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EXPERIMENTAL PIGBEL: THE PRODUCTION AND PATHOLOGY OF NECROTIZING ENTERITIS DUE TO *CLOSTRIDIUM WELCHII* TYPE C IN THE GUINEA-PIG

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Summary.—An animal model for pigbel in man was developed using guinea-pigs. Intragastric dosing with growing cultures of *Clostridium welchii* Type C only produced necrotic lesions if protease inhibitors were given as well. β toxin, which is made by the Type C organism, causes the intestinal damage and is very easily destroyed by proteases. Protease inhibitors in soybean and aprotinin were effective in inducing disease in animals on a normal diet, while inhibitors in sweet potato, which inhibit only trypsin, were only effective in animals on a low-protein diet.

In experiments using intragastric dosing, and in those where cultures and toxic filtrates were injected directly into the jejunum, the animals could be protected with an excess of pancreatic enzymes or by active or passive immunization against β toxin.

The pathology of Type C necrotizing enteritis in guinea-pigs had the macroscopic and microscopic features of pigbel in man.

These experiments suggested the basic importance of a low-protein diet and dietary trypsin inhibitors in the pathogenesis of pigbel in man.

NECROTIZING ENTERITIS caused by *Clostridium welchii* is a common problem in veterinary medicine (Blood and Henderson, 1974). In man it is much less frequent and almost always due to *Clostridium welchii* Type C (*Cl. welchii* C). In Germany, at the end of World War II there was an extensive outbreak which disappeared after a few years. The disease was named enteritis necroticans or Darmbrand (Zeissler and Rassfeld-Sternberg, 1949).

Enteritis necroticans (EN), locally called pigbel, is a persisting problem in the Highlands of Papua New Guinea (PNG) (Shepherd, 1979). It is a major cause of death and suffering in Highland children and is the most common cause of hospital death in children after the first year (Shann and Lawrence, 1979). EN has been reported from South East Asia, Africa and from China (Wright, 1966; Headington *et al.*, 1967; Shann, Lawrence and Pan Jun-Di, 1979).

The pathogenesis of the *Cl. welchii* C enterotoxaemias of veterinary experience varies in different species. In grown sheep the organism is a rumen commensal. With a change in diet from poor to richer food, conditions favour the temporary rapid overgrowth of *Cl. welchii* C. During rapid growth, β toxin, the characteristic exotoxin of *Cl. welchii* C, is formed and causes local gut damage. In the disease of neonatal piglets the organism contaminates the sow's bowel and its teats. It then multiplies in the intestinal lumen of the piglet producing β toxin which causes necrosis of the mucosa and wall (Szent-Ivanyi and Szabo, 1956); β toxin is a protein that is extremely sensitive to proteolysis (Sakurai and Duncan, 1978). This, together with the presence of colostral trypsin inhibitors in sows' milk, led Griner (1963) to suggest that protection of the toxin by the colostral trypsin inhibitors was the basic cause of the piglet disease.

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The persistence of pigbel in PNG has been attributed to reduced protease levels in the intestine from the low-protein diet and to the presence of protease inhibitors in the dietary staple sweet potato protecting β toxin from proteolysis (Lawrence & Walker, 1976). This paper describes animal experiments that led to and support this theory and compares the pathology in guinea-pigs and man.

Workers investigating the German outbreak of EN were able to produce lesions similar to the human disease in guinea-pigs by injecting cultures of *Cl. welchii* C into the small intestine, but not by intragastric dosing alone (Shütz, 1949). In PNG Egerton could not cause pigbel-like lesions in guinea-pigs by contaminating their food with *Cl. welchii* C cultures, although pathological changes similar to pigbel were readily produced in experimental animals by injecting growing cultures of *Cl. welchii* C from pigbel patients into the duodenum at laparotomy (Egerton, 1966). Other workers in the veterinary field have shown that similar pathology results from injection of bacteria-free toxic filtrates, emphasizing the importance of β toxin in pathogenesis (Field and Goodwin, 1959).

In human disease the striking banded pattern of gut damage seen in pigbel, often with well preserved or normal mucosa between patches of damage up to full-thickness necrosis, has suggested a vascular basis for the lesions. Workers in Thailand (Headington *et al.*, 1967) have reported cases of typical EN as "segmental infarcts of the small intestine"; the pathology of these cases is very similar to that of pigbel. There has been considerable discussion as to whether the pathology in pigbel results from changes in the vessels leading to infarction, or from direct damage by absorbed toxin. The area of the necrotic patches may be very small, only $\simeq$2 mm across, or they may extend over some cm of the intestine. Veins adjacent to the necrosis typically show endothelial proliferation (Cooke, 1979). Some of the experiments address this problem.

In earlier work with *Cl. welchii* C while attempting to produce experimental pigbel, soybean flour containing trypsin inhibitors was given to increase the effect a low-protein diet had in reducing protease levels in the gut. Lesions very similar to the human disease resulted. Later the importance of the heat-stable inhibitors of trypsin in sweet potato was recognised. Many attempts to induce pigbel with growing culture and raw sweet potato in guinea-pigs on a normal diet were unsuccessful. However, similar culture and raw sweet potato regularly produced disease in protein-deficient animals. This work became part of the basis of a theory explaining the persistence of human pigbel in PNG (Lawrence and Walker, 1976). The experiments reported here were designed to explore the complex relationships of organism growth, toxin production, dietary protease inhibitors and circulating immunity in the production of pigbel. For clarity they are listed together with the rationale and results in Tables I, II and III. In the first group, material was given intragastrically, simulating oral intake of food and organisms. In the second group, mixtures of culture or toxic filtrate and other materials were injected directly into the intestine at laparotomy. The third series investigates the time course of pathological changes in experimental pigbel.

MATERIALS AND METHODS

The organism used in the experiments was isolated from a case of pigbel by Mr A. MacGregor in 1972. The type A strain was also isolated from stool of a patient with pigbel. Cultures were grown in buffered cooked meat medium, pH 7·2 with 2% peptone, to which, after autoclaving, glucose was added to 1%. Large starting inocula of actively growing cultures were used. Culture fluid for experiments was decanted off after 3½ to 6 h growth at 37°.

Guinea-pigs from Australian stock and animals already living in PNG were used. Except in the case of those on a low-protein diet, they were fed a commercial ration with supplementary leaves and grass. Vitamin C supplements were given daily.

For intragastric feeding, guinea-pigs were lightly anaesthetized with ketamine and a 2mm internal diameter PVC tube on a wire sound

TABLE I.—*Intragastric experiment*

Diet	Intragastric dosing	Rationale	Results
Normal	Growing culture RSBF*	Basic experimental pigbel Culture and protease inhibitor	Pigbel
Normal	Growing culture CSBF†	Inhibitor destroyed by heat	No disease
Normal	Growing culture RSBF Pancreatin	Inhibitor plus enzyme excess	No disease
Normal	Growing Type A culture RSBF	Specificity of Type C: non-β-toxin-producing organism	No disease
Normal	Growing culture RSBF Immunized animals	Protective effect of immunity to β toxin	No disease
Normal	Growing culture Aprotinin Autoclaved sweet potato	Importance of trypsin inhibitor. Pure inhibitor	Pigbel in 2 of 3
Normal	Washed organisms RSBF	Importance of toxin produced in the gut	Pigbel in 2 of 3
Low protein	Growing culture Raw sweet potato	Low-protein diet Sweet-potato inhibitor	Pigbel
Low protein	Growing culture Autoclaved sweet potato	Inhibitor destroyed by heat	No disease
Low protein	Growing culture Raw sweet potato Pancreatin	Protective effect of proteolytic enzymes	No disease
Low protein	Growing culture Raw sweet potato Immunized animals	Protective effects of immunity	No disease

* RSBF: raw soybean flour.
† CSBF: autoclaved soybean flour.

TABLE II.—*Operative experiments*

Injected into intestine	Rationale	Result
Growing culture	Basic experiment Organism plus toxin	Pigbel
Toxic filtrate	Importance of β toxin	Pigbel
Antibiotics	Inhibit bacterial growth	
Toxic filtrate	Importance of β toxin	No disease
Pancreatin	Destroys β toxin	
Growing culture in immunized animals	Effect of immunization	No disease
Toxic filtrate in immunized animals	Effect of immunization	No disease

passed gently into the stomach and the material injected from a syringe. In the intragastric experiments 10–15 ml of sample were given. This volume was felt to represent a reasonable but not excessive meal for a guinea-pig. Tests showed that adult guinea-pigs readily ate 20–25 g of fresh leaves in a short period.

Preparation of soybean feeds.—Raw soybeans (*Soja hispida*) were powdered in a Waring blender and sieved. Powder was mixed with culture supernatant to the consistency of thin cream. Peptone powder (Bacto) was added to the mixture to 1% w/v. In control experiments the soybean flour was autoclaved at 120° for 20 min, to destroy the protease inhibitor, before mixing.

With soybean flour and growing culture the following experiments were carried out. Culture + raw soybean flour (RSBF), culture + autoclaved soybean flour (CSBF), culture + RSBF + Pancreatin, culture + RSBF in immunized animals, *Cl. welchii* Type A culture + RSBF.

Addition of pancreatic extract.—Culture/soybean, and culture/sweet potato mixtures had 300 mg of pancreatin (Cotazyme, Organon) per dose mixed in before administration.

Active immunisation.—Two doses of 0·5 ml of beta toxoid (Burroughs Wellcome) containing 100 TCPue/ml adsorbed on aluminium hydroxide were given, 1 month apart.

Experiments using purified inhibitor.—Aprotinin powder (Trasylol, Bayer) without methiolate preservative, was added to a mixture of culture supernatant, peptone and autoclaved

TABLE III.—*Results of time course experiments*

Intragastric dosing	Other	Rationale	Results
Growing culture RSBF	Passive immunization at −2, 0, 2, 4 and 7 h	Relationship of initiating meal to pathological changes	Two of 5 animals immunized at 7 h had pigbel. Others normal
Growing culture RSBS	Killed at 5 and 7 days	To compare pathology with those killed at 1–2 days and with human disease	Vascular changes with endothelial proliferation seen after 5 days, not in early material

sweet potato so that 15 ml of the mixture contained about 150,000 kiu of aprotinin.

Low-protein diet.—Guinea-pigs were fed *ad lib.* on dried shavings of sweet potato (*Ipomoea batatas*). A salt mix was added (4 g/100 g dry wt). The dry sweet potato was made more palatable, easier to handle and given a higher calorific value by adding 20 ml/100 g salad oil. Two choko leaves (*Sechium edule*) were fed daily for variety and vitamin and ascorbic acid supplements given. After 3 weeks on the diet, groups of animals were dosed with growing culture fluid mixed with either autoclaved sweet potato or powdered raw sweet potato.

Preparation of powdered sweet potato.—Fresh sweet potato was peeled, sliced, frozen and then dried for 24–36 h in an Edwards EF03 freeze dryer. The resulting discs were powdered and sieved. The powder was mixed with culture fluid to a thin fluid consistency and a small amount of sterile cooked meat and 2% peptone w/v added. In experiments requiring the thermal destruction of inhibitors the sweet potato powder was autoclaved at 121° for 20 min before mixing.

Use of washed organisms.—Fluid from a 4 h culture was centrifuged and the pelleted organisms washed twice with warm reduced medium, thus removing any toxin formed during growth, but not exposing them to adverse conditions. The organisms from 25 ml of culture were mixed with soybean flour and sterile supernatant fluid from the buffered cooked meat medium with 1% added glucose and 15 ml given to each of 3 guinea-pigs.

Passive immunization.—0·5 ml (4,200 u) of a *Cl. welchii* C antiserum (Burroughs Wellcome) raised in horses, was injected i.v. *via* the penile veins.

The injection of specific antibody was timed to obtain information on the course of events in experimental pigbel. Four animals were given antibody before dosing with RSBF and culture, 2 at the time of dosing, 4 each at 2 and 4 h and 5 at 7 h after dosing.

The animals were killed 48 h later.

Surgical experiments

Surgical procedures.—Guinea-pigs were anaesthetized with ketamine 40 mg/kg by s.c. injection. The abdominal wall was infiltrated with 1% lignocaine then the abdomen opened for injection of culture fluid or toxic filtrate into the jejunum, through a 23-gauge needle. Four to 6 ml volumes were injected in the early experiments and 8 ml in those using immunized animals.

Sterile bacteria-free toxic filtrates were prepared by centrifuging 4 h cultures and filtering the supernatant through a sterile 0·22 μm filter. For experiments requiring growing culture the uncentrifuged material was used. In one group of experiments 10^6 u penicillin G and 500 mg lincomycin were added to 100 ml toxic filtrate before use. The antibiotics were intended to prevent growth of any *Cl. welchii* already present in the intestine following toxin damage. This was done because Field and Goodwin (1959) produced experimental enterotoxaemia in piglets with an organism-free toxic filtrate, yet were still able to grow Type C organisms from the experimentally produced lesions after the animals' deaths.

Three guinea-pigs were injected with toxic filtrate that had been treated with pancreatin. Fifty ml of toxic filtrate was mixed with 300 mg of pancreatin and incubated at 37° for 15 min. Control animals were given toxic filtrate incubated for 15 min without pancreatin.

Active immunisation.—Two doses of 0·5 ml of a beta toxoid (Burroughs Wellcome) containing 100 TCPue/ml adsorbed on aluminium hydroxide were given, 1 month apart.

Two groups of 3 actively immunized guinea-pigs had either toxic filtrate or growing culture injected into the duodenal lumen while 3 immunized controls had toxic filtrate alone injected.

The time course of pathological changes in experimental pigbel.—Animals had pigbel induced with growing culture and RSBF. Six ml of the mixture was given in the hope of reducing the severity of the lesions, and of not causing death. The guinea-pigs were killed 5 and 7 days after dosing, periods similar to those seen between the initiating meal and operation in human pigbel. The findings were compared with animals killed 24–48 h after dosing.

Examination of tissues.—Experimental animals were examined after death or, more usually,

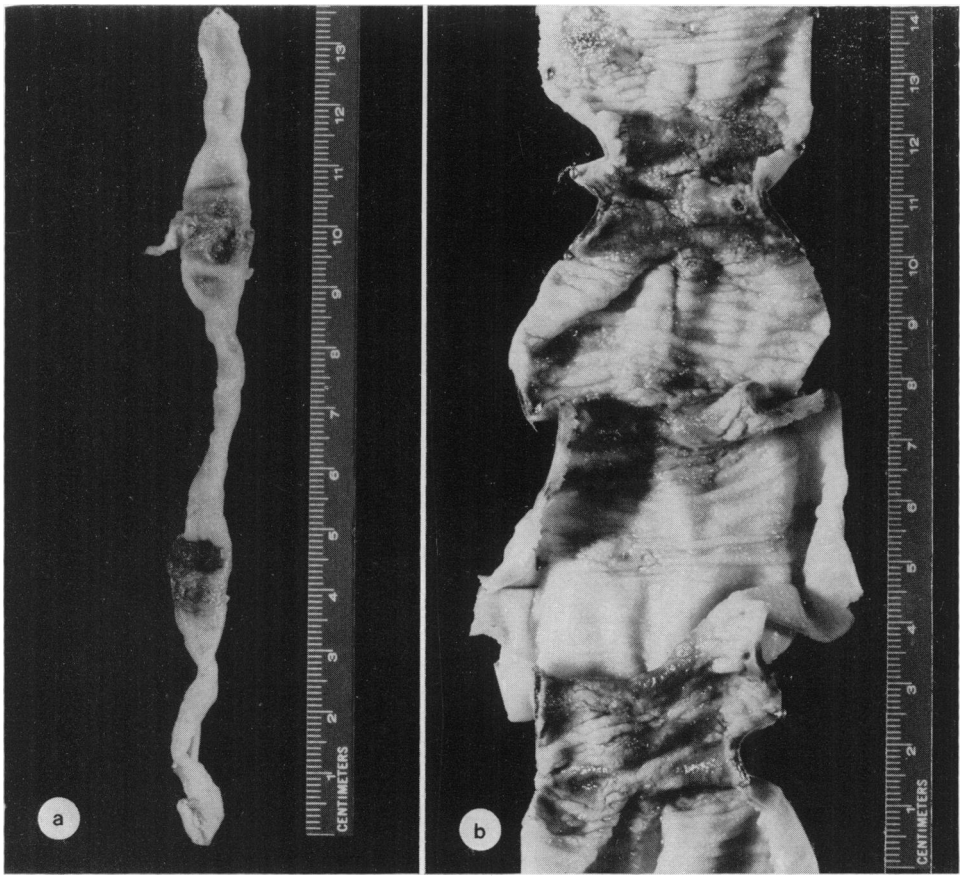

FIG. 1.—(a, guinea-pig; b, human). Macroscopic appearance of small intestine with segmental involvement of the wall.

killed when moribund after 24–48 h. The intestine was examined and removed. Tissues were fixed by opening out on a cork board in 10% formol saline. Representative pieces of intestine were sent for histological examination.

RESULTS

The clinical response of the animals varied markedly, some dying in less than 24 h after the dose, while others, although having obvious intestinal damage, would have recovered. Most animals seemed normal for about 1 day and then refused to eat or ate poorly until death or when they were killed. The macroscopic and microscopic findings were very similar to those seen in human pigbel, as can be seen in Figs 1–5.

Macroscopic findings

Lesions were confined to the small bowel apart from some minor haemorrhagic patches in the caecum in severe cases and involvement of the large bowel when bound up to affected small gut. Most of the damage was seen in the jejunum; in some severe cases the whole small bowel was affected. One animal had an isolated segment of affected ileum and in another a large proportion of the duodenum was involved with little pathology in the rest of the intestine.

Involvement was patchy, often appearing segmental as though due to infarction with normal areas of gut between (Fig. 1a). Some specimens showed yellowish areas of full-thickness necrosis on the

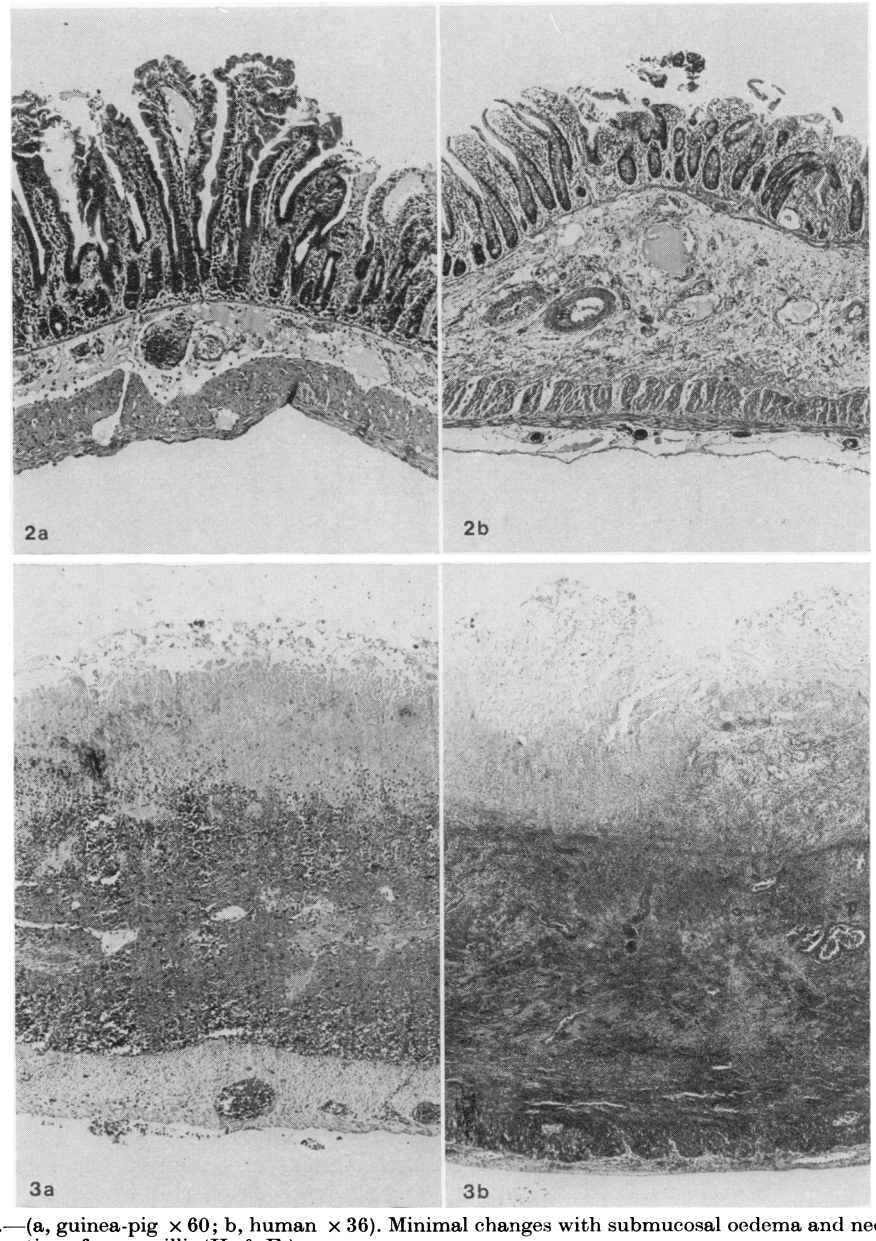

FIG. 2.—(a, guinea-pig × 60; b, human × 36). Minimal changes with submucosal oedema and necrosis of the tips of some villi. (H. & E.)

FIG. 3.—(a, guinea-pig × 60; b, human × 27). Necrosis of the whole thickness of the bowel wall. Necrotic villi at the top of the photograph. (H. & E.)

antimesenteric border of the intestine as is often seen in human pigbel. Affected segments were usually 0·5–3 cm long and looked dark red or black except where the yellowish patches of full-thickness involvement showed through. Some animals had one segment of involvement while others had up to 6.

Excess peritoneal fluid was usually present and it was frequently bloodstained.

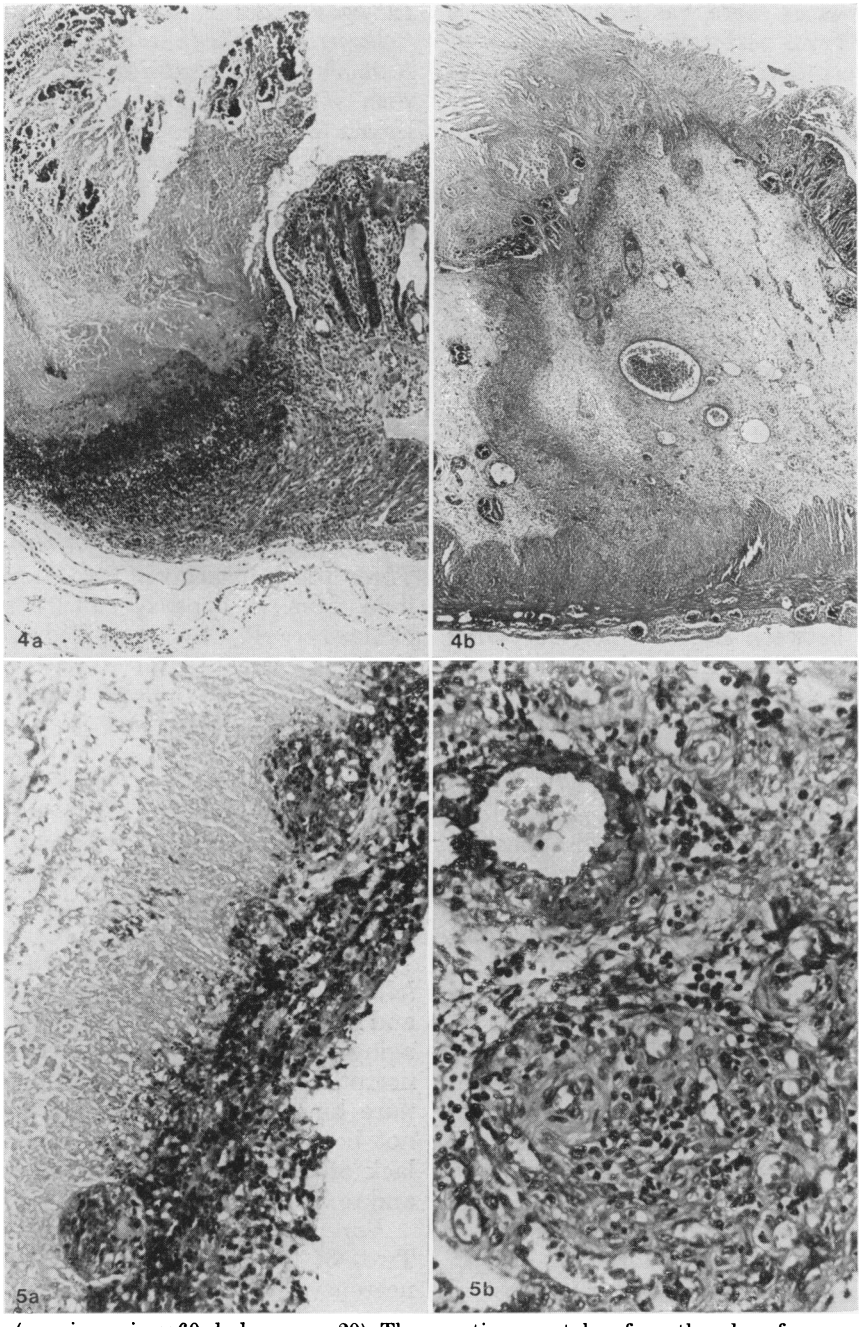

FIG. 4.—(a, guinea-pig ×60; b, human ×20). These sections are taken from the edge of a segment of necrosis including the full thickness of the bowel wall. The dark line extending from mucosa to serosa is a band of polymorphonuclear leucocytes at the junction between the necrotic bowel on the left and the viable bowel on the right. (H. & E.)

FIG. 5.—(a, guinea-pig ×180; b, human ×180). Endothelial proliferation, causing almost complete occlusion of the lumen of veins in the submucosa is a prominent feature of human cases and was present in guinea-pigs killed 5 days after dosing, but not in the lesions of those killed sooner. (Verhoff–Van Gieson.)

In a few cases there was gross peritonitis resulting from perforation. Damaged segments were covered by patches of omentum after a few days.

Like clinical pigbel the lesions seen were variable, ranging from small patches of full-thickness necrosis in the bowel wall from which the animal was recovering, to complete haemorrhagic necrosis of most of the small intestine with gas bubbles forming in the gut and mesentery.

Microscopic examination of specimens from animals killed at 24–48 h showed necrosis of mucosal epithelium with involvement of the muscle coats that varied from necrosis of the inner layer only to full-thickness necrosis of the gut wall and sometimes showed haemorrhage as well. Adjacent to segmental necrotic areas the submucosa was oedematous. Neutrophils were present in large numbers along the junction between necrotic and surviving tissue (Figs 4a and 4b) in some cases through the entire thickness of the bowel wall. Proliferating fibroblasts were seen, particularly on the serosal surface.

In the animals killed at 5 and 7 days the macroscopic changes were similar. However, the necrotic mucosa had disappeared, leaving an ulcerated area. In the ulcer base there were a few small vessels containing fibrin thrombi, developing granulation tissue and a heavy infiltrate of neutrophils. Submucosal veins showed marked endothelial proliferation with reduction in lumen size. This appearance is a striking feature of human pigbel and yet was not seen in the 24- and 48-h guinea-pig specimens although extensive necrosis was already present.

Pathologically the lesions produced in the guinea-pigs closely match those seen in human pigbel (Figs 1–5).

Control animals protected against β toxin showed no abnormalities at all, apart from the operated group where a small spot could be seen at the site of intestinal injections. The gut contents were often full of gas bubbles, suggesting that organisms had multiplied but no damage had been done.

Intragastric dosing experiments

Experiments with raw soybean flour.—Animals given growing culture mixed with RSBF developed patchy necrotic lesions of the intestine. One died 20 h later and the 3 others were killed when moribund at 36 h.

With cooked soybean flour.—The 3 animals remained well. No abnormality was found in the intestine, apart from prominent Peyer's patches, when they were killed 36 h after dosing.

With added pancreatin.—Two guinea-pigs given raw soybean powder, culture and pancreatin did not become ill and were normal at necropsy, controls given soybean flour and culture without pancreatin developed typical intestinal changes.

Experiments with type A organism.—Three guinea-pigs given RSBF and culture fluid from a Type A culture remained clinically well. One was killed: the intestine was normal. After 10 days the other 2 were given *Cl. welchii* C culture and raw soybean. Both died from intestinal necrosis.

Active immunization.—Five guinea-pigs that had been actively immunized with β toxoid were unaffected by raw soybean and culture. Four unimmunized control animals developed typical intestinal lesions.

Experiments with pure inhibitor (Trasylol).—Two of 3 guinea-pigs given aprotinin and growing culture in a medium encouraging further growth developed intestinal necrotic areas. Previous experiments using pure lima bean and soya inhibitors had not been successful, possibly owing to a lack of medium for rapid further growth and toxin production in the intestine.

Experiments using washed organisms.—Two of 3 guinea-pigs had lesions at necropsy. The third was normal.

Results in protein-deficient experiments.—Guinea-pigs on the low-protein diet steadily lost weight over the 3-week diet period. However, they remained active and bright and showed no evidence of ill health. Three animals given culture fluid mixed with autoclaved sweet potato re-

mained normal. Four guinea-pigs given raw sweet potato mixed with culture fluid at the same time developed intestinal lesions. Three died within 18 h and the fourth was killed when moribund at 26 h. There was very extensive haemorrhage and necrosis in the small bowel. Subsequent experiments with 4 animals actively immunized before the low-protein diet again demonstrated the effectiveness of immunization.

Mixing pancreatin with the sweet potato/culture before administration prevented lesions.

Operative experiments

Injection of culture or culture filtrate directly into the jejunum resulted in severe patchy necrosis in the small intestine. The banded appearance seen was very similar to that of human pigbel. All 3 animals given growing 4 h culture died from intestinal necrosis.

Three animals given toxic filtrate with penicillin and lincomycin developed typical intestinal necrosis.

Three guinea-pigs given the same dose of toxin incubated with pancreatin for 15 min before injection remained normal.

Two groups of 3 actively immunized guinea-pigs did not develop any evidence of intestinal damage after intrajejunal injection of either growing culture or toxic filtrate. Two of 3 unimmunized controls died from intestinal necrosis while the third was unaffected.

Experiments on the time relationships in pigbel

Two of the 5 animals given β antitoxin 7 h after intragastric dosing with soybean powder and culture developed patches of intestinal necrosis. The lesions were not extensive enough to have caused death. All the animals given β antitoxin earlier than 7 h were normal.

Results on the time course of pathological changes.—None of the animals died although 2 were very ill and were unable to eat for 2 days before they were killed. Macroscopically there were patches of gut-wall necrosis with omentum adherent to and sealing over the damaged areas, an appearance typical of the human disease.

DISCUSSION

This series of experiments was undertaken in an effort to explain the pathogenesis of pigbel in PNG and its relationship to Darmbrand in Germany. Although Darmbrand and pigbel seemed to be the same disease, pigbel has persisted where Darmbrand disappeared after a few years. Previous workers showed that similar pathology could be produced in the guinea-pig by injecting β toxin or growing cultures into the small intestine direct, but not by oral administration of growing cultures.

Once lesions in the guinea-pigs were produced by including raw soybean with the culture, the importance of β toxin's susceptibility to proteases was emphasized and later the role of dietary inhibitors in PNG became apparent. In experiments reported earlier (Lawrence, 1974) pigbel was produced in guinea-pigs using soybean and a *Cl. welchii* C organism, the organism recovered from the intestinal lesions and pigbel induced in another animal with the recovered organism and soybean flour. Although soybeans contain a number of toxic substances apart from protease inhibitors, control experiments showed the powdered raw soybean was innocuous when given without the growing culture.

The experiments suggest a number of important factors in pathogenesis. Firstly the central importance of β toxin and proteolytic enzymes is demonstrated by the effects of soybean, raw sweet potato and aprotinin: only in the presence of trypsin inhibitor could lesions be produced with intragastric dosing. In the case of the heat-stable sweet potato inhibitors the animals had to be protein-deficient as well. Sweet potato inhibitor inhibits trypsin but not chymotrypsin (Sugira *et al.*, 1973) whereas soybean inhibitor

inhibits both. Gyr *et al.* (1975) showed that, in the protein-deficient Patas monkey, secretion of chymotrypsin ceased completely while lowered levels of trypsin were still being produced. In the absence of chymotrypsin from the low-protein diet the sweet potato inhibitor can effectively protect β toxin. Ascaris infestation is commonly seen in pigbel patients; these parasites are known to produce trypsin inhibitors which may contribute to pathogenesis in some patients. Also, the experiments with washed organisms and the inability to produce pathology from intragastric dosing, except when substrate for further growth was included with the culture, indicates the importance of bacterial growth and toxin production within the intestine. Pigbel could not be induced by intragastric dosing with toxic filtrate and soybean inhibitor, presumably because of toxin destruction in the stomach by acid and peptic enzymes.

Pigbel is probably produced by toxin from organisms multiplying in the intestine and not by toxin already present in the initiating meal. This interpretation agrees with epidemiological evidence; some cases of pigbel follow meals, such as tinned meat, which could not have been heavily contaminated. Possibly in some cases, as in the sheep, the organism is an intestinal commensal or transient which overgrows in the changed environment caused by the high-protein meal. Whether *Cl. welchii* C specifically attaches to the mucosa like some other intestinal pathogens is not known.

Finally, active immunization with β toxoid is completely protective. Although we have not investigated the class of antibody produced in response to β toxoid in the guinea-pig it is likely, as in man, to be IgG. IgG antibody is present in the intestinal villi and leaks into the lumen, playing a part in surface immunity (Walker and Isselbacher, 1977). Passive administration of IgG is protective as long as it is given within a short time of the initiating culture meal, before the animals show any signs of illness. Rooney, Sheperd and Suebu (1979) demonstrated that treatment with antitoxin was ineffective in clinical pigbel, probably because it was always given after the onset of symptoms, long after the pathological damage had occurred.

The macroscopic and microscopic pathology of experimental pigbel in the guinea-pig is very similar to that seen in man. These findings in the guinea-pig model lend considerable support to the importance of a direct effect of absorbed toxin. Patches of necrotic bowel are present without vascular changes in animals killed at 24 to 48 h, whereas in animals killed after 5 or 7 days there is thrombosis in small veins and endothelial proliferation in larger ones similar to the pathology in operative specimens of pigbel. This indicates that these vascular changes follow the necrosis rather than cause it.

On the basis of the animal experiments it seems that a combination of the following factors is necessary for the occurrence of pigbel.

(1) presence of the organism;
(2) consumption of nutrients for rapid growth of *Cl. welchii* C;
(3) low capacity for protease (trypsin) secretion;
(4) presence of dietary or perhaps parasitic trypsin inhibitors.

In many countries these conditions may be present at times; in the PNG Highlands they are a continuing part of normal life. The diet is very low in protein, and children are often undernourished. Meat is eaten spasmodically. The dietary staple, sweet potato, which accounts for about 75% of the caloric intake, contains heat-stable inhibitors of trypsin. The organism is ubiquitous and ascaris infestation is common. In Germany, after the war, Darmbrand disappeared when nutrition improved and the people were protected from β toxin by their proteolytic enzymes. Recently we have become aware of reports of EN from Southern China. There the disease is also associated with subnutrition,

ascaris and the consumption of raw sweet potato and peanuts, which also contain trypsin inhibitors (Pan Jun-Di, personal communication). The similar epidemiological setting in China gives weight to the theories of pathogenesis in PNG. In PNG pigbel is an important health problem and, although improvements in nutrition and hygiene may reduce its incidence in time, changes in social factors are usually slow. Recently active immunization against β toxin with an adsorbed toxoid has been shown to give good protection in children (Lawrence *et al.*, 1979), as in the experimental guinea-pig. Pigbel has only been recognised in PNG by clinicians for a relatively short time (Murrell and Roth, 1963); if favourable conditions exist in other parts of the world, it may also be a preventable cause of suffering and death elsewhere.

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