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PATHOPHYSIOLOGY OF DIARRHOEA INDUCED BY A COMBINED INFECTION WITH TRANSMISSIBLE GASTROENTERITIS VIRUS AND ENTEROTOXIGENIC *ESCHERICHIA COLI* IN NEWLY-WEANED PIGLETS AND THE EFFECT OF FLURBIPROFEN TREATMENT

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ABSTRACT

Cox, E., Cools, V. & Houvenaghel, A. 1988. Pathophysiology of diarrhoea induced by a combined infection with transmissible gastroenteritis virus and enterotoxigenic *Escherichia coli* in newly-weaned piglets and the effect of flurbiprofen treatment. *Veterinary Research Communications*, **12(4–5)**, 383–399

In newly-weaned 3–4 week old piglets (n = 29) diarrhoea (100%) and vomiting (65%) were induced by inoculation with transmissible gastroenteritis virus and enterotoxigenic *E. coli* strains (O_{149}:K_{91}:K_{88ac}; LT, STa and STb enterotoxin positive). This combined infection resulted in pronounced mortality within 7 days. During this period the piglets had decreases in body weight, arterial pressure and leucocyte count and increases in heart rate and in total plasma protein concentration. The plasma pH and lactic acid concentration decreased, whereas the values for pO_2, pCO_2 and frequency of respiration did not change significantly. No significant changes in the serum concentrations of potassium, chloride or calcium were observed, whereas sodium concentration revealed a transient increase. In shocked and dying piglets an increase in haematocrit was observed, whereas base excess and bicarbonate concentration decreased.

Flurbiprofen, a potent non-steroidal anti-inflammatory drug, administered intramuscularly on 3 successive days following the combined infection at a dosage of 1 mg/kg/12 h was without beneficial effect on diarrhoea or mortality.

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) are involved in the post-weaning diarrhoea syndrome in pigs. However, experiments performed in our laboratory to induce hypersecretion diarrhoea by inoculation of newly-weaned piglets with ETEC, possessing fimbrial adhesins and producing heat-labile (LT) and heat-stable (STa and STb) enterotoxins, were not always successful (Cox *et al.*, 1986a). Post-weaning diarrhoea has a multifactorial etiology. The genetic susceptibility of the host to adhesion of ETEC as well as nutritional, environmental and immunological factors and the presence of a rotavirus infection can probably seriously influence the occurrence and the severity of the diarrhoea (Tzipori *et al.*, 1980; Lecce *et al.*, 1982; Lecce, 1983; Sellwood, 1983; 1984; Bijlsma *et al.*, 1985).

When the experimental ETEC infection was preceded by oral chloramphenicol treatment we observed an enhanced occurrence, severity and duration of diarrhoea (Cox *et al.*, 1986a). This 'colonization-resistance-suppressing' antibiotic probably makes the gut more susceptible to ETEC (Kaufman, 1984). The induced diarrhoea, however, was still insufficiently pronounced to result in significant hypovolaemia and shock.

We therefore introduced a combined viral-bacterial infection into our piglet diarrhoea

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model. Since rotavirus is enzootic in Belgian piggeries and most pigs are infected before the age of 6 to 7 weeks (Debouck, 1984), we inoculated piglets with transmissible gastroenteritis (TGE) virus, a coronavirus, prior to ETEC infection. This combined infection resulted in pronounced diarrhoea and hypovolaemia and in 100% mortality within 5 days of TGE virus inoculation (Cox *et al.*, 1986b).

We recently reported the effect of the antisecretory drugs chlorpromazine, verapamil, clonidine and propranolol on diarrhoea and survival in our piglet diarrhoea model (Cox *et al.*, 1987; Cools *et al.*, 1987). Several studies suggest the involvement of prostaglandins as important messengers in the secretory response of the enterocytes to bacterial enterotoxins (Bennett, 1971; Wise *et al.*, 1983; Powell, 1986). Non-steroidal anti-inflammatory drugs (NSAID's), which inhibit prostaglandin synthesis by blocking the cyclooxygenase pathway, have been shown to reduce cholera or ETEC-induced hypersecretion in several species (Willard, 1985; Greenough & Rabbani, 1986). However, in clinical trials in man, aspirin and indomethacin were ineffective in the treatment of cholera-induced hypersecretion diarrhoea (Greenough & Rabbani, 1986).

The purpose of the present study was to evaluate the pathological changes induced by the combined TGE virus and ETEC inoculation in newly-weaned piglets and to examine the possible effects of flurbiprofen, a potent NSAID of the arylacetic acid class, on this diarrhoea model.

MATERIALS AND METHODS

The experiments were performed on 29 newly-weaned, 3–4 week old female piglets, weighing 3.2 to 7.2 kg, from primiparous sows purchased from the same commercial farm. The piglets were of mixed breeding (Pietrain × Belgian Landrace). They were individually housed at 27°C and allowed to drink UHT sterilized whole cow's milk *ad libitum*. On day 1 of the experiment the piglets were anaesthetized with 15 mg/kg methomidate (Hypnodil®, Janssen) and 2 mg/kg azaperone (Stressnil®, Janssen) and a Silastic catheter (Dow Corning) was implanted in the left carotid artery for daily blood sampling and pressure recording. To enhance ETEC colonization of the small intestine, the piglets were pretreated on days 1, 2 and 3 with chloramphenicol (Chloromycetin®, Parke-Davis; 1,875 mg/l milk). After a starvation period during the first three hours of day 4, the piglets were orally inoculated with TGE virus (1.66×10^6 pig infective dose/animal). Twenty-four hours later (day 5) the piglets were again starved for 3 hours, after which 62 ml of a 1.4% $NaHCO_3$ solution was given intragastrically to prevent ETEC destruction. Fifteen minutes later they were each intragastrically inoculated with 10 ml of a suspension of two ETEC strains. Both strains, $0_{149}:K_{91}:K_{88ac}$, LT, STa and STb enterotoxin positive, were grown on brain heart infusion agar (Oxoid) at 37°C for 24 hours. The bacteria were suspended and diluted in sterile physiological saline to an A_{620} of 0.4, approximately 1.2×10^9 bacteria/ml, as determined by viable count.

Flurbiprofen treatment was started 5 hours after the ETEC inoculation and was performed for 3 consecutive days. Eight piglets (flurbiprofen group) were intramuscularly injected with flurbiprofen (Boots) at a dose of 1 mg/kg/12 h. Flurbiprofen was dissolved in 0.1 M NaOH (10 mg/ml) and the solution was neutralized to pH 7.4 with 0.1 M HCl. Xylocaine (2 mg) was added to reduce the pain induced by the intramuscular injection of flurbiprofen.

The other piglets (n = 21) were kept as untreated controls (control group).

Weight change, milk intake, body temperature, appearance and severity of diarrhoea, and clinical symptoms were registered daily. From day 3 onwards the following parameters were also evaluated: survival rate; frequency of respiration; arterial blood pres-

sure (Pa); heart rate (HR); haematocrit (Hct); plasma protein (Merck kit), glucose (Merck kit) and lactic acid (Boehringer kit) concentrations; arterial pH, $p0_2$ and pCO_2 (Astrup, Radiometer); base excess (BE) and bicarbonate (porcine acid-base alignment nomogram for arterial blood; Hannon, 1984); leucocyte count; serum concentrations of sodium, potassium (flame photometry), chloride (chloridometer) and calcium (atomic absorption); sodium and potassium concentrations and osmolality (cryoscopy) of faecal water, obtained as the supernatant after centrifugation of freshly collected faeces at 6,000 g for 5 min; faecal ETEC excretion; and the faecal shedding of TGE virus, rotalike- and rotavirus (enzyme-linked immunosorbent assay).

The severity of diarrhoea was evaluated by arbitrarily scoring the consistency of the faeces (0 = normal; 1 = pasty; 2 = semiliquid; 3 = watery). Scoring was always performed by the same person. The osmotic gap was calculated from the faecal water values as the difference between the measured osmolality and the estimated osmolality, based on twice the sum of the sodium and potassium concentrations (Shiau *et al.*, 1985). Secretory diarrhoea is commonly associated with a negative osmotic gap, whereas osmotic diarrhoea is associated with a positive osmotic gap of greater than 160 mosmol/l, at least in man (Shiau *et al.*, 1985). Faecal ETEC excretion was evaluated as the approximate percentage of haemolytic *E. coli* among the total number of aerobic bacteria in rectal swabs inoculated onto blood agar plates at 37°C for 24 hours and was also arbitrarily scored (0 = 0%; 1 = 0–25%; 2 = 25–50%; 3 = 50–75%; 4 = 75–100%).

The carotid arterial pressure was registered with a saline-filled polyethylene catheter connected to a miniature pressure transducer (Gaeltec). The electrocardiogram (ECG), which was used to measure HR, was monitored with five ECG electrodes fixed with rubber bands cranial to the left and right shoulder joint, above the seventh vertebral spine and on the abdomen cranial to the left and right stifle joint. Pa and ECG were recorded on a Beckman multichannel recorder.

Shocked piglets died spontaneously or were killed with an overdose of methomidate. The other piglets were euthanatized with methomidate 10 days after the start of the experiment. All piglets were examined post mortem.

The genetic susceptibility of the piglets to K_{88ac}-ETEC adhesion to their small intestinal villi was determined by the *in vitro* technique described by Girardeau (1980).

The daily diarrhoea score and faecal ETEC score are expressed as medians and ranges or individual values and were statistically analysed using the Mann-Whitney U or Wilcoxon tests. All other parameters are given as means ±SEM. A one-way analysis of variance and simple contrasts were used to assess the statistical significance between the values of the different parameters registered after the experimental infection and the values measured before infection on day 3. The Mann-Whitney U test was used to demonstrate significant differences for time-matched values of the flurbiprofen group and the control group. The percentage of survival in both groups was statistically evaluated using the Mantel modification of the Gehan generalised Wilcoxon test.

RESULTS

The post mortem *in vitro* adhesion assay revealed that the villi of all piglets from the control and the flurbiprofen group were susceptible to adhesion by K_{88ac}-ETEC to their enterocytes.

Control group

Experimental infection with TGE virus and ETEC resulted in a pronounced mortality

(71% of the animals), starting from day 5 and reaching maximal values on days 5 to 6 (Figure 1). Mortality was provoked by the pronounced watery diarrhoea, resulting in a hypovolaemic shock (Figure 2). Vomiting was observed in 65% of the animals. Hypovolaemic piglets had a poor appetite, severe depression and cyanosis of the extremities.

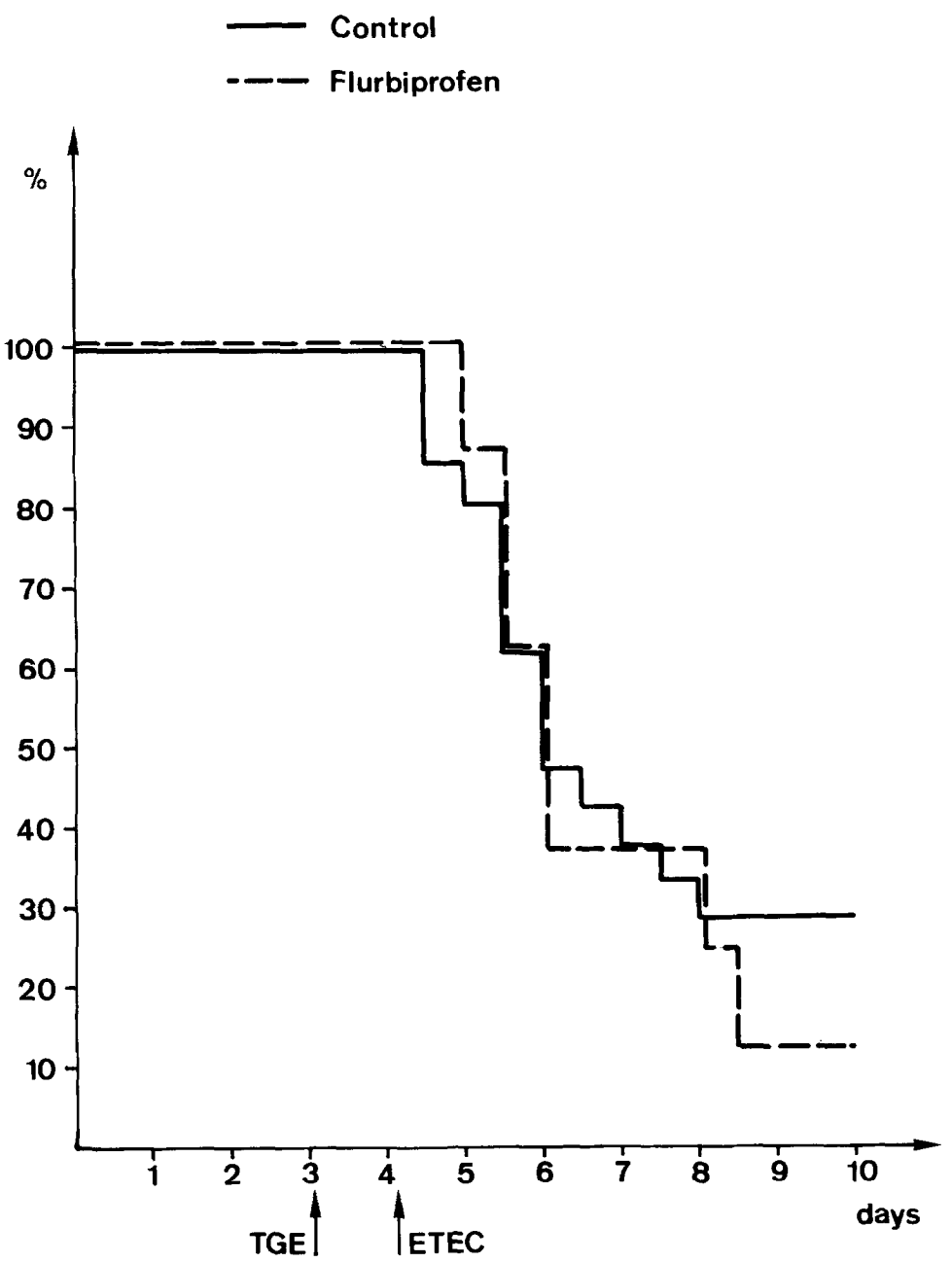

Figure 1. Survival rate following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8).

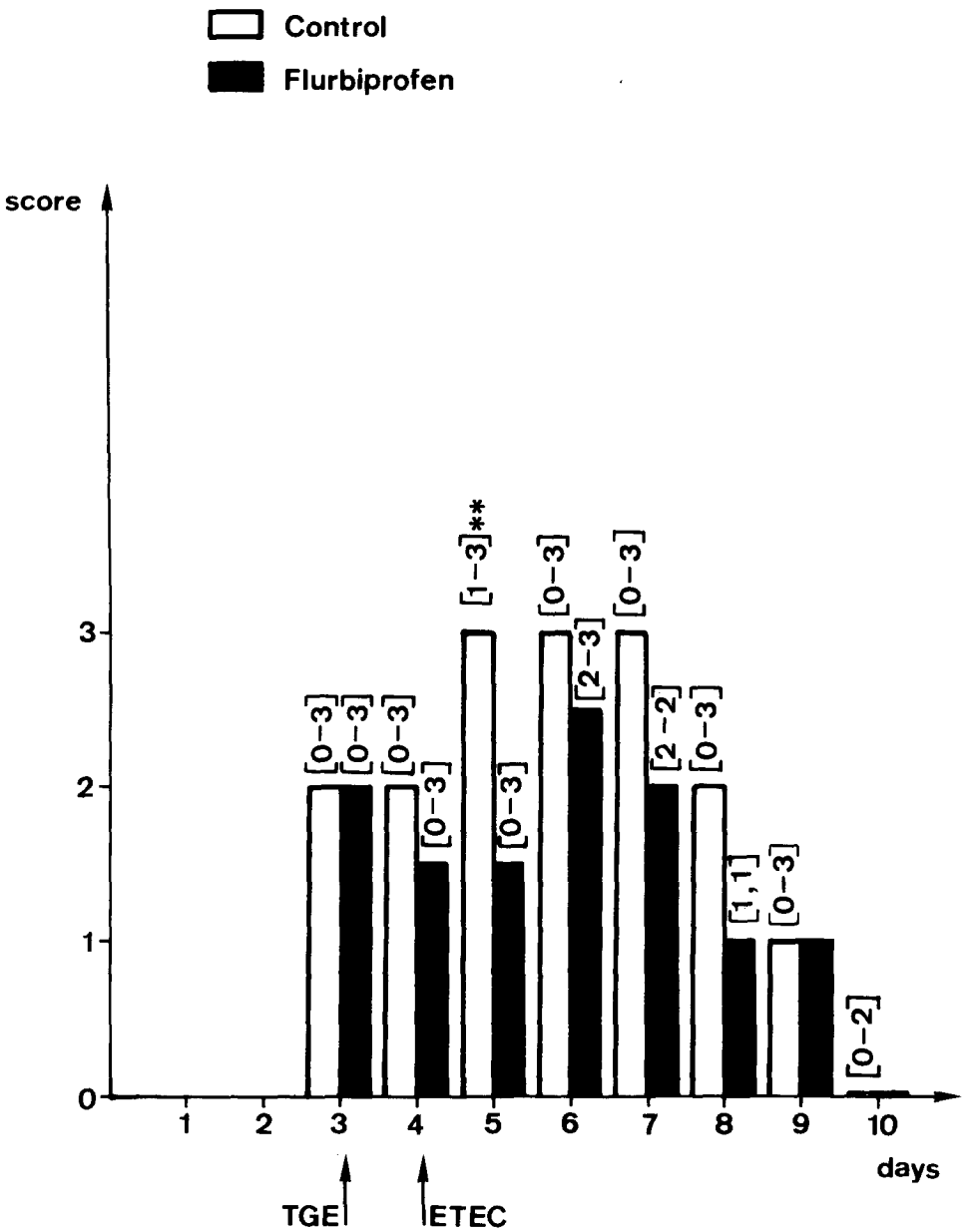

Figure 2. Diarrhoea scores (0 = normal; 1 = pasty; 2 = semiliquid; 3 = watery) following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8). Bars represent medians; ranges or individual values are given in brackets. Significant difference from the value prior to infection (day 3) is indicated by ** $P<0.01$.

In the terminal stage some pigs lay in lateral decubitus with the eyes retracted in the orbits, the flanks sunken and a prominent pelvis as a result of the severe dehydration. The piglets developed diarrhoeic faeces during the chloramphenicol pretreatment (Figure 2; day 3). Diarrhoea, however, seriously worsened following inoculation with TGE virus and ETEC ($P<0.01$). The faecal osmotic gap significantly increased from -14 ± 11.6 mosmol/l on day 3 to 53.6 ± 15.8 mosmol/l on day 5 ($P<0.01$) and 95.6 ± 27.5 mosmol/l on day 7 ($P<0.001$).

From about 20 hours following ETEC-infection a substantial number of haemolytic *E. coli* were shed in the faeces (Figure 3). TGE virus were demonstrated in the faeces of all the piglets. Rota- and rotalikevirus, were each only detected in the faeces of one pig.

Daily milk intake revealed a non-significant decrease (control value on day 3: 0.944 ± 0.106 l), followed by an increase to 1.447 ± 0.351 l on day 10. The pronounced and sustained diarrhoea, vomiting and loss of appetite resulted in a significant decrease in body weight (Figure 4).

Hct initially increased and then gradually decreased (Figure 5). In shocked and expiring piglets, however, a significant increase in Hct was observed, from $38.1\pm0.8\%$ on day 3 to $43.9\pm0.9\%$ just before death ($P<0.05$). Dehydration and hypovolaemia also resulted in a significant increase in the total plasma protein concentration from 4.6 ± 0.2 g/dl on day 3 to 5.7 ± 0.2 g/dl on day 6 ($P<0.001$) and also in HR, from 151 ± 6 beats/min on day 3 to 184 ± 9 beats/min on day 5 ($P<0.01$), and in a significant decrease in Pa (Figure 6).

Arterial pH revealed a significant decrease from 7.48 ± 0.03 on day 3 to 7.32 ± 0.14 on day 7 ($P<0.05$) and the lactic acid concentration also significantly decreased, from 21.7 ± 2.2 mg/dl on day 3 to 12.9 ± 1.6 mg/dl on day 5 ($P<0.01$). After an initial non-significant increase on day 6, the plasma glucose concentration decreased from 94.1 ± 6.1 mg/dl on day 3 to 76.7 ± 4.0 mg/dl on day 10 ($P<0.05$). The values for pO_2 (control value on day 3: 90.0 ± 3.4 mm Hg), pCO_2 (control value: 29.6 ± 1 mm Hg) and the frequency of respiration (control value: 26.3 ± 2.8/min) did not change significantly.

In shocked and expiring piglets BE decreased from -6.8 ± 2.4 mEq/l on day 3 to -24.6 ± 3.70 mEq/l on day 5 ($P<0.001$) and bicarbonate decreased from 23.3 ± 1.9 mEq/l on day 3 to 8.6 ± 1.9 mEq/l on day 5 ($P<0.001$). The corresponding value for pH on day 5 was 7.28 ± 0.05 ($P<0.01$).

Despite the pronounced dehydration there were no significant changes in the serum concentrations of potassium (control value on day 3: 4.1 ± 0.1 mEq/l), chloride (control value: 97.1 ± 1.8 mEq/l) or calcium (5.1 ± 0.2 mEq/l). Sodium concentration revealed a significant increase from 130 ± 2.5 mEq/l on day 3 to 137 ± 1.5 mEq/l on day 6 ($P<0.01$), followed by a return to near the control value on day 10 in surviving piglets.

Following the TGE virus and ETEC inoculation, the leucocyte count significantly decreased (Figure 7). In surviving piglets this parameter increased to give a significant leucocytosis on day 10 (Figure 7). After an initial, non-significant decrease the body temperature returned to near the control value and was significantly increased on day 10 in surviving animals (Figure 8).

Necropsy revealed erosions and congestion of the mucosa of the fundic region of the stomach, a congested, thin-walled small intestine, especially the ileum, with a watery content, and sometimes also congestion of the colon wall.

Flurbiprofen group

Flurbiprofen treatment was without beneficial effects on the mortality induced by the combined TGE virus and ETEC inoculation (Figure 1; $P>0.05$). As in the control group, the pigs developed severe diarrhoea during the chloramphenicol pretreatment period

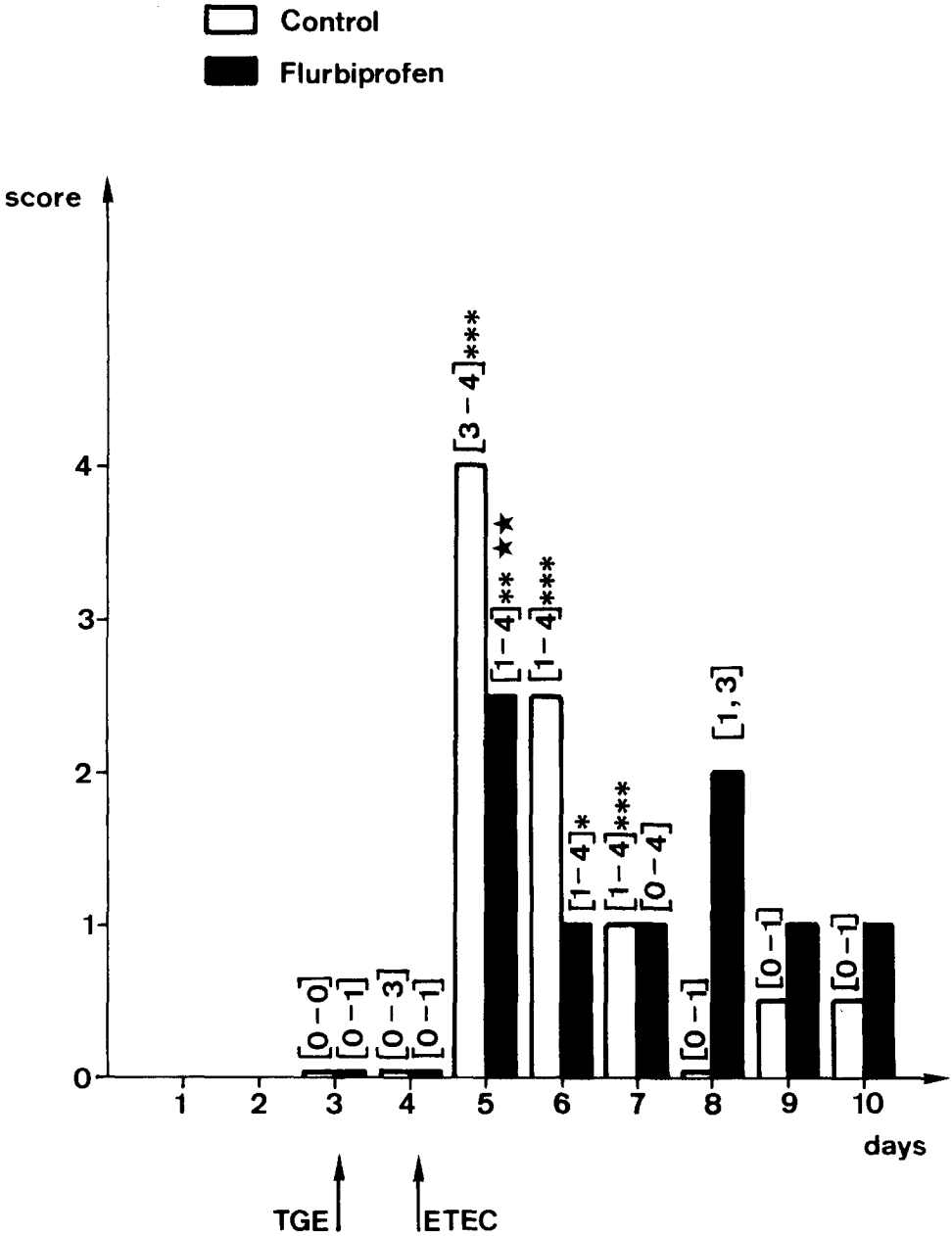

Figure 3. Faecal ETEC excretion, evaluated as the approximate percentage of haemolytic *E. coli* among the total number of aerobic bacteria in rectal swabs (0 = 0%; 1 = 0–25%; 2 = 25–50%; 3 = 50–75%; 4 = 75–100%), following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8). Bars represent medians; ranges or individual values are given in brackets. Significant differences from the values prior to infection (day 3) are indicated by * $P<0.05$; ** $P<0.01$; *** $P<0.001$. A significant difference between groups is indicated by ★★ $P<0.01$.

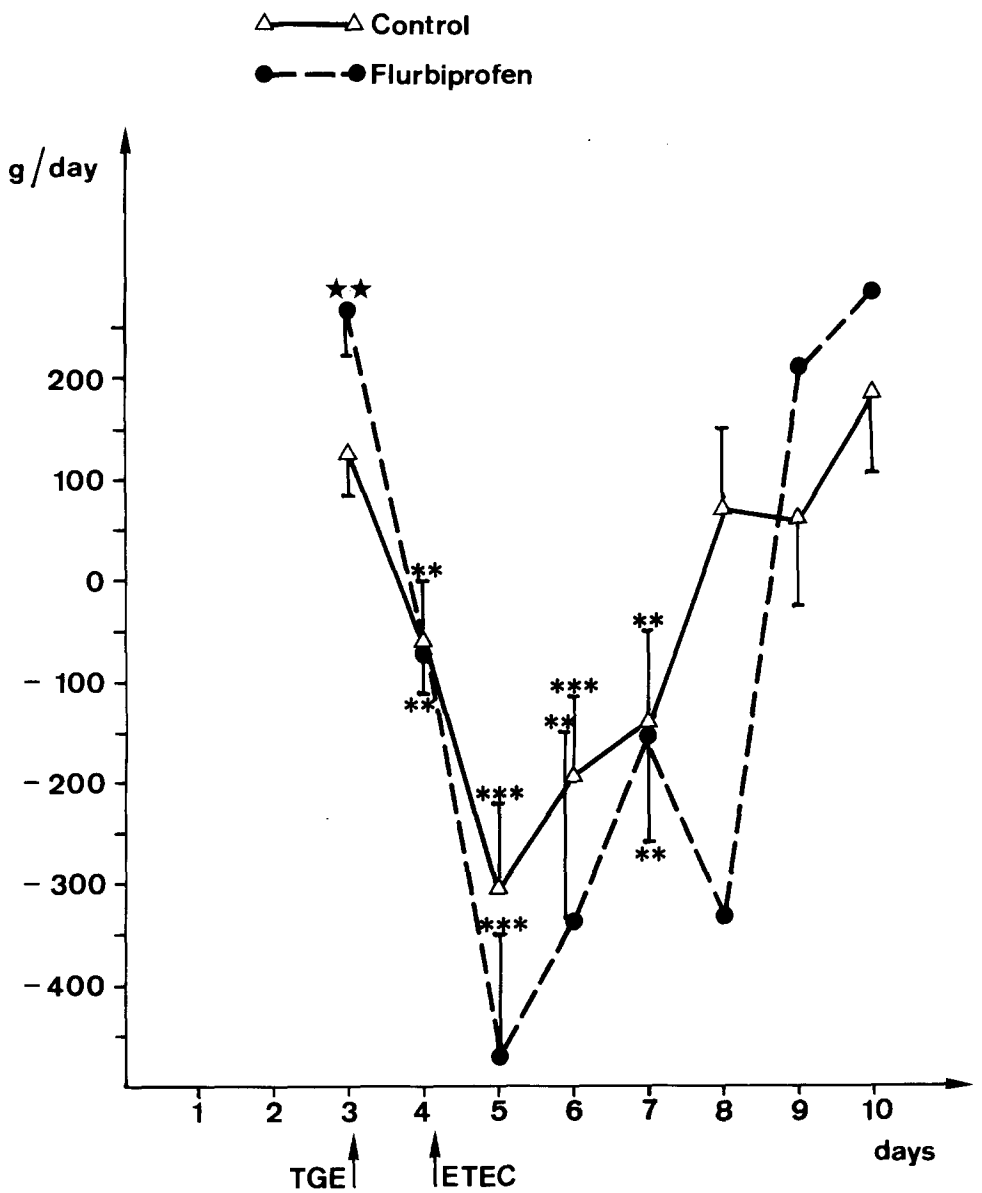

Figure 4. Weight changes following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and effect of treatment with flurbiprofen (n = 8). The values represent means ±SEM. Significant differences from the values prior to infection (day 3) are indicated by ** $P<0.01$; *** $P<0.001$. A significant difference between groups is indicated by ★★ $P<0.01$.

(Figure 2, day 3). The diarrhoea score for this group did not significantly differ from the values in the control group during the whole experimental period (Figure 2). The first administration of flurbiprofen on day 5 was followed by a temporary non-significant reduction in faecal fluid loss (Figure 2), a significant reduction in faecal shedding of

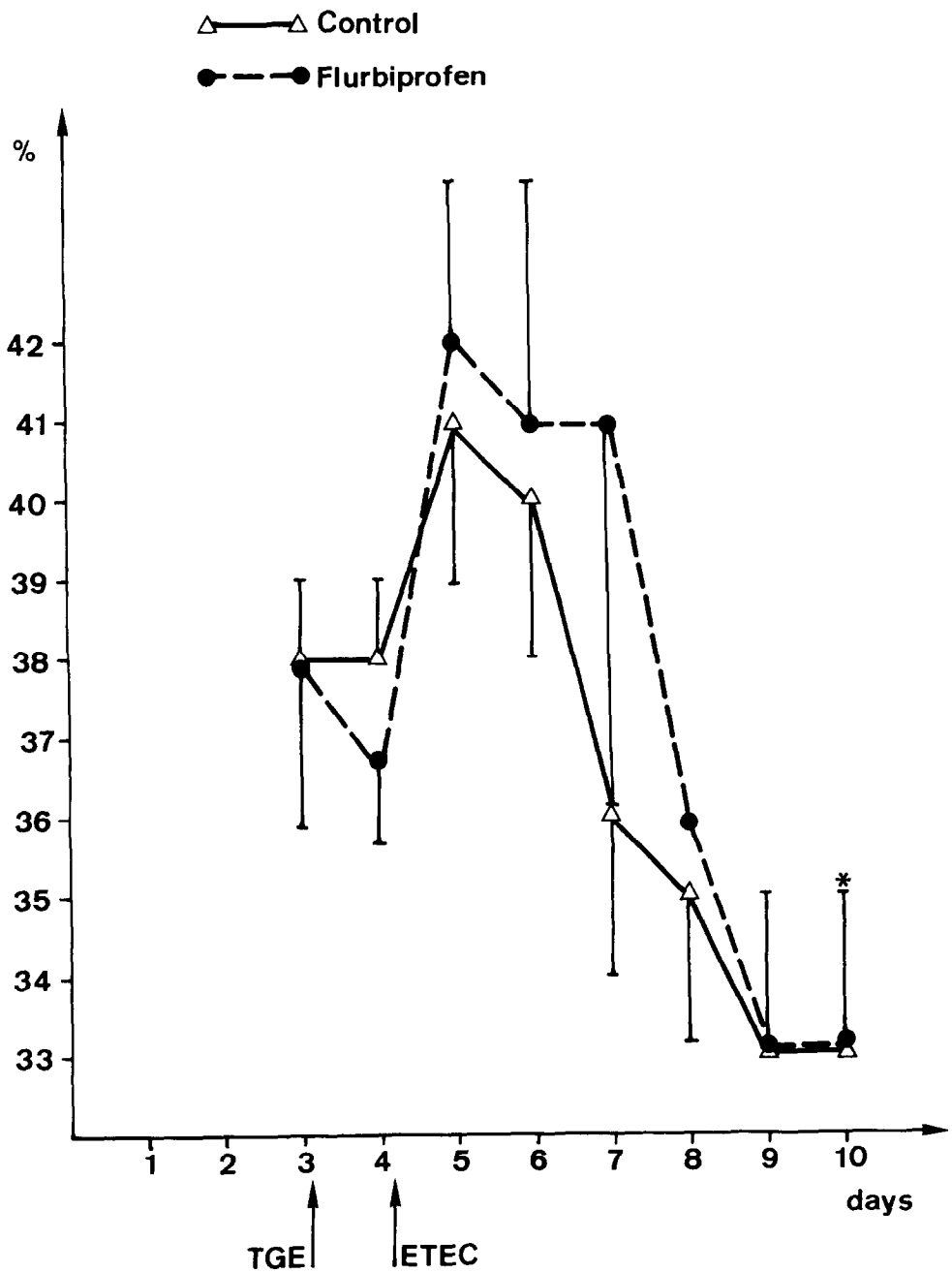

Figure 5. Changes in haematocrit (Hct) following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8). The values represent means ±SEM. A significant difference from the value prior to infection (day 3) is indicated by * $P<0.05$.

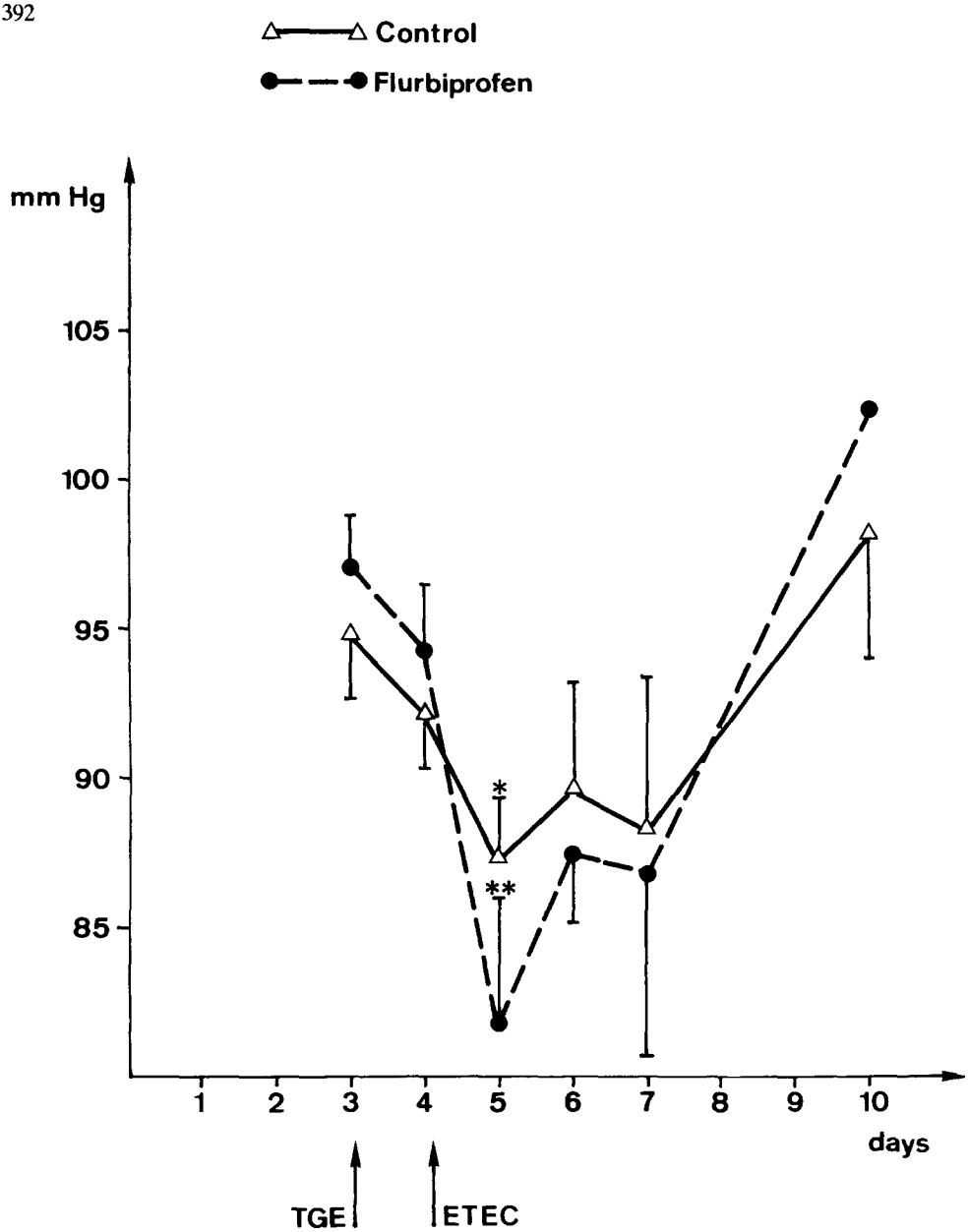

Figure 6. Changes in arterial pressure (Pa) following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8). The values represent means ±SEM. Significant differences from the values prior to infection (day 3) are indicated by * $P<0.05$; ** $P<0.01$.

haemolytic *E. coli* (Figure 3), a significantly more pronounced decrease in leucocyte count (Figure 7) and a temporary normalization of body temperature (Figure 8). After the later administrations, however, the evolution of all the parameters studied was comparable to that of the control group (Figures 3–8). Except for a decreased activity in

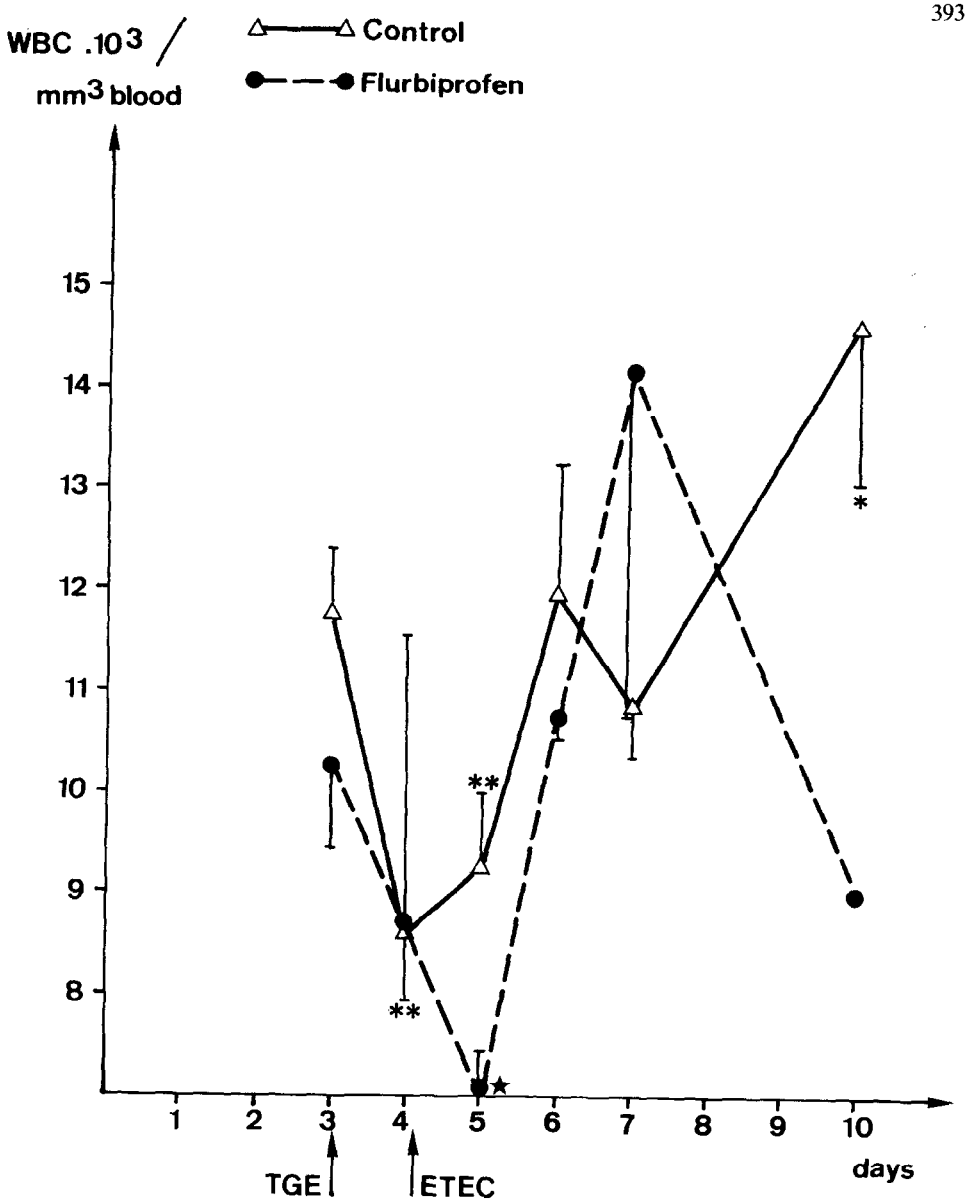

Figure 7. Changes in leucocyte counts following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8). The values represent means ±SEM. Significant differences from the values prior to infection (day 3) are indicated by * $P<0.05$; ** $P<0.01$. A significant difference between groups is indicated by ★ $P<0.05$.

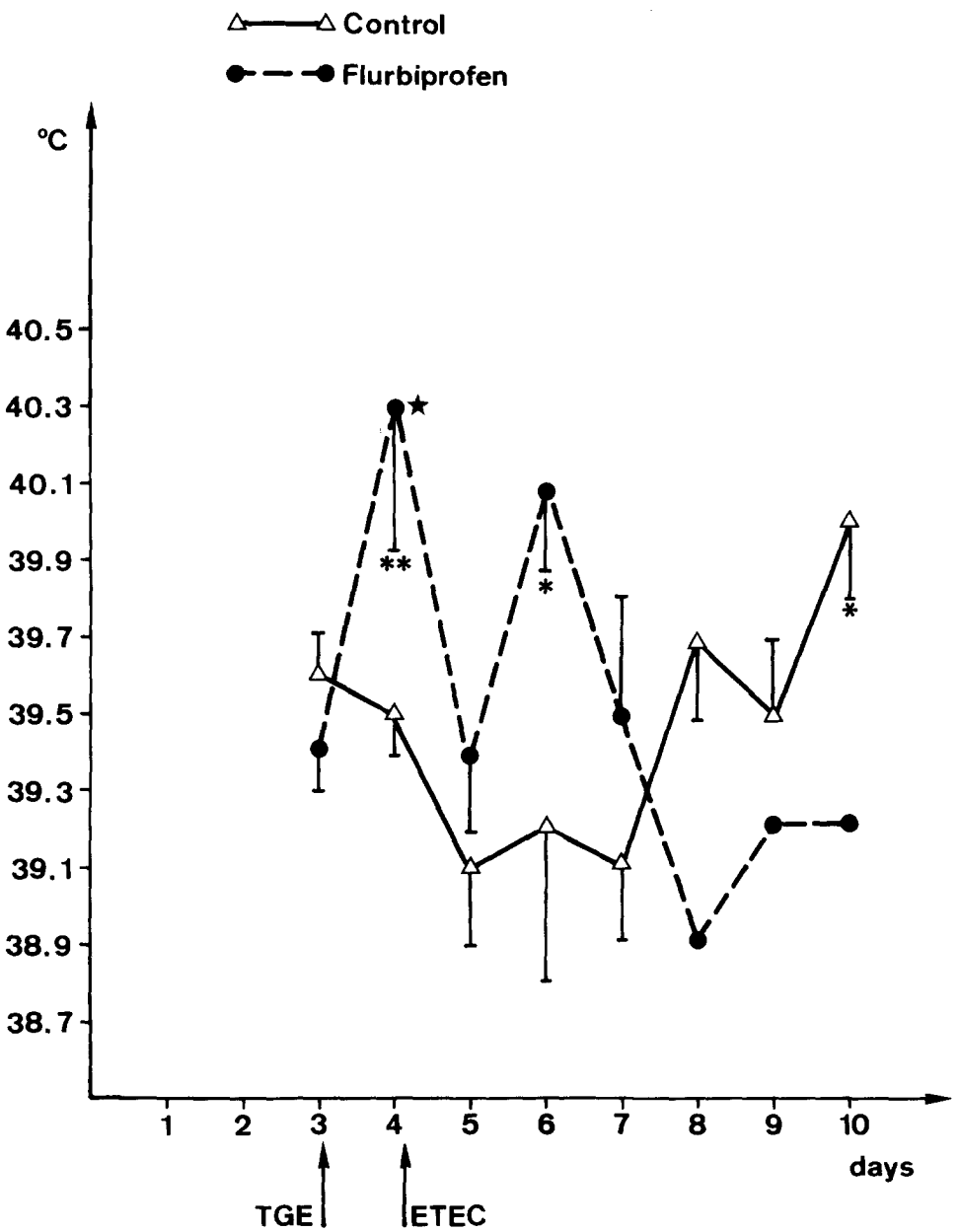

Figure 8. Changes in body temperature following a combined infection of newly-weaned piglets with TGE virus and ETEC (control group; n = 21) and the effect of treatment with flurbiprofen (n = 8). The values represent means ±SEM. Significant differences from the values prior to infection (day 3) are indicated by * $P<0.05$; ** $P<0.01$. A significant difference between groups is indicated by ★ $P<0.05$.

the flurbiprofen group there were no significant differences between the groups for any parameter not shown in the Figures.

DISCUSSION

Since the turn of the century *E. coli* has been implicated in the aetiology of porcine weaning diarrhoea (colibacillosis). The causal strains are usually haemolytic, possess fimbriae which mediate specific adherence of the *E. coli* to receptors on villous enterocytes and produce enterotoxins (Lecce, 1983). The enterotoxins LT and STa induce fluid secretion by the crypt cells, mediated by cyclic AMP and cyclic GMP respectively, whereas LT also inhibits absorption by villous cells. Although STb can induce villous atrophy, the exact mechanism through which it increases intestinal fluid loss is still unclear (Whipp *et al.*, 1987).

In the past five years there has been steady progress in research on potentially useful drugs which can reduce fluid loss in secretory diarrhoeas. In spite of promising activity in animal models, the recently tested antisecretory drugs have only reduced secretory diarrhoea in humans by 50%, chlorpromazine and nicotine having the most effect (Powell, 1986; Greenough & Rabbani, 1986).

Recently rotavirus has been shown to be causally associated with weaning diarrhoea of pigs. This virus multiplies in and destroys the absorptive cells of the villi of the small intestine, leading to malabsorption, dehydration and death in very young piglets (Lecce, 1983). In our model TGE virus, another enteropathogenic virus, was selected since rotavirus is enzootic in Belgian piggeries (Debouck, 1984), since the effects of this coronavirus in swine are well characterized and since, like rotavirus, it infects only mature epithelial cells and does not infect the undifferentiated crypt cells. TGE virus consistently induces villous atrophy and crypt hyperplasia. The disease is most acute and almost invariably fatal in the very young piglet, in which vomiting and profuse diarrhoea can lead rapidly to dehydration and death. Young pigs over 3 weeks old, as in our study, experience a debilitating diarrhoea of 10 to 14 days duration but usually survive (Aitken, 1983; Whipp *et al.*, 1985). We recently demonstrated the adhesion of the two ETEC-strains used in our present study to the atrophic villi following TGE virus inoculation in newly-weaned pigs (Cox *et al.*, in press).

In our model, dually-infected piglets experience profuse diarrhoea, vomiting, dehydration, weight loss and hypovolaemic shock, causing the death of 71% of the animals. The induced diarrhoea is probably a combination of malabsorption (TGE virus) and hypersecretion (ETEC). As a consequence of malabsorption the accumulation of non-absorbable nutrients, such as lactose, promotes a rise in luminal osmotic pressure, encouraging fluid movement from blood to lumen. At present the osmotic effect is considered to be the major component of TGE virus diarrhoea (Aitken, 1983). The evolution of the osmotic gap of the faecal water from secretory diarrhoea (day 3) to near osmotic diarrhoea (day 7) in our study is an interesting observation in this context.

The clinical signs in dying piglets in our study agree with the observations on *E. coli* infection in gnotobiotic pigs (Kenworthy & de G. Mitchell, 1976).

The diarrhoea occurring during the chloramphenicol pretreatment (days 1 to 3) in both groups studied can be explained by the change in the diet from sow's to cow's milk, by the loss of maternal immunity, by environmental changes (Tzipori *et al.*, 1980; Lecce, 1983), and probably by suppression of the gut flora by the antibiotic resulting in an increased susceptibility to infection by potentially pathogenic microorganisms (Kaufman, 1984). In healthy, nondiarrhoeal, colostrum fed neonatal calves, oral administration of chlo-

ramphenicol induces diarrhoea by partial decrease of villous height and crypt depth (Rollin *et al.*, 1986).

The high control values for arterial pH (day 3) in piglets of both groups in the present study agree with the observation on conscious immature pigs of 2 to 3 months old. Pigs of this age, however, reveal a higher pCO_2 value, a higher HCO_3^- concentration, a lower pO_2 value and a lower Hct (Hannon, 1983). Differences in our results can probably partly be attributed to the diarrhoea occurring on day 3. The decrease in Hct, following the initial increase, in surviving pigs of our study can probably be explained by a haemodilution effect, resulting from the daily extraction of blood.

When piglets developed hypovolaemia and shock some characteristic changes occurred within a few hours: an abrupt rise in Hct, a pronounced decrease in BE and a concomitant decrease in bicarbonate resulting in an acidosis. Since lactic acid did not increase but significantly decreased, other acid metabolites must be responsible for this metabolic acidosis. In experiments on ETEC-induced and spontaneous diarrhoea in neonatal piglets a metabolic acidosis with low pH and an abrupt decrease in BE and an increase in Hct was also reported in dying animals (Andrén & Persson, 1983). Kutas and Szabo (1971) likewise reported low pH and BE values in diarrhoeic piglets, resulting from experimental *E. coli* infection. In shocked animals in our study, the BE values were lower than -10 mEq/l, a critical limit below which the prognosis should be considered poor, at least in neonatal piglets (Andrén & Persson, 1983).

In experiments on gnotobiotic pigs, 27 to 34 days old when infected with ETEC-strains, animals developed a severe diarrhoea resulting in dehydration and death between 16 and 36 hours after infection. As in the piglets in our control group, no elevation of body temperature was recorded during this period. Pigs also revealed a neutropenia soon after infection and an abrupt increase in Hct (Kenworthy & de G. Mitchell, 1976). Polymorphonuclear cell infiltration in the intestinal mucosa was observed in pigs following infection with TGE virus or with STb producing ETEC strains (Pensaert *et al.*, 1970; Rose & Moon, 1985). This observation can probably partly explain the severe neutropenia observed in our study.

The post mortem findings in the piglets in the present study correspond to those observed in piglets which have died from post weaning diarrhoea.

Several studies suggest the involvement of prostaglandins in the LT and ST enterotoxin-induced hypersecretion (Berridge, 1984; Petzinger, 1984; Donowitz *et al.*, 1986). Results of studies on the effects of aspirin, indomethacin and other NSAID's are, however, somewhat inconsistent. *In vivo,* indomethacin inhibits secretion caused by *Vibrio cholerae,* cholera toxin and ST enterotoxin of *E. coli. In vitro*, this agent decreases the effects of cAMP and of drugs that stimulate the adenylate cyclase-cAMP system. *In vivo*, aspirin inhibits small intestinal secretion provoked by cholera toxin and *E. coli* LT enterotoxin but not *E. coli* ST enterotoxin (Donowitz *et al.*, 1986). In ligated calf's jejunal loops, salicylates reduced *E. coli* ST induced hypersecretion (Wise *et al.*, 1983). In the porcine jejunal loop, however, this NSAID was ineffective in reducing *E. coli* ST induced hypersecretion (Ahrens & Zhu, 1982). The inhibitory effect of the NSAID's appears to be due to increased absorption, although it is associated with some inhibition of activation of the cyclic AMP-system by LT enterotoxin of *Vibrio cholerae* and *E. coli.* (Donowitz *et al.*, 1986).

In the present experiments we studied the effect of flurbiprofen, a potent NSAID, on diarrhoea induced by combined TGE virus and ETEC infection in newly-weaned piglets. The dosage, 1 mg/kg/12 h, was derived from the oral dose in humans and from the plasma mean half-life value of approximately 7 hours after oral dosage in man (Szpunar *et al.*, 1987). In our model flurbiprofen was without significant beneficial effect on diarrhoea

score or lethality suggesting that prostaglandins are rather unimportant in the pathophysiology of the malabsorption and hypersecretion diarrhoea. Only the first administration of this NSAID resulted in a temporary amelioration of some parameters studied. The treatment, however, induced a decreased activity.

The effects of various NSAID's on cholera toxin-induced intestinal secretion in a number of animal species have generally shown them to be effective when given before but not after toxin administration (Wise *et al.*, 1983). In our experiments flurbiprofen was administered following the combined viral-bacterial inoculation. It recently appears that arachidonic acid metabolites may play an important role in invasive, inflammatory diarrhoea, but not in secretory diarrhoea (Powell, 1986; Turnberg, 1986). In our model the dual infection induced inflammatory intestinal changes (unpublished observation), as consistently occur following TGE virus and STb enterotoxin exposure (Pensaert *et al.*, 1970; Rose & Moon, 1985). No beneficial effect of the NSAID studied, however, was observed. Perhaps treatment with NSAID's would be more appropriate in diarrhoeic conditions caused by enteroinvasive bacterial strains (Petzinger, 1984).

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