clinical signs and pathological changes (Experiments 5 and 8) did not occur immediately

following infection and it therefore seems that the changes were not produced by preformed toxin in the inoculum. Further controlled experiments would be needed to evaluate this point.

The lesions seen in this study did not resemble those of C. perfringens Type C. The massive haemorrhage reported in that disease was absent. In C. perfringens Type C infection, an advancing zone of necrosis is reported in literature to proceed through the crypts, muscularis and submucosa and even eventually involve the tunica muscularis. The necrotic lesions seen in C. perfringens Type A were limited to the mucosal layer. It is clear from the studies described in Chapter 5 that the mode of action of Type A in causing enteric disease in pigs may be very different from that of Type C. The animals which died or were killed in Experiment 5 did, however show some evidence for the absorption of toxins in that rapid decomposition of the carcase was noted in the absence of systemic bacterial invasion and that pallor and fatty change was a feature of the livers of Pigs 8 and 9. It is possible also that the nervous signs noted in Pig 2008, Experiment 5, were also evidence for the absorption of toxin.

The studies described in Chapter 5 and discussed briefly here have shown that C. perfringens Type A, up till now disregarded by workers on enteric diseases of pigs, is a real pathogen of the pig. Non-immune piglets are more prone to infection than older animals. Enteric lesions are more pronounced in the small intestine than in the large intestine. Even though clinical signs caused by this

organism alone appear not to be widespread in pig herds, there seems little doubt that it is present in many herds (Chaptér 3) and could cause disease in non-immune animals.

More importantly it may contribute to and exacerbate conditions initiated by other agents and it is here that it may be most important. Its involvement in bloody gut is of interest but is clearly not causal as the infection of weaned pigs in Experiments 6, 7 and 8 failed to reproduce it. Some features of the condition, such as some mucosal congestion, some fluid secretion and some cell shedding were, however, demonstrated in Experiment 8.

The lack of attention to this organism by past workers on enteric disease of pigs might be due to the prominence given to C. perfringens Type C. The information generated in these studies which suggest that the 2 types may have different ways of causing disease in pigs should encourage investigators to look for Type A, particularly in cases of enteritis in piglets with uncertain aetiology.

CONCLUSION

In conclusion, the studies in this thesis have shown that a number of bacteria are present in the mucosa of the porcine enteric tract. Some of them are associated with specific gross and microscopic lesions, while others could not be associated with any particular lesions and their role is difficult to define. The bacteria isolated from the lesions are in many cases not considered to be pathogens of the porcine enteric tract and are considered

to belong to the normal flora of the porcine gut.

The experimental studies described in Chapters 4 and 5 demonstrated that both C. coli and C. perfringens Type A, apparently 'normal' inhabitants of the intestinal tract, could cause lesions in certain circumstances, particularly in young piglets. These findings suggest that other species found in the lesions may prove to cause or exacerbate intestinal lesions and may even cause clinical disease if tested under appropriate conditions.

In addition to the relationships between the other bacteria found and disease, C. coli and C. perfringens Type A should also be studied further along the lines suggested above. The pathogenesis of both types of infection requires further study as does their relationship to productivity. The relationship between bacteria such as C. coli and C. perfringens Type A and other agents such as viruses and parasites should also be examined. This study represents a first step in this direction.

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APPENDIX I

Detailed results of the survey in Chapter 3

Appendix 1. Detailed results of the survey

Animal Number	Farm	Age	State of Animal	Diagnosis	Bacteria Isolated	Stomach			Small Intestine			Large Intestine		
						Isol-ation	Lesions seen Macro	Micro	Isol-ation	Lesions seen Macro	Micro	Isol-ation	Lesions seen Macro	Micro
1	1	1W	(D)	Enteritis	Non-haemolytic E.coli.	+	HF+P	ND	+	S	ND	+	FC	ND
					B-haemolytic E.coli.	+	N		+	N		+	N	
					Streptococcus faecalis.	+			+			+		
					Clostridium perfringens.	-			+			+		
2	1	5d	(D)	Enteritis	Non-haemolytic E.coli.	+	EM	ND	+	S	ND	+	FC	ND
					B-haemolytic E.coli.	-	N		+	LCO		+	N	
					Streptococcus faecalis.	+			+			+		
					C.perfringens.	-			+			+		
3	1	8d	(D)	Enteritis	Non-haemolytic E.coli.	+	HF		+	S	ND	+	FC	ND
					B-haemolytic E.coli.		N			MCO			N	
					Streptococcus faecium.									
					C.perfringens.									

Key to symbols on final page.

Appendix 1 (Continued)

4	1	6d	(D)	Enteritis Septicaemia Arthritis	Non-haemolytic E.coli. Streptococcus faecalis.	+ +	EM n	ND	+ +	VS SCO	+	+ +	PC	+
5	3	3½w.	(D)	Enteritis *	Campylobacter coli. Non-haemolytic E.coli. B-haemolytic E.coli. S.faecalis.	- + - +	EM LCO	ND	+ + + +	VS CO	ND	+ - + +	S LCO	+
6	1	3w	(D)	Pneumonia Pericarditis Enteritis	Non-haemolytic E.coli. S.faecalis.	+ -	F N	ND	+ -	S LCO	+	+ +	FD N	-
7	1	3w	(D)	Enteritis	B-haemolytic E.coli. Non-haemolytic E.coli. C.perfringens. S.faecium.	- +	HF CO	ND	+ +	S CO	+	+ +	FC LCO	+
8	1	2d	(D)	Starvation	Non-haemolytic E.coli. S.faecium.	+ -	EM N	ND	+ +	NC N	ND	+ +	FC N	ND

Key to symbols on final page.

Appendix 1 (Continued)

9	1	1d	(D)	Overlaying internal bleeding	Non-haemolytic E.coli.	-	EM N	ND	+	NC N	ND	+	FC N	ND
10	1	2d	(D)	Starvation	Non-haemolytic E.coli.	-	EM N	ND	+	EM N	ND	+	EM N	ND
11	1	3½w	(D)	Enteritis Pneumonia	C.perfringens. Type A.	-	EM	ND	+	VS	+	+	PC	+
					Non-haemolytic E.coli.	+	N		+	SCO		+	MCO	
					Pasteurella multocida.	+			+			-		
					S.faecalis.	-			-			+		
12	3	2w	K	Enteritis *	C.coli.	-	EM		+	S	+	+	FC	-
					S.faecalis.	+	N		+	MCO		-	N	
					Non-haemolytic E.coli.	+			+			-		
					Staphylococcus epidermidis.	+			+			+		
13	1	4w	(D)	Enteritis	B-haemolytic E.coli.	-	EM		+	VS		+	PC	+
					Non-haemolytic E.coli.	+	N		-	LCO		-	CO (necrosis)	
					C.perfringens.	-			+			+		
					S.faecalis.	-			-			+		
					S.epidermidis.	-			-			+		

Key to symbols on final page.

Appendix 1 (Continued)

14	1	3w	(D)	Pneumonia	B-haemolytic E.coli.	-	EM	ND	+	S	ND	+	FC	ND
					Non-haemolytic E.coli.	-	N		+	LCO		-	N	
					S.faecalis.	+			+			+		
					C.perfringens Type A.	-			+			+		
15	1	3½w	(D)	Enteritis Pneumonia	B-haemolytic E.coli.	+	HF	-	+	S	+	+	FC	-
					S.faecium.	-	N		+	CO		+	N	
					Non-haemolytic E.coli.	+			+			+		
16	1	2d	(D)	Enteritis	B-haemolytic E.coli.	+	HF		+	S		+	FC	
					S.faecalis.	+	N		+	CO		+	N	
					Non-haemolytic E.coli.	+			+			+		
17	1	2d	(D)	Enteritis	B-haemolytic E.coli.	-	EM	-	+	S	+	+	FC	+
					S.faecalis.	-	N		-	CO		+	N	
					Bacillus licheniformis.	-			+			-		
18	1	2d	(D)	Enteritis	B-haemolytic E.coli.	-	HF	ND	+	S	ND	+	FC	ND
					S.faecalis.	+	N		+	CO		+	N	

Key to symbols on final page.

Appendix 1 (Continued)

19	1	3½w	(D)	Enteritis	B-haemolytic E.coli.	-	EM	-	+	S	+	+	F	-
					Non-haemolytic E.coli.	+	N		+	LCO		-	N	
					S.faecium.	-			+			-		
					Peptostreptococcus spp.	-			-			+		
20	1	2½w	(D)	Peritonitis Enteritis Pericarditis	S.faecalis.	+	HF	+	+	VS	+	-	S	+
					Pasteurella multocida.	-	CO		+	CO		-	CO	
					Non-haemolytic E.coli.	+			+			-		
					Peptostreptococcus spp.	-			+			+		
					Bacillus licheniformis.	+			-			-		
21	1	3w	(D)	Pneumonia Enteritis	Non-haemolytic E.coli.	+	EM	-	+	VS	+	+	PC	+
					B-haemolytic E.coli.	+	N		+	CO		+	LCO	
					S.faecalis.	-			-			+		
					B.licheniformis	-			+			+		
					Clostridium sporogenes.	+			-			+		

Key to symbols on final page.

Appendix 1 (Continued)

22	3	6w	(D)	Multiple Arthritis Enteritis	B-haemolytic E.coli.	-	HF	-	+	S	+	+	FC	-
					Non-haemolytic E.coli.	+	N		-	MCO		-	N	
					S.faecium.	+			+			-		
					Peptostreptococcus spp.	-			-			+		
23	1	17w	(D)	Pneumonia	Non-haemolytic E.coli.	+	HF	-	+	S	+	+	FC	-
					S.faecalis.	+	N		+	MCO		+	N	
					Peptostreptococcus spp.	-			-			+		
24	1	6w	(D)	Pneumonias	Non-haemolytic E.coli.	+	EM	+	-	VS	+	+	PC	+
					C.perfringens Type A.	-	LCO		+	MCO		+		
25	1	1d	(D)	Traumer and Starvation	Non-haemolytic E.coli.	+	EM N	ND	+	EM N	ND	+	EM N	ND
26	1	1d	(D)	Overlaying	Non-haemolytic E.coli.	+	EM N	ND	+	EM N	ND	-	EM N	ND
27	1	1d	(D)	Overlaying	Non-haemolytic E.coli.	+	EM	ND	+	EM	ND	+	EM	ND
					S.faecalis.	-	N		+	N		+	N	

Key to symbols on final page.

Appendix 1 (Continued)

28		1d	(D)	Overlaying	S.faecalis.	+	EM	+	+	S	+	+	FC	-
	1				C.perfringens.	-	LCO		+	LCO		+	N	
29		2w		Pneumonia	B-haemolytic E.coli.	-	HF	ND	+	S	+	+	PC	+
	2			Enteritis	Non-haemolytic E.coli.	+	N		-	CO		-	MCO	
					C.perfringens Type A.	-			+			+		
					S.faecium.	+			+			-		
					Peptostreptococcus intermedius.	-			-			+		
30		1w		Pericarditis	B-haemolytic E.coli.	-	HF	+	+	S	+	+	FC	+
	2			Pneumonia Enteritis	Non-haemolytic E.coli.	+	CO		-	MCO		+	N	
					C.perfringens.	-			+			+		
					S.faecalis.	-			-			+		
31		1½w		Peritonitis	B-haemolytic E.coli.	-	HF	+	+	VS	+	-	PC	+
	2			Enteritis	Non-haemolytic E.coli.	+	MCO		+	CO		+	LCO	
					Streptococcus spp.	+			+			+		
					C.coli.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

32	2	2w	(D)	Enteritis	S.epidermidis.	+	EM	+	+	VS/B	+	-	S	+
					B-haemolytic E.coli.	-	CO		+	CO		-	LCO	
					Non-haemolytic E.coli.	+			+			+		
					C.perfringens Type A.	-			+			+		
					C.coli.	-			+			+		
33	2	4w	(D)	Pneumonia	B-haemolytic E.coli.	-	F	-	+	VS	+	-	PC	+
					Non-haemolytic E.coli.	+	N		+	CO		+	CO	
					S.faecalis.	+			+			-		
					C.coli.	-			+			+		
34	2	Adult	(D)	Haemorrhagic bowel syndrome Pericarditis Pneumonia	Erysipelothrix rhusiopathiae.	-	FF	+	+	FF/B	+	-	FF/B	+
					C.perfringens. Type A.	-	N		+	SCO		-	CO	
					Corynebacterium pyogenes.	-			+			+		
					Non-haemolytic E.coli.	-			-			-		
					S.faecalis.	+			+			+		
					Lactobacillus spp.	-			+			+		
					P.intermedius.	-			-			+		
					C.coli.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

35	1	1w	(D)	Enteritis	C.coli.	+	HF	-	+	VS	+	+	S	+
					C.perfringens Type A.	-			+			+		
					Non-haemolytic E.coli.	-	N		+	CO		+	LCO	
					S.faecium.	+			+			+		
36	1	7d	(D)	Enteritis	C.coli.	-	HF	ND	+	VS	+	+	PC	+
					Peptostreptococcus spp..	-	N		+	SCO		+	CO	
					Non-haemolytic E.coli.	-			+			+		
					C.perfringens Type A.	-			+			+		
37	1	7d	(D)	Enteritis Apical Pneumonia	S.epidermidis.	+	EM	-	-	S	+	+	PC	+
					S.faecalis.	+			+			+		
					C.coli.	-	N		+	CO		+	N	
					C.perfringens Type A.	-			+			+		
38	1	2w	(D)		C.perfringens Type A.	-	HF	ND	+	VS	+	+	S	+
					Non-haemolytic E.coli.	-	N		+	CO		+	CO	
					Proteus mirabilis.	-			+			+		
					S.faecalis.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

39	1	4w	(D)	Pneumonia	Non-haemolytic E.coli.	-	HF	ND	+	S	ND	+	FC	ND
					S.faecalis.	+	N		+	CO		+	N	
					C.perfringens Type A.	-			+			+		
40	1	7d	(D)	Enteritis	C.coli.	-	EM	-	+	VS	+	+	S	+
					B-haemolytic E.coli.	-			+			-		
					Non-haemolytic E.coli.	+	N		-	CO		+	CO	
					S.faecalis.	-			-			+		
41	3	10w	(K)	Swine Dysentery	Non-haemolytic E.coli.	+	F	ND	+	S	-	-	S	+
					C.perfringens.	-	N		+	N		+	CO	
					T.hyodysenteriae.	-			-			+		
					Bacteroides vulgatus.	-			-			+		
					Lactobacillus spp..	-			-			+		
					Pseudomonas aeruginosa.	-			+			+		
42	3	12w	K	Enteritis	Fusobacterium necrophorum.	-	F	ND	-	S	+	+	PC	+
					C.perfringens.	-	N		+	LCO		+	LCO	
					Non-haemolytic E.coli.	-			+			+		
					C. pyogenes.	-			+			-		
					S.faecalis.	+			+			+		
					S.epidermidis.	+			-			-		

Key to symbols on final page.

Appendix 1 (Continued)

43	3	12w	K	Enteritis Swine Dysentery	T.hyodysenteriae.	-	F	ND	-	S	+	+	PC	+
					C.perfringens. Type A.	-			+			+		
					Non-haemolytic E.coli.	+	N		+	MCO		+	CO	
					S.faecalis.	+			+			+		
					S.epidermidis.	+			-			-		
					Bacteroides fragilis.	-			-			+		
44	3	12w	K	Swine Dysentery Enteritis	T.hyodysenteriae.	-	F	ND	-	VS	+	+	S	+
					B.vulgatus.	-			-			+		
					Non-haemolytic E.coli.	+	N		-	CO		+	CO	
					S.faecalis.	+			+			+		
					C.perfringens.	-			+			+		
45	3	12w	K	Enteritis	T.hyodysenteriae.	-	F	ND	-	NC	-	+	PC	+
					B.fragilis.	-			-			+		
					Non-haemolytic E.coli.	-	N		+	N		+		
					S.faecalis.	+			+			+		
					S.epidermidis.	+			+			-		
46	3	12w	K	Enteritis	B.vulgatus.	-	F	ND	-	HC	+	+	PC	+
					C.perfringens.	-	LCO		+	N		+	CO	
					T.hyodysenteriae.	-			-			+		
					Non-haemolytic E.coli.	+			+			+		
					S.faecalis.	+			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

47	3	12w	K	Enteritis Swine Dysentery	T.hyodysenteriae.	-	F	ND	-	S	+	+	S	+
					C.coli.	-			+			+		
					Lactobacillus spp.	-	N		+	CO		+	CO	
					Non-haemolytic E.coli.	+			+			+		
					S.faecalis.	+			+			+		
					Peptostreptococcus spp..	-			-			+		
					F.necrophorum.	-			-			+		
48	3	13+w	K	Enteritis Apical Pneumonia	Bacillus mycoides.	+	F	ND	+	S	+	-	PC	+
					C.coli.	-			+			+		
					T.hyodysenteriae.	-	N		-	CO		+	LCO	
					S.faecalis.	+			+			+		
					Non-haemolytic E.coli.	+			+			-		
49	3	13+w	K	Enteritis	T.hyodysenteriae.	-	F	ND	-	N	-	+	PC	+
					Non-haemolytic E.coli.	+	N		+	N		+	LCO	
					S.faecalis.	+			+			+		
50	3	12+w	K	Enteritis	T.hyodysenteriae.	-	F	ND	-	S	+	+	S	+
					C.coli.	-			+			+		
					Non-haemolytic E.coli.	+	N		+	CO		+	CO	
					Streptococcus spp..	+			+			+		
					B.vulgatus.	-			-			+		
					Lactobacillus spp.	-			-			+		

Key to symbols on final page.

Appendix 1 (Continued)

51	3	12+w	K	Enteritis	B.vulgatus.	-	F	ND	+	S	+	+	PC	+
					Lactobacillus spp.	-			+			+		
					T.hyodysenteriae.	-	N		-	MCO		+	CO	
					C.coli.	-			+			+		
					S.faecalis.	+			+			+		
52	3	12w	K	Enteritis Pneumonia	Non-haemolytic E.coli.	+	F	ND	+	NC	-	+	NC	-
					C.perfringens.	-	N		+	N		+	N	
53	1	3w	(D)	Pneumonia	Non-haemolytic E.coli.	-	F	ND	+	S	ND	-	PC	ND
					Bacillus spp..	-			+			-		
					Corynebacterium	-			+			-		
					S.faecium.	+			+			+		
					S.epidermidis.	+			-			+		
					C.perfringens Type A.	-			+			+		
54	1	2w	(D)	Enteritis	C.coli.	-	F	ND	+	VS	ND	+	S	ND
					Lactobacillus spp.	-			+			+		
					S.faecalis.	+	N		+	CO		+	LCO	
					S.epidermidis.	-			-			+		
55	1	6d	(D)	Enteritis	C.perfringens Type A.	-	HF	ND	+	S	+	+	PC	+
					F.necrophorum.	-	N		-	SCO		+	CO	
					S.epidermidis.	-			-			+		
					S.faecalis.	-			+			+		
					Non-haemolytic E.coli.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

56	1	3w	(D)	Pneumonia Enteritis	B-haemolytic E.coli.	-	F		+	S	ND	-	PC	ND
					P.mirabilis.	+	N		+	LCO		-	LCO	
					S.faecalis.	-			+			+		
					B.vulgatus.	-			-			+		
57	4	3½w	(D)	Enteritis	C.perfringens Type A.	-	HF	+	+	VS/B	+	+	vs/B	+
					C.pyogenes.	+	CO		+	CO		-		
					Pasteurella multocida.	+			+	(necrosis)		-		
					C.coli.	-			+			+		
					S.faecalis.	-			+			+		
					S.epidermidis.	-			+			+		
					F.necrophorum.	-			-			+		
58	4	3w	K	Meningitis Septicaemia Pneumonia	Streptococcus suis	+	EM	+	+	S	+	+	PC	+
					Non-haemolytic E.coli.	-	LCO		+	CO		+	MCO	
					C.pyogenes.	+			+			-		
					C.coli.	-			+			+		
					Bacillus cereus.	-			-			+		
					S.epidermidis.	+			-			+		
59	4	13d	(D)	Enteritis Pneumonia	C.coli.	-	F	ND	+	S	ND	+	FC	ND
					Lactobacillus fermentum.	+			+			+		
					S.suis.	+	N		+	CO		+	N	
					C.perfringens.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

60	4	3w	(D)	Pneumonia Enteritis	C.perfringens Type A.	+	F	+	+	VS/B	+	+	S	+
					Non-haemolytic E.coli.	+	LCO		+	CO	(necrosis)	+	CO	
					S.faecium.	+			+			+		
					F.necrophorum.	-			-			+		
					C.pyogenes.	+			+			+		
61	1	5d	(D)	Retro-peritoneal haemorrhage Enteritis Starvation	B-haemolytic E.coli.	+	EM	ND	+	S	ND	+	S	ND
					Non-haemolytic E.coli.	+	N		+	MCO		-	LCO	
					S.faecalis.	+			+			-		
					B.licheniformis.	-			-			+		
					C.perfringens.	-			+			+		
62	1	1d	(D)	Overlaying	Non-haemolytic E.coli.	+	EM	ND	+	NC	ND	+	FC	ND
					S.faecalis.	+	N		+	N		-	N	
					B.licheniformis.	-			+			+		
63	1	3d	(D)	Enteritis	Non-haemolytic E.coli.	+	EM	-	+	S	+	+	PC	-
					B-haemolytic E.coli.	+	N		+	MCO		-	N	
					S.faecium.	+			+			-		
					S.epidermidis.	+			+			+		
					B.licheniformis.	-			-			+		
64	1	10d	(D)	Enteritis Pneumonia Pericarditis	B-haemolytic E.coli.	+	EM	ND	+	S	ND	+	PC	ND
					Lactobacillus spp...	-	N		+	LCO		+	N	
					S.faecalis.	-			-			+		

Key to symbols on final page.

Appendix 1 (Continued)

65	1	1d	(D)	Stillborn	Streptococcus spp..	+	EM N	ND	-	EM N	ND	-	EM N	ND
66	1		(D)	Stillborn	Non-haemolytic E.coli.	+	EM N	ND		EM N	ND	-	EM N	ND
67	1	7d	(D)	Enteritis	C.coli. Streptococcus spp..	- +	F N	ND	+ +	S MCO	ND	+ +	FC LCO	ND
68	1	12d	(D)	Peritonitis Pneumonia Enteritis	P.multocida. C.pyogenes. S.faecalis. P.aeruginosa. Non-haemolytic E.coli.	+ + + - -	EM LCO	+	+ + + + +	S CO	+	+ + - + -	FC MCO	+
69	1	2d	(D)	Enteritis Pericarditis	B-haemolytic E.coli. B.licheniformis.	- -	EM N	ND	+ +	NC MCO	ND	+ +	FC N	ND
70	1	3d	(D)	Enteritis	C.perfringens Type A. B.licheniformis. Non-haemolytic E.coli. S.faecalis.	- - - +	HF N	+	+ + + +	VS/B SCO	+	+ - + +	S LCO	+
71	1	3d	(D)	Internal Bleeding	Non-haemolytic E.coli. Streptococcus spp..	- -	EM N	ND	+ +	EM N	ND	+ +	EM N	ND

Key to symbols on final page.

Appendix 1 (Continued)

72	1	22d	(D)	Atresia ani	Non-haemolytic E.coli.	+	F	ND	+	S	ND	+	PC	ND
					S.faecalis.	+	CO		+	CO		+	CO	
73	1	3w	(D)	Enteritis	Non-haemolytic E.coli.	-	EM	ND	+	VS	+	-	S	+
					S.faecalis.	+	N		+	CO		+	CO	
					B.vulgatus.	-			-			-		
					F.necrophorum.	-			-			-		
74	1	2w	(D)	Enteritis	Non-haemolytic E.coli.	-	EM	+	+	S	+	+	PC	+
					S.faecalis.	+	CO		+	SCO		+	SCO	
					Lactobacillus spp.	-			+			+		
75	1	3w	(D)	Gastric ulceration enteritis	C.coli.	-	FF	+	+	VS	+	+	S	+
					S.faecalis.	+			+			+		
					Bacillus spp..	+	UL		+	CO		+	LCO	
76	3	13w	K	Enteritis	Non-haemolytic E.coli.	-	N	-	+	S	ND	+	PC	ND
					C.coli.	-			+	MCO		+	LCO	
77	3	12w	K	Enteritis	Peptostreptococcus spp..	-	F	ND	-	S	+	+	F	+
					Lactobacillus spp..	-	N		+	MCO		+	LCO	
					Non-haemolytic E.coli.	+			+			-		
					C.coli.	-			+			+		
					S.faecalis.	+			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

78	3	12w	K	Swine Dysentery	T.hyodysenteriae.	-	F		-	S	+	+	PC	+
					F.necrophorum.	-			-			+		
					Bacillus spp..	-	N		+	MCO		+	CO	
					Non-haemolytic E.coli.				+			+		
79	3	14w	K	Swine Dysentery Enteritis	T.hyodysenteriae.	-	F	ND	-	S	+	+	S	+
					C.coli.	-			+			+		
					C.perfringens.	-	N		+	MCO		+	CO	
					Non-haemolytic E.coli.	-			+			-		
					S.faecalis.	-			+			+		
80	3	12+w	K	Enteritis	T.hyodysenteriae.	-	F	ND	-	S	+	+	PC	+
					C.coli.	-			+			+		
					Bacillus spp..	-	N		-	MCO		+	CO	
					S.faecalis.	-			+			+		
					Non-haemolytic E.coli.	+			+			-		
81	3	12+w	K	Swine Dysentery Enteritis	T.hyodysenteriae.	-	F	ND	-	S	+	+	S	+
					B.vulgatus.	-			-			+		
					C.coli.	-	N		+	CO		+	CO	
					Clostridium spp.	-			+			+		
					Non-haemolytic E.coli.	+			+			-		
82	3	12w	K	None	Lactobacillus spp..	-	F	ND	+	NC	-	+	PC	-
					Non-haemolytic E.coli.	+	N		+	N		+	N	

Key to symbols on final page.

Appendix 1 (Continued)

83	3	13w	K	Swine Dysentery	S.faecalis.	-	F	ND	+	S	+	-	PC	+
					Non-haemolytic E.coli.		N		+	LCO		-	CO	
					T.hyodysenteriae.				-			+		
84	3	13w	K	Swine Dysentery	Non-haemolytic E.coli.	+	F	-	+	S	+	+	PC	+
					Lactobacillus spp..	-	N		+	MCO		+	CO	
					C.coli.	-			+			+		
85	3	12+w	K	Swine Dysentery	T.hyodysenteriae.	-	F	-	-	S	+	+	PC	;
					S.faecalis.	+	N		+	MCO		+	CO	
					P.aeruginosa.	-			+			+		
					Non-haemolytic E.coli.	+			+			+		
86	3	12w	K	None	Non-haemolytic E.coli.	-	F	ND	+	NC	-	+	FC	-
					S.faecalis.	-	N		+	N		+	N	
87	3	13w	K	Swine Dysentery	T.hyodysenteriae.	-	F	-	-	S	+	+	PC	+
					F.necrophorum.	-			-			+		
					C.coli.	-	N		+	MCO		+	CO	
					C.perfringens Type A.	-			+			+		
					Non-haemolytic E.coli.	+			+			+		
					S.faecalis.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

88	3	13w	K	Pneumonia	Non-haemolytic E.coli.	+	F	-	+	NC	-	+	FC	-
					Lactobacillus spp..	-	N		+	N		+	N	
89	3	13w	K	Swine Dysentery	T.hyodysenteriae.	-	F	-	-	S	+	+	PC	+
					C.coli.	-			+			+		
					S.faecalis.	+	N		+	MCO		+	CO	
90	3	12+w	K		Lactobacillus spp..	-	F	-	+	NC	-	+	FC	-
					S.faecalis.	+			+			+		
					Non-haemolytic E.coli.	+	N		+	N		+	N	
91	1	3w		Enteritis Pneumonia	C.perfringens Type A.	-	HF	-	+	VS	+	+	FC	+
					S.faecalis.	+	N		+	CO		-	MCO	
					Non-haemolytic E.coli.	+			+			+		
92	5	2w	K	Enteritis Streptococci Meningitis	B-haemolytic E.coli.	+	EM	-	+	S	+	+	FC	+
					Non-haemolytic E.coli.	-	N		+	CO		-	CO	
					S.suis.	+			+			+		
93	5	1-2w	K	Enteritis Streptococci Meningitis	Non-haemolytic E.coli.	+	EM	-	+	S	+	+	FC	+
					S.suis.	+	N		+	SCO		+	CO	

Key to symbols on final page.

Appendix 1 (Continued)

94	2d	K	Enteritis	Non-haemolytic E.coli.	+	EM	-	+	VS	+	+	PC	+
1				C.coli.	+	N		+	SCO		+	CO	
95	1w	K	Enteritis	S.faecalis.	-	EM	-	+	VS	+	+	FC	+
5			Streptococci Meningitis *	Non-haemolytic E.coli.	+	N		+	SCO		+	CO	
96	4½w	(D)	Bloody gut Enteritis	C.perfringens Type A.	-	EM	-	+	VS	+	+	FC	+
2				B-haemolytic E.coli.	+	N		+	SCO		+	CO	
				Non-haemolytic E.coli.	+			+			+		
				P.aeruginosa.	-			+			+		
				S.faecalis.	+			+			-		
97	3w	(D)	Internal Bleeding	S.faecalis		EM	ND	+	S	ND	+	FC	ND
1				Non-haemolytic E.coli.		N		+	LCO		+	N	
98	8w	(D)	Enteritis	Weakly haemolytic spirochaete.	-	F	-	-	S	+	+	PC	+
1				Bacteroides melaninogenicus.	-	N		+	CO		+	CO	
				C.coli.	-			+			+		
				S.faecalis.	+			+			-		
				Non-haemolytic E.coli.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

99	2w		Peritonitis	C.coli.	-	HF	-	+	S	+	+	FC	+
	1		Pneumonia	C.perfringens.	-			+			+		
				Non-haemolytic E.coli.	-	N		+	CO		+	CO	
				S.faecalis.	+			+			+		
100	2w	(D)	Pneumonia	C.perfringens Type A.	-	N	-	+	S	+	+	PC	+
	1		Enteritis	C.coli.	-			+	CO	+	+	LCO	
				Non-haemolytic E.coli.	-			+			+		
				S.faecalis.	+			+			-		
101	4w	(D)	Bloody gut	P.haemolytica.	+	CO	+	+	S	+	+	PC	+
	3		Pneumonia	C.perfringens Type A.	-			+			+		
				S.faecium.	+			+	CO		+	CO	
				Non-haemolytic E.coli.	+			+			+		
				C.pyogenes.	-			+			+		
102	2w	(D)	Enteritis *	Non-haemolytic E.coli.	-	N	-	+	S	+	+	FC	+
	3			S.faecalis.	+			+	SCP		-	SCO	
103	3w	(D)	Peritonitis	C.pyogenes.	-	HF	-	+	S	ND	+	FC	ND
	1			S.aureus.									
				F.necrophorum.	-	N		+	LCO		+	LCO	
				S.faecalis.	+			+			-		
				P.aeruginosa.	+			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

104	3w	(D)	Enteritis	B-haemolytic E.coli.	+	EM	-	+	NC	ND	+	FC	ND
1				Non-haemolytic E.coli.	-	N		+	LCO		+	N	
105	2w	(D)	Enteritis	B-haemolytic E.coli.	+	F	ND	+	S	ND	+	PC	ND
2				Non-haemolytic E.coli.	-	N		+	LCO		+	LCO	
				C.coli.	-			+			+		
106	3w	(D)		B-haemolytic E.coli.	-	N	ND	+	LVO	ND	+	N	ND
2				S.faecalis.	+			+			-		
107	4w	(D)	Enteritis	C.coli.	-	F	ND	+	S	+	+	FC	+
1			Inguinal hernia	Non-haemolytic E.coli.	-			+			+		
			Pneumonia	P.multocida.	-	N		+	CO		+	LCO	
				Streptococcus spp..	+			+			+		
108	1w	(D)	Enteritis	C.perfringens Type A.	-	HF	ND	+	S	+	+	PC	+
1				F.fusiformis.	-	N		+	CO		+	LCO	
				S.faecium.	+			+			+		
				Non-haemolytic E.coli.	-			+			+		

Key to symbols on final page.

Appendix 1 (Continued)

109	2w	(D)	Pneumonia	B-haemolytic E.coli.	-	F	ND	+	S	ND	+	PC	ND
	1		Enteritis										
			Septicaemia	Non-haemolytic E.coli.	-	N		+	LCO		+	LCO	
				P.aeruginosa.	-			+			+		
				S.faecium.	+			+			+		
110	4w	(D)	Pneumonia	B.fragilis.	-	F	ND	+	VS	+	+	S	+
	1			C.perfringens Type A.	-	N		+	CO		+	CO	
				Non-haemolytic E.coli.	-			+			+		
				S.faecalis.	+			+			-		
111	6w	(D)	Pneumonia	C.coli.	-	F		+	S		+	PC	
	1			B-haemolytic E.coli.	-			+			+		
				Non-haemolytic E.coli.	-	N		+	LCO		+	LCO	
				S.faecium.	-			+			+		
112	4w	(D)	Enteritis	C.coli.	-	F	ND	+	S	ND	+	FC	ND
	1			S.faecalis.	+	N		+	LCO		-	N	
113	3½w	(D)	Gastritis	B-haemolytic E.coli.	+	F	ND	+	VS	+	+	PC	+
	1		Enteritis			N			SCO			SCO	
114	6w	(D)	Enteritis	C.coli.	-	F	ND	+	S	ND	+	FC	ND
	1		Pneumonia	B.mycoides.	-			+			+		
				Non-haemolytic E.coli.	+	N		+	LCO		+	N	

Key to symbols on final page.

Appendix 1 (Continued)

115	7d	(D)	Enteritis	Non-haemolytic E.coli.	-	F	-	+	S	+	+	S	+
6				B-haemolytic E.coli.	-	N		+	CO		+	CO	
				C.coli.	-			+			+		
116	6d	(D)	Enteritis	Non-haemolytic E.coli.	+	F	+	+	S	+	+	S	+
6				B-haemolytic E.coli.	-	N		+	CO		+	CO	
				C.coli.	-			+			+		
117	9d	(D)	Enteritis	C.perfringens Type A.	-	F		+	S	+	+	S	+
2				Non-haemolytic E.coli.	+	N		+	CO		+	CO	
				S.faecium.	-			+			+		
118	3w	(D)	Pneumonia Enteritis	C.perfringens Type A.	-	F	ND	+	S	+	+	FC	+
1				C.coli.	-	N		+	CO		+	LCO	
				B.fragilis.	-			+			+		
				S.faecalis.	+			+			-		
119	13d	(D)	Enteritis	Streptococcus.	-	F	-	+	S	+	+	FC	+
1				C.coli.	-			+			+		
				Clostridium spp.	-	N		+	LCO		+	LCO	
				B.mycoides.	-			+			+		
				B.licheniformis.				+			+		

Key to symbols on final page.

Appendix 1 (Continued)

120	3d	(D)	Enteritis	C.coli.	-	F	ND	+	S	+	+	PC	+	
	1			B-haemolytic E.coli.	-			+			-			
				Non-haemolytic E.coli.	+	N		+	LCO		+	LCO		
				S.faecalis.	+			+			-			
121	3d	(D)	Enteritis	C.coli.	-	F	ND	+	S	+	+	PC	+	
	1			B-haemolytic E.coli.	-			+			+			
				Non-haemolytic E.coli.	+	N		+	LCO		+	LCO		
				S.faecium.	+			+			+			
122	6w		Enteritis	B-haemolytic E.coli.	-	F	ND	+	S	+	+	FC	+	
	1			S.faecalis.	+	N		+	CO		-	LCO		
				Non-haemolytic E.coli.	+			+			+			
123	3w	(D)	Enteritis	B.vulgatus.	-	EM	+	+	S	+	+	PC	+	
	1			S.faecalis.	+			+			+			
				C.coli.	-	CO		+	CO		+	N		
				Lactobacillus fermentum.	+			+			+			
				Non-haemolytic E.coli.	+			+			-			
124	2½w	(D)	Enteritis	C.coli.	+	HF	+	+	S	+	+	PC	+	
	1			Non-haemolytic E.coli.	-	LCO		+	CO		+	LCO		

Key to symbols on final page.

Appendix 1 (Continued)

125	3	8w	K	Rectal Stricture Enteritis Pneumonia	C.coli.	-	F	+	+	VS	+	+	PC	+
					P.intermedius.	-	SCO		+	SCO		+	SCO	
					S.faecalis.	-			+			+		
					Torulopsis glabrata	+			+			+		
126	7	8w	(D)	Enteritis Pneumonia Swine Dysentery	C.sputorum ss. mucosalis	-	HF	+	+	S	+	+	PC	+
					C.coli.	-	LCO		+	CO		+	CO	
					C.sporogenes.	-			+			+		
					Lactobacillus catenaforme.	-			+			+		
					T.hyodysenteriae.	-			-			+		
					B.vulgatus.	-			-			+		
					Non-haemolytic E.coli.	+			+			+		
127	7	8w	K	Enteritis Pneumonia (PIA) Swine Dysentery	C.sputorum ss. mucosalis.	-	EM	ND	+	S	+	+	S	+
					C.coli.	-			+			+		
					C.sporogenes.	-			+			+		
					Non-haemolytic E.coli.	+			+			+		
					T.hyodysenteriae.	-			-			+		
					S.faecium.	-			-			+		
128	2	5d	K	Bloody gut	C.perfringens Type A.	-	HF	ND	+	VS/B	+	+	S/B	+
					B-haemolytic E.coli.	-	N		+	SCO		+	SCO	

Key to symbols on final page.

Appendix 1 (Continued)

129	5w 8	K	Exudative Epidermitis Enteritis	S.hyicus.	+	HF	ND	+	S	ND	+	S	ND
				Clostridium spp.. (unidentified)	-			+			+		
				Non-haemolytic E.coli.	-	N		+	LCO		-	LCO	

KEY

HF	=	Half filled
F	=	Filled
FF	=	Filled with fluid
EM	=	Empty
N	=	Normal mucosa
MCO	=	Mildly congested mucosa
LCO	=	Locally congested mucosa
SCO	=	Severely congested mucosa
ND	=	Not done
UL	=	Ulcerated mucosa
*	=	Presence of viral particles in intestinal contents.
CO	=	Congested mucosa
PIA	=	Porcine intestinal adenopathy
SD	=	Swine dysentery
S	=	Soft content
FC	=	Firm content
VS	=	Very soft content
PC	=	Pasty content
NC	=	Normal content
B	=	Presence of blood
(D)	=	Died
K	=	Killed
d	=	Days
w	=	Weeks
+	=	Bacteria isolated or presence of lesions
-	=	Bacteria not isolated or absence of lesions

APPENDIX II

Papers and other publications describing work described in this thesis

Papers and Articles

A re-examination of the role of *Campylobacter fetus* subspecies *coli* in enteric disease of the pig

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Veterinary Record (1981) 109, 112-115

Campylobacter fetus subspecies _coli_ was isolated from the small intestines of 17 piglets less than six weeks of age submitted for diagnosis. Sixteen of these animals had enteritis and in five of them no other probable bacterial cause of the enteric lesions was identified. Changes including congestion of the small intestinal mucosa, reduction in the height of the villi, thickening of the terminal ileum and histological evidence for inflammatory change in the small intestine were seen at post mortem examination. _C f_ subsp _coli_ was also isolated from the large intestinal mucosa of all the infected pigs. In a further study, the organism was recovered from the colonic mucosa of 10 weaned animals treated for experimental swine dysentery. Two of these animals which had had a persistent mucoid diarrhoea were found to have large intestinal lesions resembling those of mild swine dysentery. No spirochaetes could be demonstrated in or isolated from the lesions seen in these two animals or from the other eight. The possibility that _C f_ subsp _coli_ may be a cause of enteritis in unweaned piglets and produce changes primarily in the small intestine is discussed. Evidence that _C f_ subsp _coli_ may cause large intestinal lesions in weaned pigs under certain circumstances is also discussed.

Campylobacter fetus subspecies *coli* (formerly *Vibrio coli*) has long been associated with enteritis in the pig. It has been shown not to be the initiating agent of classical swine dysentery as numerous attempts to reproduce that disease in pigs by feeding pure cultures of *C f* subsp *coli* have failed (Boley and others 1951, Warner 1965, Terpstra and others 1968, Andress and Barnum 1968, Andress and others 1968). Subsequent work by Taylor and Alexander (1971) and Harris and others (1972) has shown that a large spirochaete is responsible for swine dysentery. In spite of this failure to associate *C f* subsp *coli* with swine dysentery a number of reports exist in the literature to suggest that the infection of experimental weaned pigs with pure cultures of this organism has resulted in the production of diarrhoea (Doyle 1944, Roberts 1956).

This paper records the occurrence of *C f* subsp *coli* in lesions of the small and large intestines in pigs with mucoid diarrhoea from which *Treponema hyodysenteriae* and other spirochaetes could not be isolated or otherwise demonstrated.

Materials and methods

Seventeen of the pigs used in this study were submitted for post mortem examination from two farms. One was alive when submitted but the remainder had died within the previous 24 hours. All of these pigs were aged six weeks or less and the majority had a history of diarrhoea. The remainder of the animals described in the study had been purchased from a third farm and used in a study of the treatment of experimental swine dysentery. They were between 10 and 12 weeks old at the time of slaughter and examination.

Post mortem examination of the pigs submitted for diagnosis was carried out and the findings recorded. Particular attention was paid to the alimentary tract and to the appearance of the serosal surface of the intestines, the draining lymph nodes and the contents of each region of the intestine. The mucosa of the stomach, jejunum, ileum, caecum and colon was examined after rinsing in sterile physiological saline and its appearance was recorded. Samples of mucosa from the ileum were placed in sterile physiological saline and the morphology of the intestinal villi was examined using a dissecting microscope.

The pigs used in the experimental study were not examined in such detail but the whole large intestine was opened and the mucosa was rinsed before examination took place. Only the serosal surface of the remainder of the gastrointestinal tract was examined macroscopically in these animals.

Samples intended for histological examination were taken from the colon and caecum of all live pigs in the drug trial and from the small and large intestine of the live diarrhoeic pig submitted for diagnosis and from similar sites in two other animals which had died shortly before examination. All samples were fixed in 10 per cent formol saline. Sections were stained with haematoxylin and eosin.

Bacteriological examination was carried out on material from the series of animals submitted for diagnosis in the following way. Smears were made from loopfuls of the lumenal surface of the washed mucosa after searing and were Gram stained. Samples of the mucosa taken in a similar fashion were used to inoculate sheep blood agar and MacConkey agar plates for aerobic incubation. Seven per cent horse blood agar medium for the isolation of *C f* subsp *jejuni* (Skirrow 1979) containing a campylobacter supplement SR69 (Oxoid) and the selective medium described by Lawson and Rowland (1974) were inoculated and incubated under microaerophilic conditions. Seven per cent horse blood agar plates and spectinomycin blood agar plates were inoculated and incubated anaerobically. Anaerobic atmospheres were produced using anaerobe jars containing a mixture of 95 per cent hydrogen and 5 per cent carbon dioxide with cold catalysts. Microaerophilic conditions were produced in the same way but without the inclusion of the catalyst.

The small and large intestines and any local lesions were examined in this way in the pigs submitted for diagnosis but only the large intestinal mucosa was examined for catalase-positive campylobacters and spirochaetes (spectinomycin blood agar) in the pigs from the experimental study.

All aerobic cultures were examined after 24 and 48 hours' incubation and all microaerophilic and anaerobic cultures after 48 hours' incubation. The presumptive identity of the colonies present was recorded and the identity of those not easily assigned to species was confirmed using the methods of Cowan and Steele (1974). Campylobacters were identified using the methods described by Veron and Chatelain (1973), Smibert (1974, 1978) and Skirrow (1977). Spirochaetes were identified by the indirect fluorescent antibody and growth inhibition tests using specific absorbed antisera against *T hyodysenteriae*.

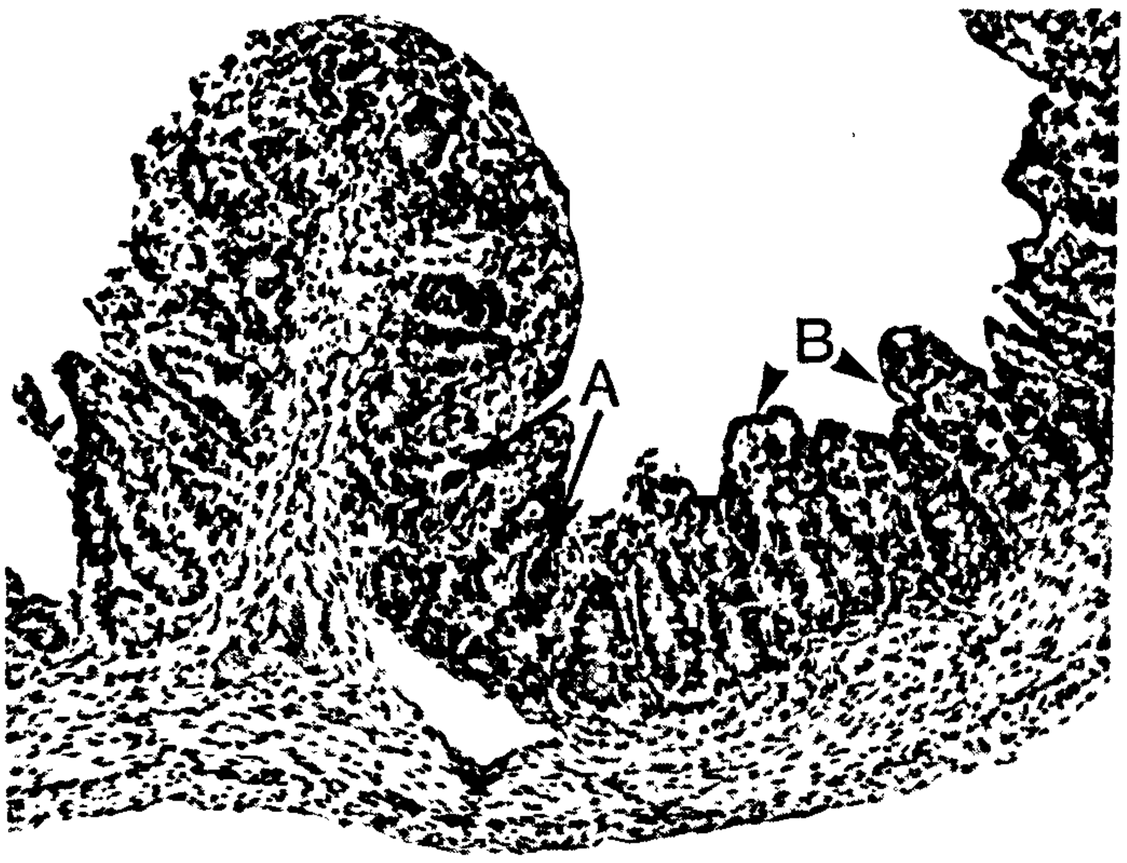

FIG 1: Jejunal mucosa from pig 4. Note the blocked crypts (A), villous atrophy and lowered villous epithelium (B). H & E × 70

Results

Pigs submitted for diagnosis

Campylobacters were isolated from the small intestinal mucosa of the 16 animals submitted for diagnosis. Campylobacters were isolated only on the selective medium containing Oxoid supplement. None was isolated on the selective medium intended for the isolation of *C sputorum* subsp *mucosalis*. Isolates from each pig tested were found to be catalase positive, grew at 42°C and 44°C, failed to grow at 25°C, failed to produce hydrogen sulphide on triple sugar iron medium and grew on brilliant green agar 1:100,000 and in media containing 8 per cent glucose. They were therefore identified as *C f* subsp *coli*. Large numbers of colonies with the morphology of *C f* subsp *coli* grew on the selective medium for catalase-positive campylobacters inoculated from small intestinal mucosa. In no case was *C f* subsp *coli* isolated in pure culture from the small intestine. Other bacteria such as Gram-positive cocci, Gram-negative bacilli and, on some occasions, Gram-positive rods were seen in smears made from the mucosa from which *C f* subsp *coli* was later isolated. Beta-haemolytic *Escherichia coli*, non-haemolytic *E coli*, faecal streptococci and *Clostridium perfringens* type A were also isolated in most cases. Where β-haemolytic *E coli* were identified in the small intestine, a diagnosis of *E coli* enteritis was recorded. Neither β-haemolytic *E coli* nor *C perfringens* type A were isolated from pigs 1, 9, 10, 16 and 17.

FIG 3: Colonic mucosa from pig 13. Some crypts (C) are dilated and debris is visible adjacent to the lumenal epithelium (arrow). H & E × 70

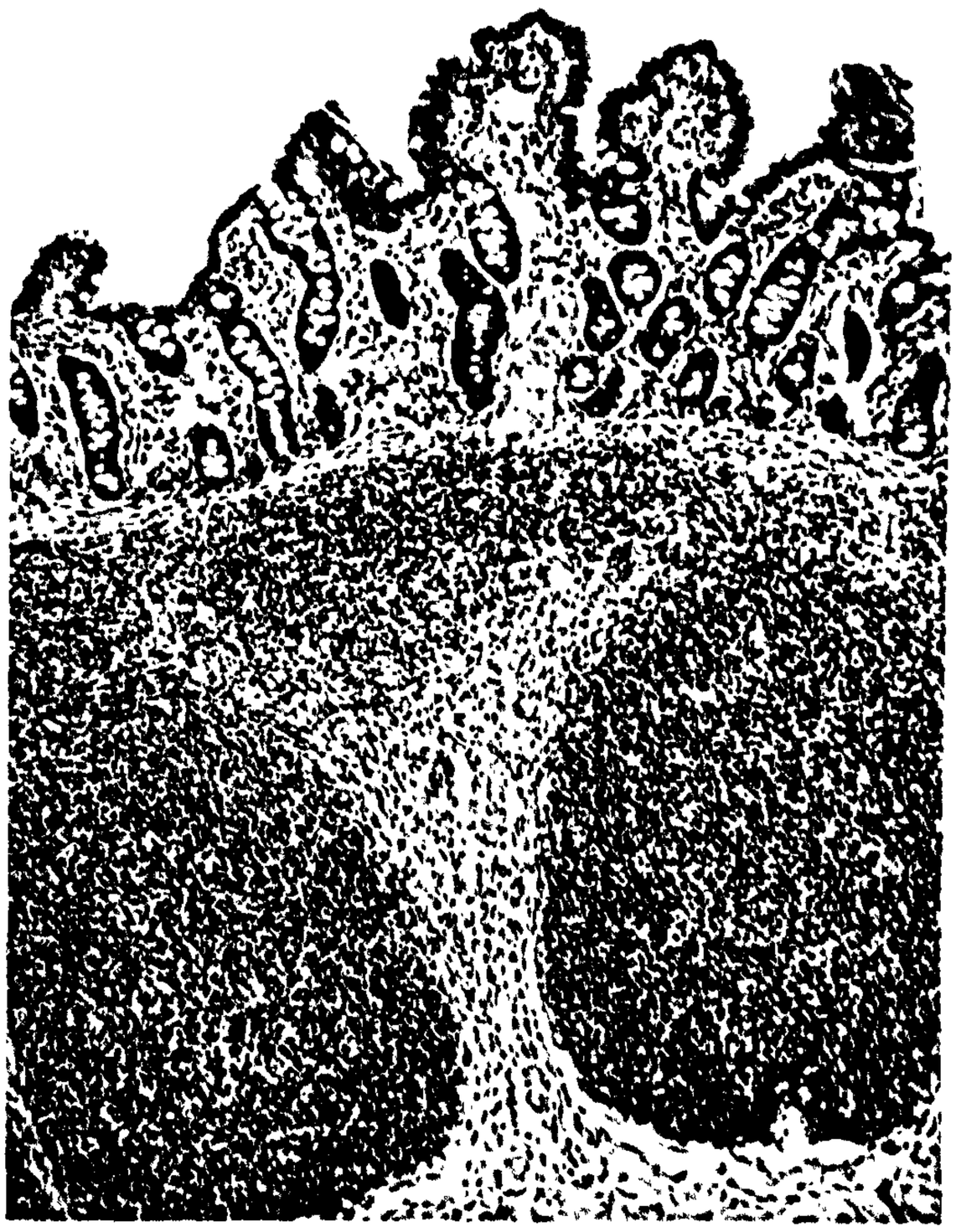

FIG 2: Ileal mucosa from pig 13. Note the massive lymphoid accumulation in the submucosa (L). H & E × 92

In 16 of the animals from which *C f* subsp *coli* was isolated gross changes were noted in the small intestine or its contents. In the remaining animal (pig 7) no gross lesions were found in the gastrointestinal tract. The contents of the small intestine were fluid in consistency in the majority of cases and the mucosa was inflamed or congested throughout its length. In eight animals (pigs 3, 4, 9, 11, 12, 13, 14 and 17) the villi were stunted in both the jejunum and the ileum. In these eight animals the wall of the ileum was thickened, particularly in the terminal portion. These changes were not noted in the remaining pigs.

Histological examination was carried out on pigs 4, 13 and 14. In all three animals the height of the villi in the jejunum was reduced and fusion of villi had taken place. In pigs 13 and 14 the villous epithelium was intact but in pig 4 the cells of the villous epithelium were flattened or absent and the crypts contained accumulations of inflammatory cells (Fig 1). Changes seen in the ileum included shortening and fusion of the villi, the presence of dilated lacteals and infiltration of the lamina propria with polymorphonuclear leucocytes and eosinophils. Mitoses were commonly seen in the crypts. Lymphoid follicles were prominent in the submucosa (Fig 2).

Fluid contents with no obvious blood or excess mucus were present in the colons of 10 pigs and those of the remainder were empty or contained normal contents (pig 7). The large intestinal mucosa was inflamed in 16 pigs, in two cases (pigs 10 and 16) areas of mucosa with adherent contents were seen and in one animal (pig 1) necrosis of the mucosa was noted. In histological sections from pig 4, dilated crypts, dilated capillaries and inflammatory cells were seen in the lamina propria which showed considerable post mortem change. Dilated crypts were the major abnormality noted in pig 13 (Fig 3). The results of this study are summarised in Table 1.

Experimental pigs

Seven of the experimental pigs examined were clinically

TABLE 1: Post mortem findings in 17 pigs in which *C f* subsp *coli* was isolated from the small intestine

Pig number and farm of origin (A or B)	Age (days)	Diarrhoea present at death	Gross lesions present: in small intestine	in large intestine	*C f* subsp *coli* isolated from LI	Diagnosis
1A	24	+	+	+	+	Enteritis
2A	7	+	+	+	+	*E coli* enteritis
3A	14	+	+	+	+	*E coli* enteritis
4A	6	+	+	+	+	Enteritis
5A	21	+	+	+	+	Gastroenteritis
6A	28	−	+	+	+	*E coli* enteritis
7A	21	−	−	−	−	Ruptured liver, overlying
8A	14	+	+	+	+	Enteritis
9A	7	+	+	+	+	Peritonitis, enteritis
10A	35	+	+	+	+	Enteritis
11A	14	+	+	+	+	Enteritis
12A	21	+	+	+	+	Peritonitis, enteritis
13A	21	+	+	+	+	*E coli* enteritis
14A	21	+	+	+	+	Pneumonia and *E coli* enteritis
15A	42	+	+	+	+	*E coli* enteritis
16A	28	+	+	+	+	Enteritis
17B	21	+	+	+	+	Enteritis

LI Large intestine

normal, two had mucoid diarrhoea which contained no blood and one had loose faeces. At post mortem examination changes were seen in the large intestine of seven animals. These varied from mild reddening of the large intestinal mucosa to localised areas of inflammation with adherent colon contents, 1 to 2 × 2 to 3cm in diameter. More severe changes were seen in three animals in which the large intestinal contents were fluid. These changes were most severe and widespread in the two animals with diarrhoea and consisted of thickening of the large intestinal mucosa and the presence of fibrin, excess clear mucus and necrotic debris on the lumenal surface. No blood was present.

When wet smears of the colonic mucosal epithelium were prepared and examined by phase contrast microscopy, no spirochaetes were seen. In the three most severely affected animals, large numbers of trichomonads and motile vibrios were seen. *C f* subsp *coli* was isolated from the large intestinal mucosa in each case but no salmonellae or spirochaetes of either the *T hyodysenteriae* or non-*T hyodysenteriae* types could be isolated.

Histological sections of the lesions were examined and the large intestinal mucosa was found to be thickened and had dilated crypts (Fig 4). In some cases capillary dilation and lowering of and loss of the mucosal epithelium were seen.

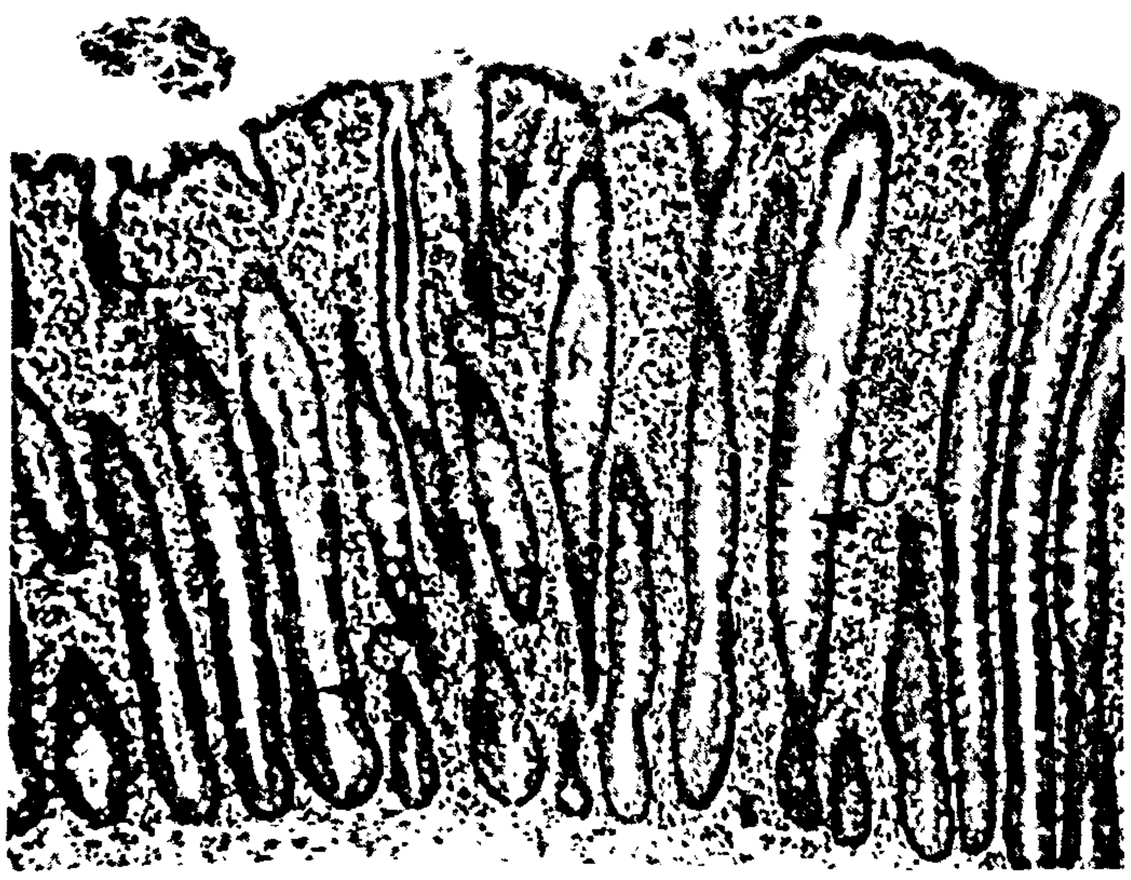

FIG 4: Colonic mucosa from an experimental pig with mucoid diarrhoea (pig 51). Note the thickened lamina propria (LP) and the dilated crypts (C). H & E × 70

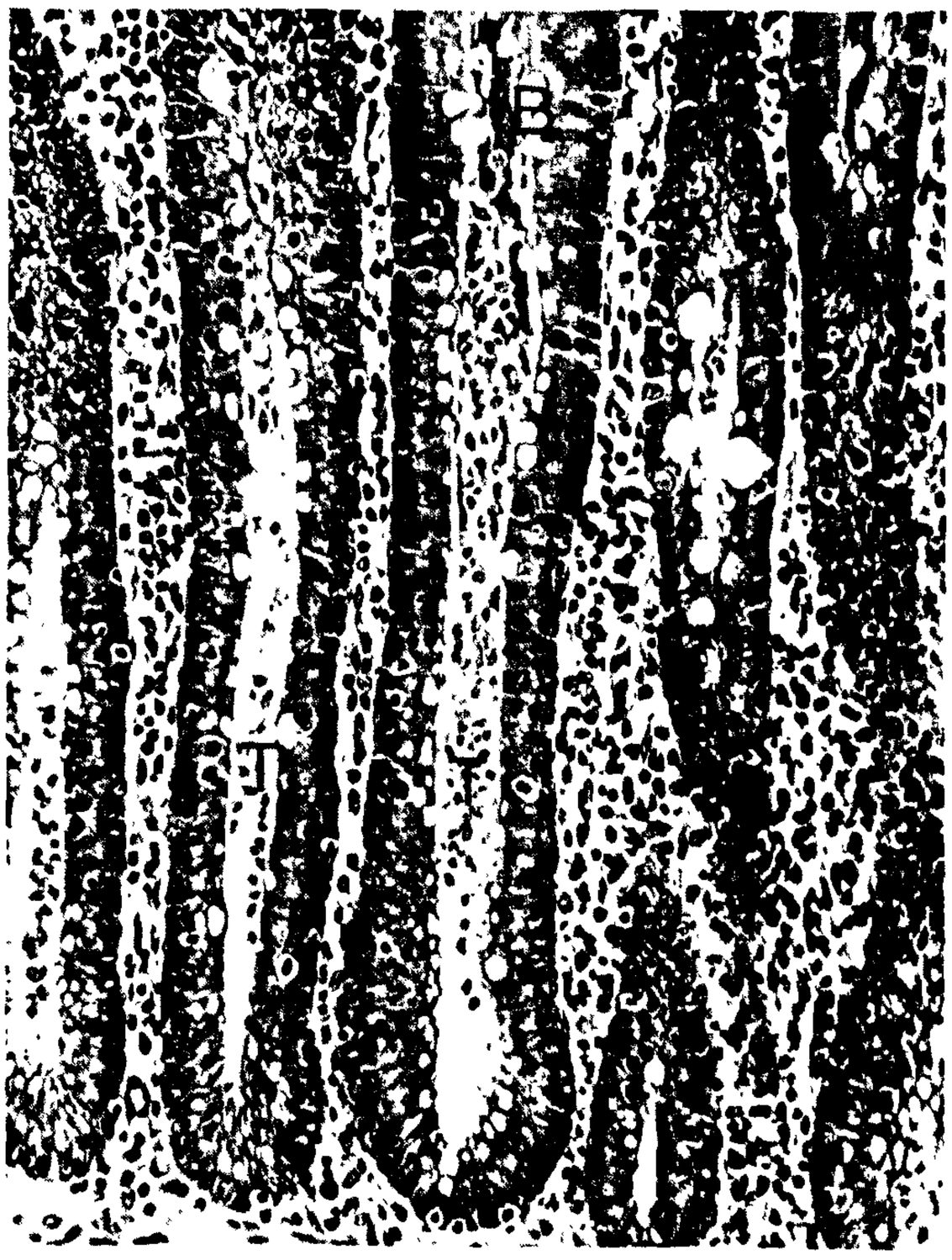

FIG 5: High power view of crypts in Fig 4. Accumulations of bacteria are present towards the mouth of the crypt (B), and bodies resembling trichomonads (T) are present in the bases of the crypts. H & E × 210

Organisms and cellular debris were seen in the crypts in some sections and organisms resembling *Trichomonas* species were present in large numbers in some dilated crypts (Fig 5).

Discussion

The isolation of *C f* subsp *coli* in profuse culture from both the small and large intestines of piglets in the diagnostic series is of particular interest. *C f* subsp *coli* has been recorded in the porcine small intestine by Deas (1960) who isolated the organism from the terminal ileum of weaned pigs with diarrhoea. He produced transient diarrhoea in weaned pigs by feeding pure cultures of his isolate in experimental studies. Lawson and Rowland (1974) recorded the presence of *C f* subsp *coli* in the small intestines of pigs with intestinal adenomatosis but considered that they played no part in that syndrome. Birrell (1957) described a syndrome in which yellowish diarrhoea occurred in piglets of three days of age onwards. Mild catarrhal enteritis was noted in the small intestine but vibrios were only isolated from the large intestine. The isolation of such large numbers of organisms from the small intestine of unweaned piglets with enteritis does not appear to have been reported previously.

The evidence for considering *C f* subsp *coli* as a possible cause of the enteritis seen in piglets includes the isolation data described above, especially in cases 1, 9, 10, 16 and 17 in which no other possible bacterial pathogens were isolated. In the absence of virological and parasitological examination of the intestine and its contents in this series this evidence must be interpreted with caution.

Further evidence for the possibility that *C f* subsp *coli* may cause enteritis in piglets is provided by recent work on *C f* subsp *jejuni*, which differs from *C f* subsp *coli* in two minor biochemical characters. In man (Butzler and Skirrow 1979) and in cattle (Al-Mashat and Taylor 1980) *C f* subsp *jejuni* is capable of causing lesions in the small intestine. The lesions seen in this series of cases in the piglet resembled those described in cattle by Al-Mashat and Taylor (1980) and in pigs by Andress and others (1968). In particular, the stunting of the villi, the thickening of the ileal mucosa and the presence of cellular exudate in the crypts resemble the changes described in cattle. They also show some similarity to certain changes seen in proliferative intestinal adenomatosis but the mucosal epithelial changes characteristic of that syndrome were not seen in the three cases examined in this series. *C s* subsp *mucosalis* was not isolated from any of these pigs but this in itself is not evidence for its absence. The literature on *C f* subsp *coli* and its relationship to enteric disease in pigs is extensive but does not contradict the view discussed above that the organism may be a cause of enteritis, particularly of the small intestine in piglets. Both the presence of *C f* subsp *coli* in the small intestine and its presence in baby pigs have been almost completely ignored.

The association of *C f* subsp *coli* with large intestinal disease is less clear although Prescott and Barker (1980) reported that *C f* subsp *jejuni* caused large intestinal lesions in gnotobiotic dogs. Large intestinal changes were noted in the pigs submitted for diagnosis. In view of the small intestinal changes seen, little significance can be ascribed to the presence of *C f* subsp *coli* in the colonic mucosa and in lesions. The changes seen histologically were not sufficiently distinctive to be attributable to any other aetiological agent and it may be that *C f* subsp *coli* merely represents an opportunist invader in this site. Invasion of the large intestinal mucosa may, however, be an integral part of an enteritis initiated by or associated with *C f* subsp *coli* infection.

The identification of *C f* subsp *coli* in inflammatory lesions in the large intestines of the experimental pigs is probably of limited significance in itself as the small intestines of these pigs were not examined in detail. The absence of *T hyodysenteriae* or other spirochaetes from the cases of mucoid diarrhoea observed and the presence of changes of mild colitis are, however, of interest and may suggest that previous authors (Doyle 1944, Roberts 1956) may have been correct in attributing to *C f* subsp *coli (V coli)* a causal role in large intestinal disease in pigs.

The lesions seen in the most severely affected experimental animals resembled those of mild swine dysentery or chronic spirochaetal diarrhoea although neither *T hyodysenteriae* nor other spirochaetes were demonstrated. It is possible that the antimicrobial treatment given may have allowed colonisation of the gut by organisms (including *C f* subsp *coli*) which had previously been present in low numbers, which were previously absent from the individual concerned or to which immunity had waned.

The findings discussed above suggest that *C f* subsp *coli* may be a cause of diarrhoea in both sucking piglets and weaned pigs in certain circumstances and that in the sucking piglet the infection of the small intestine is important. The observations by authors such as Doyle (1944) that *C f* subsp *coli (V coli)* could cause disease when fed in pure culture to experimental pigs have been disregarded by workers on enteric disease in pigs in recent years. This lack of attention was partly due to the failure of workers such as Andress and Barnum (1968) to reproduce swine dysentery by feeding pure cultures of *C f* subsp *coli* to weaned pigs and partly due to the demonstration that a spirochaete initiated swine dysentery (Taylor and Alexander 1971). It is therefore possible that *C f* subsp *coli* is a primary pathogen and may cause diarrhoea in the unweaned non-immune piglet and that the target organ is the small intestine. Mucoid diarrhoea may also occur in non-immune weaned pigs, perhaps following drug treatments. Experimental studies to clarify these points are currently in progress.

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NATURAL AND EXPERIMENTAL INFECTION OF PIGS WITH CAMPYLOBACTER COLI.

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Introduction

The possibility that Campylobacter coli could cause enteric disease in pigs was examined by Taylor and Olubunmi (1981) who had found the organism in the inflamed small intestinal mucosa of piglets with enteritis and in inflammatory large intestinal lesions of pigs incompletely treated for swine dysentery. Their studies, coupled with recent results of the experimental infection of calves with the closely-related C. jejuni (Al-Mashat and Taylor 1980) suggested that, in non-immune pigs C. coli might cause a mild, mucoid diarrhoea and that some of the small intestinal changes noted in piglets in their survey might have been entirely due to infection by C. coli.

In order to test this hypothesis, pure cultures of C. coli were fed to pigs in a number of controlled experiments. They were fed to milk-fed hysterectomy-derived, colostrum-deprived (HDCD) piglets and to weaned HDCD pigs, to conventional sucking piglets and to weaned conventional piglets of the same stock.

Materials and Methods

The pigs used were all from a minimal disease herd and were a commercial hybrid. As the herd carried C. coli, hysterectomy was carried out on 2 sows and the piglets reared until required to produce HDCD pigs for Experiments 1 and 2. Experimental HDCD piglets were housed in isolators. Sucking piglets were housed with the sow in conventional accommodation and weaned pigs on straw or shavings and fed on a standard weaner ration which contained no non-nutrient additives. One study each was carried out with HDCD piglets, HDCD weaned pigs, conventional piglets and conventional weaned pigs.

The inoculum was prepared by harvesting the surface growth from 48 hour blood agar plate cultures of C. coli and suspending it in saline. The isolate used had been obtained from the small intestine of a 7-day old piglet which had died from diarrhoea. It had been cloned twice and was stored freeze-dried. The density of organisms present was counted and each pig received 2-4 x 10^{10} organisms on a single occasion after feed had been withheld overnight.

Clinical and bacteriological observations were carried out daily and post mortem examinations were carried out at the end of each study. C. coli was isolated using blood agar containing campylobacter supplement SR69 (Oxoid).

The presence of agglutinating antibody to C. coli of the inocular strain was determined using the test described by Butzler and Skirrow (1979) on sera taken at the beginning and end of each study.

Results

1. Infections in HDCD piglets

Inoculation of 4-day old piglets was followed by a rise in rectal temperature to 41.1°C within 3 days and this was maintained for the remaining 10 days of the study. Yellowish diarrhoea developed on day 2 post inoculation and from day 3 this was accompanied by mucus which sometimes contained blood. C. coli was isolated daily from day 2 onwards. No diarrhoea or C. coli were recorded in the faeces of the controls. At post-mortem examination, the infected piglets were in poor bodily condition with thickening of the ileum and enlargement of the mesenteric lymph nodes. The jejunal contents were yellowish, contained excess clear mucus and the mucosa was hyperaemic in patches. In the ileum these changes were accompanied by thickening. The caecal and colonic contents were pasty and adherent. Inflammatory changes were present at all levels of the intestine and lymphoid proliferation was found to be prominent in the ileum. C. coli was isolated from the jejunum, ileum, caecum and colon of all infected piglets and antibody was present at titres of 1:160 in their sera. No evidence of C. coli infection was found in the controls.

2. Weaned HDCD pigs

When two six-week old, weaned pigs were inoculated with the same organism a similar rise in rectal temperature was seen. There were few faecal changes except for occasional looseness and the presence of C. coli and excess mucus on the faeces. Affected pigs were dull for 3-4 days post infection. Lesions resembling those described in the piglets were present in the intestinal tract of infected pigs. Antibody was present at slaughter at titres of 1:640. None of these changes were seen in the controls.

3. Conventional piglets

Pasty faeces were passed by these piglets within 2-6 days post infection and C. coli was isolated from their faeces from 2 days post infection. Lesions resembling those seen in the HDCD piglets were found in their intestinal tracts at post-mortem examination and C. coli was isolated from the same sites in the HDCD piglets. Antibody levels of up to 1:640 were present.

Infection with C. coli did not develop until day 9 in litters farrowed normally in the farm of origin.

4. Conventional weaned pigs

As with the weaned HDCD pigs, few clinical signs developed other than mild fever and the presence of clear mucus on the surface of formed motions. C. coli was isolated daily from all pigs of the infected group and occasionally from the faeces of the controls. The infected group all showed thickening of the terminal ileum and enlargement of the mesenteric lymph nodes at post-mortem examination and all had serum antibody to the inocular strain at levels of 1:320 - 1:640 compared with 1:20 and 1:40 pre-inoculation.

Discussion

There seems to be little doubt that C. coli can initiate a mucoid diarrhoea which may contain blood in non-immune piglets, and, in animals of all ages, can infect the jejunum, ileum, caecum and colon to cause inflammatory change and lymphoid hyperplasia. The studies described here did not suggest that C. coli caused death even in piglets but its presence may contribute blood, mucus and some diarrhoea to enteric syndromes and some inflammatory changes in the jejunal, ileal, caecal and colonic mucosa to the pathology of some enteric diseases. The effects of infection on productivity still need to be studied in more detail.

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ENTERIC DISEASE IN SUCKING AND WEANED PIGS INITIATED BY AND ASSOCIATED WITH CLOSTRIDIUM PERFRINGENS TYPE A.

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Introduction

C. perfringens Type A is known to cause food poisoning in man (Hobbs 1965), necrotic enteritis in chickens (Al Sheikhly and Truscott 1977) and has been considered to cause diarrhoea in lambs (Hauschild 1967) and calves (Niilo and Dorward 1971). Disease associated with C. perfringens in pigs is usually considered to be associated with Type C and to occur in piglets (Barnes and Moon 1964) although Type A has been isolated from pig faeces and intestinal contents on many occasions (Amtsberg et al. 1976). This paper describes the isolation of C. perfringens Type A from inflammatory lesions in the intestinal tracts of piglets dying from a number of disease conditions and experimental studies carried out to test the pathogenicity of one isolate of the organism for hysterectomy-derived, colostrum-deprived piglets and for conventional weaned pigs.

Materials and Methods

116 piglets aged 3 days to 3 weeks were obtained from 3 farms for post mortem examination. Most were dead but a few were chronically or severely ill and were killed. Gross and microscopical changes were recorded and bacteria present in the intestinal mucosa were isolated and recorded.

An isolate of C. perfringens Type A obtained from inflammatory small intestinal lesions in a 3-day old diarrhoeic piglet was used to infect 3 HDCD piglets in a controlled experiment and 10 conventional weaned pigs in 2 controlled experiments. In a further study, the development of the changes following infection was examined by killing 6 inoculated weaned pigs at daily intervals with appropriate controls. Inoculum was prepared from a low passage freeze dried culture of the isolate and given orally to pigs after overnight fasting. Approximately 10^9 organisms were fed on each occasion. Clinical signs and daily liveweight gain were recorded in the two weaned pig studies and the presence of C. perfringens Type A in the faeces was monitored by culture on reinforced clostridial medium. A thorough post mortem examination was carried out on animals at the end of the study.

Results

C. perfringens was isolated from 12/116 piglets from 3 farms in the survey. It was isolated from the jejunum, ileum, caecum and colon, particularly from piglets which had died from diarrhoea within 10 days of birth. Lesions at sites from which it was isolated included congestion of the mucosa and areas of focal haemorrhage and necrosis. The gut contents were in most cases fluid, often creamy in consistency and sometimes contained flecks of blood. Agents also identified in piglets from these farms included coccidia, cryptosporidia, coronavirus of the epidemic diarrhoea type, rotavirus and Campylobacter coli. Entero pathogenic E. coli were rare.

When inoculated into HDCD piglets a transient rise in temperature to 40°C occurred and a profuse, creamy diarrhoea containing flecks of blood developed. C. perfringens resembling the inocular strain was isolated in profuse culture only from the inoculated animals. The inoculated animals died or were killed in extremis within 72 hours of inoculation and were found to be in poor bodily condition with sunken eyes and evidence of dehydration. The thoracic pericardial and abdominal cavities contained varying amounts of fluid. The liver was pale, but the most marked changes were seen in the small and large intestines. The serosal surface was congested and the intestine was flaccid with fluid or pasty contents. In the small intestine the contents were fluid and contained specks of blood and small pieces of necrotic debris. In the large intestine the contents were creamy in colour, pasty and contained flecks of blood. The mucosa of the small intestine was congested with pinpoint haemorrhages and small areas of necrosis. There was villous atrophy. Localised areas of inflammation were seen in the large intestinal mucosa. The histological changes were those of congestion, destruction of the mucosal architecture and necrosis. C. perfringens Type A was isolated from the jejunum, ileum, caecum and colon of these inoculated piglets but not from the controls.

In the first two studies carried out with weaned pigs the clinical signs were restricted to a variable rise in rectal temperature (to 40.1°C), depression, dullness and in one case transient incoordination. Loose faeces with varying amounts of mucus, some of which contained blood, was passed from days 3 to 9 post inoculation. Feed conversion efficiency was depressed in one study. C. perfringens Type A was isolated only from the faeces of infected animals. At slaughter 21 days after inoculation changes were restricted to the small intestine which was flaccid with mucoid contents and congested, mildly necrotic mucosa. C. perfringens Type A was isolated from these areas in every case.

When pigs were killed at daily intervals the most prominent changes were seen in the jejunum between days 2 and 4 post inoculation. The small intestine was flaccid, congested with brownish fluid contents and the mucosa was pale on day 2 but became progressively more congested. The histological changes were principally those of villous atrophy with apical villous damage and oedema of the submucosa. C. perfringens Type A was isolated from both the intestinal contents and the mucosa.

Discussion

These studies suggest that C. perfringens Type A can be found in diarrhoeic syndromes in sucking piglets, but, because of the number of other agents which may have been present their significance was difficult to assess. In experimental infections, creamy faeces flecked with blood are passed by non-immune piglets and blood and mucus may occur in the faeces of weaners. The clinical signs are less severe in older pigs. The main site of the infection appears to be the small intestine and inflammatory and necrotic changes appear to result from infection. Mortality may occur in non-immune piglets and it is possible that productivity may be affected in older pigs.

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Production of enteritis in pigs by the oral inoculation of pure cultures of *Campylobacter coli*

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Pure cultures of an isolate of *Campylobacter coli* obtained from small intestinal lesions in a seven-day-old piglet were used to inoculate hysterectomy derived, colostrum deprived piglets, conventional sucking piglets and conventional weaned pigs in three separate controlled experiments. Rectal temperatures rose to 40°C in inoculated hysterectomy derived, colostrum deprived pigs within four days of infection and a mucoid yellowish diarrhoea containing occasional flecks of blood developed. *C coli* was isolated only from the faeces of the infected pigs and from all levels of their intestines at post mortem examination 12 days after inoculation. The small intestine was flaccid, pale and thickened in all cases, the contents were mucoid, the mucosa was hyperaemic and the mesenteric lymph nodes were enlarged. Mild villous atrophy and inflammatory changes were seen in the small intestines of the inoculated pigs. Mild colitis was present in both inoculated animals and controls. Agglutinating antibody to the inocular strain of *C coli* was present only in sera from the inoculated pigs at titres of up to 1:640. Similar changes were seen in conventional sucking piglets but in the weaned pigs no definite clinical signs were observed although the pathological changes were present. Both *C coli* and other enteric pathogens were present in the herd of origin of the conventional pigs.

CAMPYLOBACTER coli (formerly *Vibrio coli)* was isolated from the small intestines of diarrhoeic piglets which had died by Taylor and Olubunmi (1981), who called it *C fetus* subspecies *coli*. Profuse cultures were isolated from the inflamed mucosa. The ages of the pigs in the survey ranged from two days to three weeks old. The organism was also isolated from the inflamed large intestinal mucosa of the same pigs and from the large intestinal mucosa of pigs with colitis but without any evidence of *Treponema hyodysenteriae* infection.

These findings and other accounts in the literature of the production of diarrhoea or dysentery following experimental inoculation of pigs with pure cultures of the organism (Doyle 1944, Roberts 1956) suggest that *C coli* could be capable of initiating enteritis in pigs. This point of view was discussed by Taylor and Olubunmi (1981).

In order to ascertain whether this was the case, pure cultures of the organism were fed to hysterectomy derived, colostrum deprived piglets and conventional piglets and to weaned conventional pigs in three controlled experiments.

Materials and methods

All pigs used in this study were derived from stock of minimal disease status (Northern Pig Development High Health Status) and were obtained from the University of Glasgow animal husbandry department.

For experiment 1, hysterectomy-derived, colostrum deprived piglets were produced from a sow obtained from the above herd. Ten piglets were reared, of which six were used in this study. Piglets were housed individually in isolators in a temperature controlled room. The three infected and three control piglets were maintained in separate blocks of cages. They were fed on evaporated tinned milk (Carnation) supplemented on the first two days of life with a multivitamin supplement (Abidec Multivitamins; Parke-Davis). Iron was given to the piglets by intramuscular injection (1 ml Imposil; Fisons) when two days old.

For experiment 2, one litter of 10 conventional piglets was farrowed in isolation in a loosebox with background and creep heating, access to the sow and sawdust bedding. One millilitre of iron was given when the piglets were two days old. No supplementary feeding or antibiotic treatment was given. A similar sized control litter was monitored for the presence of *C coli* and other enteric pathogens while undergoing normal farm management in the farrowing house. This management included iron injections on the first day and oral ampicillin (Penbritin; Beecham) treatment of diarrhoeic animals on the third day of life.

Ten, eight-week-old pigs were used in the weaned pig study (experiment 3). They were divided into two groups (infected and control) of five pigs each housed in separate pens bedded on straw and received ad libitum a barley-based ration containing no non-nutrient additives standard to pigs of that age on the farm of origin. Water was freely available.

All pigs other than those in the control conventional group of piglets (experiment 2) were individually identified by ear numbers.

Pigs were inoculated with pure cultures of an isolate of *C coli* isolated from the small intestine of a seven-day-old piglet which had died of diarrhoea (piglet 9A, Taylor and Olubunmi 1981). This strain had been cloned twice, identified as *C coli* by methods used by Taylor and Olubunmi (1981) and was hippurate negative (Skirrow and Benjamin 1982). A culture, freeze-dried by the method of Garvie (1967) was reconstituted for each experiment, passaged once on horse blood agar and used to prepare the inoculum. Thickly inoculated blood agar plates were incubated for 48 hours in microaerophilic conditions at 37°C. The surface growth was harvested in sterile physiological saline and used to inoculate the experimental animals. Viable counts of organisms present in the suspension were determined using the method of Miles and Misra (1938).

Inoculation was carried out using 10 ml of the bacterial suspension. Hysterectomy derived and weaned pigs, but not sucking animals, were fasted for 24 hours before inoculation. Hysterectomy derived and conventional piglets were four days old when inoculated. The inoculum was given orally. In experiment 1 (hysterectomy derived piglets) $2{\cdot}1 \times 10^{10}$ organisms were given, in experiment 2 (conventional sucking piglets) $3{\cdot}0 \times 10^{10}$ organisms were given and in experiment 3 (weaned pigs) $3{\cdot}5 \times 10^{10}$ organisms were given to each piglet.

In experiment 1, three hysterectomy derived piglets were infected and three acted as controls. In experiment 2, one piglet was killed before infection and nine were infected. In the control group 10 piglets were observed. Five weaned pigs were infected in experiment 3 and five acted as uninfected controls.

The appearance of the pigs, the consistency of their faeces and their rectal temperatures were recorded daily in all groups other than the control group of experiment 2 in which these parameters were recorded on days 2, 4, 7, 9 and 12 after

birth. Feed consumption and weight gain were measured in the pigs of experiment 3.

Rectal faecal samples were taken from animals with diarrhoea and rectal swabs from all other animals on each occasion that clinical observations were made and also before inoculation. All samples were cultured on sheep blood and MacConkey agar plates and incubated aerobically to detect the presence of β-haemolytic *Escherichia coli*. All were cultured on horse blood agar and on horse blood agar containing campylobacter supplement (SR69 Skirrow; Oxoid) with a final concentration of 100 iu/ml of nystatin (Mycostatin; Squibb) and incubated under microaerophilic conditions. All were also cultured on horse blood agar and incubated under anaerobic conditions. Diarrhoeic samples were also examined for the presence of *Salmonella* species.

Rectal faecal samples from some piglets in experiment 1 and the farm-maintained conventional piglets were negatively stained and examined for the presence of viral particles by electron microscopy.

Clotted blood samples were taken post mortem from all pigs in experiments 1 and 3 and from the pigs killed in experiment 2. Samples were also taken from the pigs of experiment 3 before inoculation. Serum was stored at −20°C and tested for agglutinating antibody to the inocular strain by the method described by Butzler and Skirrow (1979). The antigen suspension was standardised at tube 8 using Wellcome opacity tubes (Wellcome Regeants).

All the pigs from experiments 1, 3 and the infected group of experiment 2 were killed and examined post mortem. Three pigs (17, 18 and 19) from the control group of experiment 2 were also killed, on days 4, 9 and 12 of life. Pigs were killed with intravenous pentobarbitone sodium (Euthatal; May & Baker). The gross findings were recorded and particular attention was paid to the gastrointestinal tract. The appearance of the contents and mucosa was recorded and samples of mucosa were also examined in physiological saline under a dissecting microscope.

Samples of the duodenum, jejunum, ileum, caecum, colon and mesenteric lymph node, liver and gall bladder were taken for bacteriological examination, as described above. Similar samples, with the exception of the gall bladder, were taken for histological examination and any other lesions noted were included. Direct smears were made from the surface of the intestinal mucosa and stained by Gram's method.

Bacteriological examination was carried out by the methods described above on samples taken from the mucosa after the surface had been seared. Bacterial colonies which developed were identified presumptively by their colonial morphology and their identity confirmed using the methods of Cowan and Steele (1974). Campylobacters were identified by the methods used by Taylor and Olubunmi (1981).

Results

Experiment 1

On the second day after inoculation, two of the three inoculated animals (pigs, 1, 2 and 3) passed soft yellowish faeces and on the third day, obvious clear mucus was present in the soft faeces of all three piglets and fresh blood was present in the mucus of one animal (pig 2). The faeces remained soft, yellowish and contained obvious mucus (Fig 1) for the remainder of the period of the experiment (Table 1). Blood was only seen on one subsequent occasion (pig 2, day 11 post infection). The inoculated piglets appeared depressed on the third day after inoculation and remained so for the duration of the experiment. Their flanks became hollow, their coats hairy, their eyes sunken and their bodily condition deteriorated. Soiling of the perineum occurred. The appearance of the faeces is shown in Fig 2. Forty-eight hours after inoculation rectal temperatures of 39·4°C to 40·7°C were

FIG 1: Soft faeces passed by pig 2, 12 days after inoculation. Note the tag of mucus

recorded and rectal temperatures of all of the inoculated animals remained elevated above those of the controls for the remainder of the study.

Two of the control piglets remained clinically normal throughout the period of the experiment. A third (pig 4) was destroyed on humane grounds on day 4 because of a congenital foot abnormality. The faeces of the control pigs remained firm for the period of the experiment.

C coli was isolated from the faeces of one inoculated piglet (pig 1) 24 hours after inoculation and daily from the faeces of all inoculated piglets from day 2 after inoculation to the end of the experiment (Table 1). It was never recovered from the faeces of the control piglets. Non-haemolytic *E coli* and a non-haemolytic *Clostridium* species which was not identified, were also isolated from the faeces of both groups of piglets. No salmonellae were isolated. No virus particles were seen in negatively stained preparations from the faeces of pig 2 (day 3) and pigs 1 and 2 (day 11) when viewed by electron microscopy.

At post mortem examination 11 days after inoculation, the

TABLE 1: Changes in faecal consistency in hysterectomy derived, colostrum deprived piglets following infection with pure cultures of *C coli* and isolation of the organism from the faeces (experiment 1)

	Day post inoculation												
Pig number	0	1	2	3	4	5	6	7	8	9	10	11	12
Infected group													
1	N	N	N	DM	DM	DM	DM	D	D	D	D	D	K
	−	+	+	+	+	+	+	+	+	+	+	+	+
2	N	N	D	DM	DM	DM	DM	D	DM	D	D	D	K
	−	−	+	+	+	+	+	+	+	+	+	+	+
3	N	N	D	DM	DM	DM	DM	D	D	D	D	D	K
	−	−	+	+	+	+	+	+	+	+	+	+	
Control group													
4	N	N	N	N	N	K							
	−	−	−	−	−								
5	N	N	N	N	N	N	N	N	N	N	N	N	K
	−	−	−	−	−	−	−	−	−	−	−	−	
6	N	N	N	N	N	N	N	N	N	N	N	N	K
	−	−	−	−	−	−	−	−	−	−	−	−	

N Normal
D Diarrhoea/soft faeces
M Mucus present in faeces
K Killed
± Positive or negative for *C coli* on faecal culture

FIG 2: Perineal region of pig 2 at the end of the experiment, 12 days after inoculation, showing texture of a typical motion

infected piglets were in poor bodily condition with soiled hindquarters, sunken eyes, hollowed flanks and hairy coats. Gross changes were confined to the abdominal viscera. The livers of all three piglets were slightly pale, the mesenteric lymph nodes were pale and enlarged and the jejunum and ileum appeared pale and thickened. The serosa of the large intestine and the other abdominal organs appeared grossly normal. The mesocolon was oedematous in pig 1. The jejunal and ileal contents were yellowish and contained excess mucus. Stunted villi were present in both areas and the mucosa was thickened and locally hyperaemic, particularly in the terminal portion of the ileum. The contents of the caecum and colon were yellowish in colour, pasty and contained obvious mucus which in pig 1 was streaked with fresh blood. The contents were adherent to the mucosa which was locally hyperaemic. Similar changes without stunting of the villi were seen in pigs 2 and 3.

The control animals were in good bodily condition. Pig 4 had contracted tendons of the hindlimb and erythema of the tail and limbs consequent upon this and was killed on day 4 of the experiment. Pericarditis was present in two (pigs 4 and 5), oedema of the mesocolon in pigs 5 and 6, umbilical oedema in pig 4 and excess clear peritoneal fluid in pig 6. The gastrointestinal tract, its mucosa and contents were all normal in appearance.

FIG 3: Jejunal mucosa of pig 2, with lymphoid accumulation in the submucosa (L) and the presence of a few cells in the crypts and between the villi. Haematoxylin and eosin × 35

Histologically, the small intestinal mucosa appeared normal for the hysterectomy derived, colostrum deprived control piglets, with vacuolation of the ileal mucosal epithelium, the presence of some lymphoid tissue in the ileum and eosinophils in the lamina propria of the ileum. Bacteria were seen in food debris and adjacent to the mucosal epithelium in some sites. In the infected animals stunting of the villi was noted in the jejunum of one (pig 2). In that pig polymorphonuclear leucocytes were present in the lumina of some crypts and some small lymphoid foci were seen (Fig 3). The ileal mucosa of all three infected pigs resembled that of the controls except for the greater prominence of submucosal lymphoid tissue and cellular infiltrate in the lamina propria. In pig 1 some eosinophils were present in the lamina propria of the ileum.

In all six animals changes were present in the large intestinal mucosa. They varied from lowering of the mucosal epithelium with adherent bacteria to the presence of massive cellular exudate and apparent polymorphonuclear cell infiltration of the lamina propria. This latter change was noted in individual control and infected piglets.

C coli was isolated only from the gastrointestinal tract of the inoculated piglets. It was isolated in small numbers from the duodenum and in more profuse culture from the mucosa of the jejunum, ileum, caecum and colon in all three pigs. Gram-negative curved bacteria with the morphology of campylobacters were seen in direct smears of the mucosa of the four lower sites in the gut. It was only isolated from the mesenteric lymph nodes in pig 2 and was not isolated from the liver, gall bladder or gastric mucosa in any of the piglets. Non-haemolytic *E coli* were isolated from the gastrointestinal tracts in all three inoculated piglets and a non-haemolytic *Clostridium* species resembling that found in the faeces was present in the ileum and large intestine of all three pigs.

E coli and *Clostridium* species were also present in the alimentary tracts of the controls. In addition non-haemolytic *E coli* was recovered from the pericardial fluid in pigs 4 and 5 and from the liver in pig 4. Antibody to the inocular strain of *C coli* was only demonstrated in the sera taken from the inoculated animals at the end of the experiment at titres of 1:160 (pigs 1 and 2) and 1:640 (pig 3).

Experiment 2

The faeces of all piglets in the infected group in this study were normal before infection. One piglet (pig 7) was found to have splayleg and had difficulty in sucking. It was killed at two days of age.

The inoculated piglets were killed at the intervals following infection shown in Table 2. A slight rise in body temperature to a maximum of 40°C occurred within 48 hours of infection

TABLE 2: Occurrence of faecal changes in nine conventional piglets following inoculation with a pure culture of *C coli* (experiment 2)

Pig number	Days −2	−1	0	1	2	3	4	5	6	7	8	9	10
7	N	K											
8	N	N	NK										
9	N	N	N	N	N	S	SMK						
10	N	N	N	N	N	N	SM	SMK					
11	N	N	N	N	N	N	S	S	SMK				
12	N	N	N	N	N	N	S	S	S	SK			
13	N	N	N	N	N	N	S	S	S	S	S	S	SK
14	N	N	N	N	N	N	N	SM	SM	SM	S	S	SK
15	N	N	N	N	N	S	S	S	S	S	S	S	SK
16	N	N	N	N	N	N	N	N	S	S	S	S	SK

N Normal
S Soft
M Mucus present
K Killed

TABLE 3: Summary of clinical and bacteriological findings in the 10 conventional control piglets (experiment 2)

Day	Rectal temperature in excess of 39°C	Number with diarrhoea	*C coli* isolated	Virus demonstrated
2	ND	2/10*	0/10*	1/3* tested
4	1/10	3/10	0/10	—
7	0/8	3/8	0/10	—
9	1/8	1/8	4/8	—
12	0/7	3/7	5/8	—

* Number of pigs affected/number healthy
ND not done

and body temperatures fluctuated thereafter in the range 38·7 to 39·9°C. Soft, pale faeces were passed by two piglets on the third day after infection and clear mucus was seen on the poorly formed motions by the fourth day after infection. Faecal changes were noted in all piglets within six days of inoculation. The details of the appearance of the faeces are shown in Table 2.

C coli was isolated in profuse culture from the faeces of seven of the piglets on the second day after inoculation and from those of each piglet from the third day onwards. It was not isolated from the faeces of these piglets before inoculation. No beta haemolytic *E coli* were isolated from the faeces of this group, and no tests for enteropathogenicity were carried out on the coliforms isolated. Faecal streptococci and a non-haemolytic clostridium were also isolated.

The clinical and microbiological findings in the control group of 10 piglets were less well documented, being restricted to those made on the five visits to the farm and by the lack of individual identification of the piglets. They are summarised in Table 3. Watery diarrhoea in which rotavirus was demonstrated was noted on day 2; animals with splayleg were present. Soft mucoid faeces, greyish in colour was seen on days 4, 7 and 12. *C coli* was isolated in small numbers from the faeces of the proportion of pigs shown in Table 3 on days 9 and 12.

In the infected group, at post mortem examination, no gross changes were seen in the piglets killed at two days of age and four hours after infection. A number of gross changes were seen in all the infected piglets killed from the fourth day after inoculation. The thoracic viscera, liver, kidneys and spleen were grossly normal. There was some milk in the stomach and the small intestine appeared pale and thickened. The mesenteric lymph nodes appeared pale and were enlarged in all cases. Some reduction in the height of the villi was noted in the duodenum, which was more pronounced in the jejunum and ileum. The contents of the jejunum and ileum varied in consistency but were generally fluid and contained obvious clear mucus. Hyperaemia was present locally on the mucosal surface and the wall of the jejunum was thickened and fleshy. These changes were more pronounced in the ileum, particularly in its distal portion. The large intestinal contents were soft or pasty, greyish in colour and contained mucus. The mucosa appeared to be grossly normal in most cases, although adhesion of contents was noted in pigs killed early in the study.

Histological changes were most obvious in the two pigs killed four and five days after infection. Changes in the jejunum included shortening of the villi, the presence of cells in the lumen and the presence of neutrophils in the lamina propria. In the ileum, submucosal lymphoid hyperplasia was present, eosinophilic and neutrophilic polymorphonuclear leucocytes were prominent in the lamina propria, there were mitoses in the bases of the crypts and bacteria were seen in large numbers adjacent to the mouths of the crypts. The crypts of the colonic and caecal mucosa were mildly dilated and contained some bacteria and eosinophilic debris. Inflammatory cells were present in the lamina propria.

In the animals killed at seven and 10 days after infection, changes were most prominent in the ileum and were most marked in those killed after 10 days. Lymphoid hyperplasia and bacteria in the crypts were seen. There were mild inflammatory changes in the colon and caecum in which dilated crypts and hypercellularity of the lamina propria were noted.

C coli was not isolated from the liver, gall bladder or stomach of any piglet. Small numbers of colonies were isolated from the duodenum of all infected pigs and from the jejunum of all but pig 8. Profuse cultures of the organism were recovered from the ileum, caecum and colon in all infected piglets, except pigs 8 and 10, from which no *C coli* was isolated from the colons. A few colonies were recovered from the mesenteric lymph node in pig 10.

Three piglets from the control litter were killed and examined post mortem. The animal killed on day 4 (pig 17) had splayleg and enteritis. Villi were absent from the jejunum and those in the ileum were stunted. Rotavirus was demonstrated in the caecal contents. No beta haemolytic *E coli* or campylobacters were isolated. The second piglet (pig 18) was killed on day 9 and appeared grossly normal with no thickening of the intestinal wall or mesenteric lymph node enlargement. A few colonies of *C coli* were isolated from the ileum and caecum only. The third animal (pig 19) was killed on day 12 and had mucoid diarrhoea. The jejunal serosa was pale but not thickened and the contents fluid. The ileal contents were mucoid and adherent to the mucosa as were those of the caecum. Intestinal villi were reduced in height.

The control piglets killed in this study all showed histological changes. The piglet killed at four days of age was found to have lesions of the small intestine consistent with those of rotavirus infection and the animal killed at nine days of age had lesions in the ileum containing coccidial bodies. The animal killed at 12 days of age had small intestinal changes resembling those of late rotavirus or coccidial infection, although neither agent was demonstrated. Dilated crypts were present in the colon. Few colonies of *C coli* were isolated from the ileum, caecum and colon. A non-haemolytic mucoid *E coli* was isolated in mixed culture with large numbers of faecal streptococci. No virological examination was carried out.

The pathological and bacteriological findings in experiment 2 are summarised in Table 4.

Antibodies to the inocular strain of *C coli* were present at slaughter in the sera of the inoculated pigs killed from four days after infection. Titres were low in the controls (1:10 in pigs 17, 18 and 19) but rose with time after infection in the infected group (1:40, day 4), (1:80, day 5) (1:160 and 1:320, day 7) and at day 10 were as follows: 1:160 (one), 1:320 (four), 1:640 (two).

Experiment 3

Clinical changes in the infected group were minimal. A transient rise in body temperature to 40 to 40·2°C occurred in all five inoculated animals within 24 hours of inoculation. Forty-eight hours after infection no difference between the body temperature of control and infected pigs could be detected. Faecal changes were minimal. There was no obvious change in faecal consistency but clear mucus was noted on the surface of formed motions passed by the infected pigs from day 3 onwards. No such mucus was seen on the faeces of the controls. No depression of appetite or loss of condition was noted in the infected animals. Their feed conversion ratio (2·8) was similar to that of the controls (2·9) and their mean rate of daily liveweight gain (543 g) was greater than that of the controls (489g) over the same period.

C coli was isolated from one pig in both groups before inoculation and intermittently from all pigs in the control

TABLE 4: Summary of the pathological and bacteriological findings in experiment 2

Number of pigs	Age when killed (days)	Day after infection	Distal small intestine thick walled with mucus	Enlarged mesenteric lymph node	*C coli* isolated from:					
					Duodenum	Jejunum	Ileum	Caecum	Colon	Mesenteric lymph nodes
Infected group										
1	2	–	–	–	–	–	–	–	–	–
1	4	(4 hours)	–	–	+	–	–	–	–	–
1	8	4	+	+	+	+	++	++	++	–
1	9	5	+	+	+	+	++	++	–	+
2	11	7	+	+	+	+	++	++	++	–
4	14	10	+	+	+	+	++	++	++	–
Control group										
1	4	–	–	–	–	–	–	–	–	–
1	9	–	–	–	–	–	+	+	+	–
1	12	–	–	+	–	–	+	+	+	–

No *C coli* colonies were isolated from the stomach in any case

group throughout the experiment. The organism was isolated daily from the faeces of all pigs in the infected group from the second day after infection.

At post mortem examination all the pigs were in good bodily condition and, apart from some scarring of the liver, gross changes were restricted to the gastrointestinal tract and its associated lymph nodes. In all animals of the infected group the serosa of the posterior jejunum and the ileum was pale and flaccid with the exception of the terminal ileum which appeared fleshy. The mesenteric lymph nodes were enlarged and pale when compared with those of the controls. The jejunal and ileal contents were yellowish and fluid and the caecal contents were pasty. Patchy hyperaemia was noted on the surface of the mucosa of the duodenum, jejunum and the whole of the ileum and the wall of the distal small intestine was thickened and fleshy. Changes in the terminal ileum were most pronounced in pig 21 in which clear excess mucus was present on the mucosal surface of the terminal ileum. The villi of the distal part of the small intestine were lowered when viewed using the dissecting microscope. The remainder of the gastrointestinal tract and its contents appeared grossly normal. In the five control pigs, the only gross abnormalities were slight congestion and reduction of height of the villi of the jejunum of pig 28 and pinpoint haemorrhages in the mucosa of the ileum of pig 27.

Changes seen in histological sections of the infected group were most marked in the ileum and colon. In the ileum changes included stunting of the villi, cellular infiltration of the lamina propria with eosinophils and neutrophil polymorphs, mild capillary dilatation and lymphoid hyperplasia. In pigs 20 and 23 inflammatory cells were seen in the crypts. Colonic changes included marked dilatation of the crypts and the presence of organisms within them. Mild capillary dilatation was also seen.

In the control animals the above changes were much less marked. Inflammatory cells were present in crypts in the duodenal mucosa of pig 25 and the villi were shortened in all sections of small intestine. Capillary dilatation was present in the lamina propria of the ileum of pig 27 and mild inflammatory change was noted in all ilea. Cryptosporidia were seen in the mucosal epithelium of the ileum of pig 28. Mild colonic changes of crypt dilation were seen in all pigs and bacteria were present in these crypts. *C coli* was isolated from the duodenum, jejunum and mesenteric lymph nodes of the infected animals but not from these sites in the controls. Profuse cultures were isolated from the mucosa of the ileum, caecum and colon in the infected animals but in small numbers only from the ileum and colon of the control animals (Table 5). The organism could not be recovered from the liver or gall bladder in any pig in this study.

Serum antibody to the inocular strain was present in pre-inoculation sera at titres of 1:20 (eight out of 10) and 1:40 (two out of 10). Antibody to the inocular strain was present in sera from the infected pigs at titres of 1:320 (three out of five) and 1:640 (two out of five) at slaughter but only at 1:10 (one out of five) and 1:20 (four out of five) in the controls.

Discussion

The results of experiment 1 suggest that *C coli* is capable of initiating clinical signs in non-immune pigs. The faeces of the inoculated pigs were bulky, soft, pale and contained mucus and, on occasion, fresh blood. The changes were not dramatic

TABLE 5: Summary of the pathological and bacteriological findings in experiment 3

Pig number	Infected	Distal small intestine thick walled with mucus	Enlarged mesenteric lymph node	*C coli* isolated from:					
				Duodenum	Jejunum	Ileum	Caecum	Colon	Mesenteric lymph nodes
20	+	+	+	–	+	++	++	++	+
21	+	+	+	–	+	++	++	++	+
22	+	+	+	+	+	++	++	++	+
23	+	+	+	+	+	++	++	++	–
24	+	+	+	+	+	++	++	+	–
25	–	–	–	–	–	+	–	+	–
26	–	–	–	–	–	–	–	–	–
27	–	–	–	–	–	+	–	+	–
28	–	–	–	–	–	–	–	–	–
29	–	–	–	–	–	–	–	+	–

and in this respect they resembled the changes produced in cattle by Al Mashat and Taylor (1980, 1981) in experimental infections with other *Campylobacter* species. The incubation period of two to three days resembled that found in cattle, as did the slight rise in rectal temperature. The rectal temperatures of the inoculated group never rose more than 1°C above those of the controls. In this small scale study the condition of the infected pigs was markedly reduced when compared with controls. It is unlikely that the isolation of *E coli* and clostridium from both the control and infected groups was connected with the clinical signs observed.

The clinical signs in the conventional animals were much less obvious although features noted in the non-immune piglets (mild fever and softening of the faeces with occasional mucus) were seen in experiment 2. Once again infection did not result in death and in this case, with no immediate control, bodily condition did not appear markedly impaired. In the weaned pigs clinical changes were very mild and apart from slight fever and softening of the faeces with some excess mucus no other clinical changes were seen. The slight nature of the changes was underlined by the similarity of feed conversion ratio and liveweight gain in the two groups.

In all three experiments *C coli* was recovered from the faeces of the infected pigs within two days of inoculation and it was regularly recovered thereafter, suggesting that it had colonised these animals. In the conventional pigs small numbers of *C coli* were isolated from the faeces of members of the control litter of piglets from nine days of age, from the weaned pigs in experiment 3 before infection and in the control group in that experiment. No attempt was made to distinguish between faecal *C coli* organisms of the inocular and resident strains in the infected animals. The regular recovery of profuse cultures of *C coli* from the faeces of inoculated animals in all three experiments and the rise in antibody levels following infection in experiment 3 suggest that many of the organisms isolated were likely to belong to the inocular strain.

Gross pathological changes attributable to *C coli* infection were seen in infected animals in all three experiments. The changes seen were observed 10 to 14 days after infection in experiments 1 and 3 and in four pigs in experiment 2. They were, therefore, late or chronic lesions; in this study earlier lesions were only examined in experiment 2 in which piglets were killed four to seven days after infection. The changes seen in all three experiments were most striking in the non-immune piglets of experiment 1. The ileum was pale and fleshy in appearance and the contents were fluid with some excess clear mucus. The cut surface of the ileal mucosa was thickened and there was patchy erythema of its lumenal surface. The mesenteric lymph nodes were enlarged and pale. These findings resembled those seen by Taylor and Olubunmi (1981) in field cases of pigs from which *C coli* was isolated and those found by Al Mashat and Taylor (1980) in calves experimentally infected with *C jejuni*, a close relative of *C coli*. The changes noted in the large intestines of the pigs in this series were more difficult to attribute to *C coli*, particularly in experiment 1. The histological changes were slight in most cases. In all cases, however, lymphoid hyperplasia and cellular infiltration of the lamina propria of the ileum was a feature of the infected animals and appears to account for the gross thickening and pallor of the small intestinal wall. In some studies, particularly in experiment 1, this type of change was not observed in the controls but in the conventional pigs it was present to some degree. Other changes associated with campylobacter infection, such as stunted villi, the accumulation of inflammatory cells in crypts, capillary dilatation and polymorphonuclear leucocyte infiltration of the lamina propria, were also seen in varying degrees in control animals in which other agents had been identified. The difference between control and infected animals was most obvious in the pigs of experiment 1.

The large intestinal changes were either present in both control and infected pigs (experiment 1) or not very marked (experiment 2). In experiment 3 dilated capillaries and dilated crypts filled with bacteria were seen in the infected animals but were less common in the controls.

C coli was recovered from the mucosa of the ileum and large intestine in large numbers and less frequently from the jejunum and mesenteric lymph nodes. The latter site was the only one from which *C coli* was isolated outside the gastrointestinal tract. The numbers of colonies recorded in control conventional pigs was markedly less than in infected animals although counts would be needed to provide objective information on this point.

The results discussed above suggest that *C coli* can initiate clinical signs and pathological changes when fed orally to hysterectomy derived, colostrum deprived piglets and can initiate some features of the syndrome observed in them when given both to conventional piglets and to weaned pigs. The changes seen in the conventional pigs were less marked and more difficult to interpret than those seen in the hysterectomy derived, colostrum deprived piglets because of the presence of other infectious agents in the herd of origin and in control litters. The agents demonstrated in the conventional animals (rotavirus and coccidia in the piglets and cryptosporidium in the weaned pigs) may have affected the pathological findings. It is not completely clear what effect the size of the inoculum had on the changes seen, particularly in the pigs of experiment 1. The delay of 24 to 48 hours before the onset of faecal changes, the duration of those changes and their failure to appear in conventional piglets of the same age given an inoculum of similar size suggests that they were of little importance. The rise in serum antibody levels in experiment 3 and the presence of high levels of such antibody only in the sera of the inoculated piglets of experiment 1 and 2 suggest further that the changes seen were associated with infection with the inocular strain.

These studies have therefore confirmed the supposition of Taylor and Olubunmi (1981) that *C coli* can initiate a clinical and pathological syndrome in non-immune piglets and cause similar but much less marked changes in conventional milk fed and weaned piglets. The changes seen appear to be distinct from those of proliferative intestinal adenopathy but may be confused with those seen in coccidial or cryptosporidial infection. There seems little doubt that small intestinal infection and lesions do occur in experimental disease. However, the association of *C coli* with colonic lesions was not conclusively shown in the experiments described here, although Prescott and others (1982) suggested that colonic lesions did occur in gnotobiotic pigs following infection with the closely related *C jejuni*.

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