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Increased metabolism of arachidonic acid in an immune model of colitis in guinea-pigs

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1 Inflammation of the guinea-pig colon was produced by skin sensitization and subsequent intracolonic challenge with the chemical hapten, dinitrochlorobenzene.

2 Metabolism of [^{14}C]-arachidonic acid by homogenates of control colon was very low, although metabolites co-migrating on thin layer chromatography (t.l.c.) with prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGD_2, 6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TXB_2), HHT and 11-, 12-, 15-HETE were formed.

3 There was an overall 3 fold increase in metabolism of [^{14}C]-arachidonic acid by homogenates of inflamed mucosa. The greatest increase in metabolite formation was of PGE_2, with smaller increases in HHT, 11-,12-,15-HETE, PGD_2, TXB_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$. The formation of these metabolites was inhibited both by indomethacin and the dual pathway inhibitor, BW755C.

4 The formation of immunoreactive PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ was also increased in homogenates of inflamed guinea-pig colon. The small level of immunoreactive LTB_4 detected in control colon was not changed in inflamed colonic tissue.

5 The dinitrochlorobenzene model of colitis offers a means of studying arachidonic acid metabolism in an immune-mediated inflammatory response in intestinal tissue.

Introduction

Ulcerative colitis is a recurrent inflammatory and ulcerative disease of the colon and rectum, characterized clinically by rectal bleeding, diarrhoea, cramping, abdominal pain, anorexia and weight loss. The aetiology of this inflammatory bowel disease is unknown although immunoregulation and lymphocyte function in patients with ulcerative colitis has been reported to be abnormal, suggesting that it may be an autoimmune disorder (for reviews, see: Kirsner & Shorter, 1982a,b; Jewell & Rhodes, 1983).

Metabolites of arachidonic acid have been implicated in the pathogenesis of ulcerative colitis. There is an increased level of prostaglandins in the faeces (Gould, 1976), blood (Gould *et al.*, 1981) and rectal dialysate (Rampton *et al.*, 1980) from patients with active disease. In addition, the *in vitro* synthesis of prostanoids such as prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, thromboxane B_2 (TXB_2) and 6-keto-$PGF_{1\alpha}$ is increased in inflamed colonic mucosa, taken during relapse from patients with ulcerative colitis (Ligumsky *et al.*, 1981). As well as an increased formation of these cyclo-oxygenase metabolites by inflamed human colonic mucosa, an increase in the arachidonate lipoxygenase metabolites, 5-HETE, 12-HETE, 15-HETE and leukotriene B_4 (LTB_4) has also recently been reported (Boughton-Smith *et al.*, 1983; Sharon & Stenson, 1984).

To elucidate further the involvement of arachidonate metabolites in ulcerative colitis, we have investigated the metabolism of arachidonic acid in inflamed colonic tissue taken from an animal model of inflammatory bowel disease (IBD). An immune model of colitis was used (Bicks *et al.*, 1965; 1967) in which a delayed hypersensitivity reaction was induced in guinea-pigs by skin sensitization and subsequent intracolonic challenge with the chemical hapten dinitrochlorobenzene (DNCB). In a previous study, the level of PGE_2 was elevated in inflamed colonic mucosa taken from guinea-pigs with DNCB-induced colitis (Norris *et al.*, 1982a). In the present study we have investigated the changes in formation of the arachidonic acid, cyclo-oxygenase and lipoxygenase metabolites formed by the guinea-pig colon. Arachidonic acid metabolism was assessed by use of both conversion of exogenous radiolabelled arachidonic acid ([^{14}C]-AA) and measuring the formation of TXB_2, PGE_2, 6-keto-$PGF_{1\alpha}$ and LTB_4 formed from endogenous substrate by specific radioimmunoassays.

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A preliminary account of some of these data has been presented to the British Society of Gastroenterology (Boughton-Smith & Whittle, 1984).

Methods

Model of colitis

Inflammation of the guinea-pig colon was produced by a method similar to that of Bicks *et al.* (1965). Guinea-pigs (male, 300–350 g) were sensitized by applying to a shaved area on the back a 2.5% ethanolic solution of DNCB (50 μl) once daily for 3 consecutive days; control animals received ethanol (50 μl) alone. After a further 9–12 days, the animals were challenged by intracolonic application (10 FG catheter, 4.5 cm in length, rounded at tip) of a 1% preparation of DNCB in Orabase (1 ml per application) once daily for 3 consecutive days.

In preliminary experiments, a 1% preparation of DNCB was found to produce a reproducible colitis in sensitized animals, but not in non-sensitized guinea-pigs. At a lower concentration of DNCB (0.25%) the colitis was not as reproducible, whereas a 4% preparation of DNCB had a direct inflammatory action on the colon of guinea-pigs not previously sensitized to DNCB.

Tissue preparation

Guinea-pigs were killed by cervical dislocation 24 h after the final challenge with DNCB. The distal 5–8 cm of descending colon was dissected out and any faeces removed. The colorectal segment was cut open lengthwise, rinsed in saline (0°C) and visually assessed for inflammation. In some experiments, segments of colon were prepared for histological evaluation. Any colon from the DNCB-sensitized and challenged guinea-pigs not showing gross signs of inflammation was rejected (< 10%); otherwise pooled tissue from DNCB or control animals was weighed, finely chopped with scissors and homogenized (30 s Ultraturax) in 50 mM Tris buffer (pH 7.4 at 0°C) to give a 100 mg ml^{-1} (10% w/v) homogenate.

Incubations

Aliquots (1 ml) of homogenate were preincubated (15 min, 37°C) with enzyme inhibitors or Tris buffer (100 μl) before adding [^{14}C]-AA (0.25 μCi, 1.3 μg) and incubating for 30 min in a shaking water bath (37°C). Samples for radioimmunoassay (RIA) of PGE_2, 6-keto-$PGF_{1\alpha}$, TXB_2 and LTB_4 were incubated in an identical manner except that there was no addition of [^{14}C]-AA. Following incubation, the RIA samples were centrifuged (2000 r.p.m., 10 min) and the supernatant stored (−20°C).

Extraction and thin layer chromatography (t.l.c.)

The unmetabolized [^{14}C]-AA and [^{14}C]-AA metabolites were extracted by a method similar to that described by Salmon *et al.* (1982). After adding acetone (2 ml, −20°C) the samples were vortexed and centrifuged (10 min, 2000 r.p.m.). Replicate samples were then treated in two different ways: (1) In samples in which the prostaglandins were to be later separated by t.l.c. using solvent system B (see below) the supernatant was washed with hexane (2 ml). (2) In samples in which the mono and dihydroxy acid metabolites were to be separated using solvent system A (see below), to allow more efficient extraction of these metabolites into the aqueous phase, the pH of the supernatant was first adjusted to pH 9.5–10, with 1 M NaOH, before adding hexane (2 ml). This also resulted in a much greater extraction of unmetabolized [^{14}C]-AA into the aqueous phase.

The samples were then treated in an identical manner. Following vortexing and centrifugation (10 min, 2000 r.p.m.) the upper hexane layer was discarded and, after acidifying (pH 3.5) the remaining sample with formic acid, chloroform (2 ml) was added. After a further vortexing and centrifugation (10 min, 2000 r.p.m.) the lower chloroform layer was dried (50°C) under N_2, redissolved in 60 μl chloroform: methanol (2:1) and applied to multilane t.l.c. plates (Whatman LK 5D). In each experiment, duplicate t.l.c. plates were prepared and developed using two solvent systems (Figure 1).

Solvent system A (ether : hexane : acetic acid, 60 : 40 : 1 v/v/v) facilitates the separation of the lipoxygenase derived mono- and dihydroxy acids from the prostaglandins and thromboxane (which run as a single band).

Solvent system B (the organic phase of ethyl acetate : trimethylpentane : water : acetic acid, 110 : 50 : 100 : 20, v/v/v/v), separates the individual prostaglandins and thromboxane.

The radioactive metabolites were located by autoradiography (DEF-2 Xray film Kodak, 3 days contact) and identified by co-chromatography with authentic cold standards, visualized by phosphomolybdic acid spray and heating (80°C), and in addition by co-chromatography with the standardized [^{14}C]-AA metabolites formed from incubation with rabbit polymorphonuclear-leucocytes (Randall *et al.*, 1980) and human washed platelets. Radioactive bands were scraped from the t.l.c. plates and quantitated by standard liquid scintillation counting techniques in a Packard Tri-carb scintillation counter (model B2450) interfaced with a Commodore micro computer (model 4016). The radioactivity (d.p.m.) from each band was expressed as a percentage of the total d.p.m. recovered from the t.l.c. plate (% conversion).

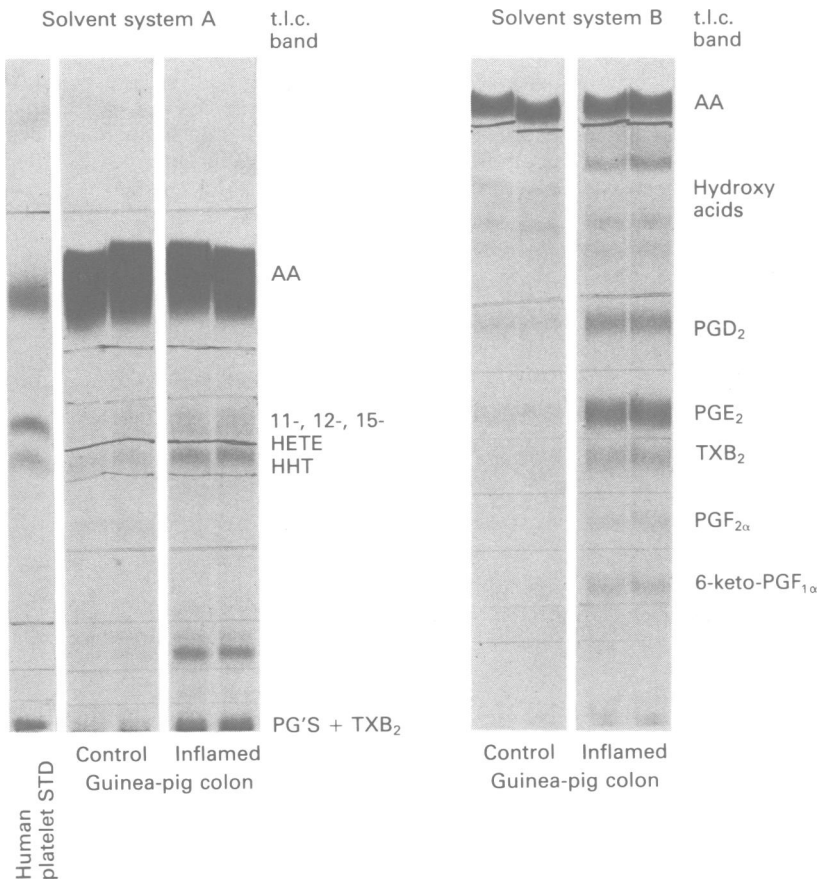

Figure 1 Autoradiogram of radiolabelled metabolites of [^{14}C]-arachidonic acid ([^{14}C]-AA) formed by homogenates of control and inflamed guinea-pig colon. The solvent system A was used to separate lipoxygenase products from the prostaglandins, and solvent system B was used to separate the different cyclo-oxygenase products. The radiolabelled products formed from incubating [^{14}C]-AA with human washed platelets are shown for comparison.

Radioimmunoassay

Supernatants from guinea-pig colonic homogenates were measured without prior extraction or chromatography by specific radioimmunoassays (RIA's) for LTB_4 (diluted 1 : 2 to 1 : 5), PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ (diluted 1 : 10 to 1 : 100 in assay buffer) which were carried out by Dr J.A. Salmon, Dept. Prostaglandin Research, Wellcome Research Laboratories (Salmon, 1978; Salmon & Flower 1979; Salmon *et al.*, 1982). The amount of eicosanoid formed is expressed as ng $100\,mg^{-1}$ tissue.

Histology

To confirm the visual assessment of inflammation, segments of inflamed and control colon were fixed in 10% neutral buffered formalin and processed for either wax or plastic (spurr resin) histology. The wax sections (5 μm) were stained with haematoxylin and eosin and the plastic sections (1 μm) with methylene blue-azure 11-basic fuchsin. The prepared sections were examined by light microscopy (kindly performed by Dr N.G. Read, Department of Toxicology, Wellcome Research Laboratories).

Materials

Dinitrochlorobenzene (1-chloro-2 : 4-dinitro-benzene; B.D.H. Ltd) was prepared for colonic application by dissolving 500 mg in 0.5 ml of acetone, adding 4.5 ml of olive oil (Boots Co.) and thoroughly mixing with 45 g of Orabase protective paste (Squibb & Sons Ltd) a compound which adheres to mucous membranes. Indomethacin (Sigma Chemical Co. Ltd) was dissolved in 5% $NaHCO_3$ and diluted in 50 mM Tris

buffer. BW755C (3-amino-1[m-(trifluoro methyl)-phenyl]-2-pyrazoline) as the hydrochloride (Wellcome Research Laboratories, Beckenham) was dissolved in water. [1-^{14}C]-arachidonic acid, 59.3 mCi mmol^{-1} (Amersham Int. p.l.c.). LK50 multilane t.l.c. plates (Whatman Inc.) and DEF-2 X-ray film (Kodak) were used. All organic solvents used were of Analar grade.

Statistical analysis

Results are expressed as mean ± s.e.mean of (n) experiments. Differences between control and inflamed tissue conversion and inhibition of conversion (calculated as % control) were compared within each experiment and statistical significance calculated by Student's t test for paired data (two-tailed), the level of statistical significance being taken as $P < 0.05$.

Results

Inflammation of the colon in guinea-pigs skin sensitized to DNCB and challenged by an intracolonic preparation of 1% DNCB was characterized by gross hyperaemia and oedema of the colonic mucosal surface which had been exposed to the DNCB. Histological examination of the colons showed inflammatory cell infiltration, mucus depletion, oedema and hyperaemia as described by others (Bicks *et al.*, 1967). In control animals which had not been sensitized to DNCB, but had received intracolonic DNCB, there was no gross hyperaemia, although in some animals, slight oedema was apparent. There were no histological signs of inflammation in these control colons.

[^{14}C]-arachidonic acid metabolism

In the experiments in which the metabolites were separated by solvent system B, overall conversion of [^{14}C]-AA to products by control colon was low (12.6 ± 4% of recovered radioactivity, $n = 3$). The prostanoids comprised 49 ± 15% of the total products and were identified by their chromatographic mobility as, $PGD_2 > 6\text{-keto-PG } F_{1\alpha} > TXB_2 > PGE_2 > PGF_{2\alpha}$ (Figure 2). Of the remaining metabolites separated in solvent system B, the 'hydroxy acids' comprised 49 ± 21% of total products formed (6.2 ± 2.7% of recovered radioactivity). In inflamed colon the overall conversion of [^{14}C]-AA to metabolites was significantly greater than control (38.3 ± 7.5% of recovered radioactivity, $n = 3$, $P < 0.05$) representing an overall 3 fold increase in metabolite formation. The prostanoids comprised

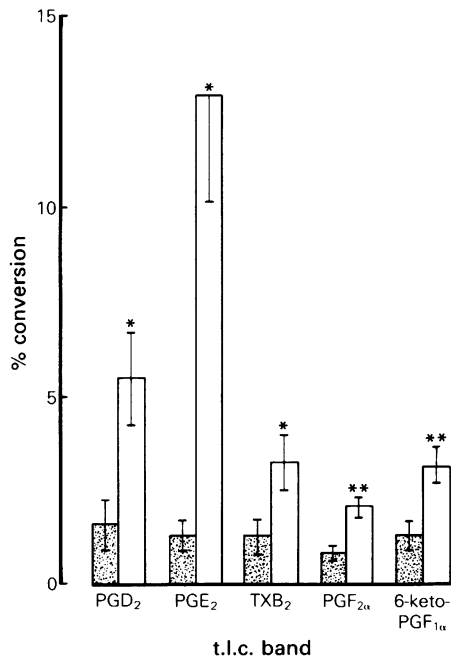

Figure 2 Metabolites of [^{14}C]-arachidonic acid formed by homogenates of control (stippled columns) and inflamed (open columns) guinea-pig colon and separated by t.l.c. in solvent system B. Results expressed as % conversion (% total d.p.m. recovered from t.l.c. plate) are from 3 experiments (in quadruplicate); s.e.means indicated by vertical lines. The level of statistical significance is shown as $*P < 0.05$; $**P < 0.01$.

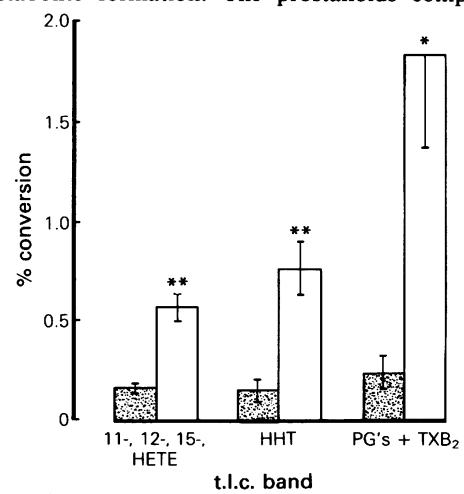

Figure 3 Metabolites of [^{14}C]-arachidonic acid formed by homogenates of control (stippled columns) and inflamed (open columns) guinea-pig colon and separated by t.l.c. in solvent system A. Results expressed as % conversion (% total d.p.m. recovered from t.l.c. plate) are from 5 experiments (in duplicate); s.e.means indicated by vertical lines. The level of statistical significance is shown as $*P < 0.05$; $**P < 0.01$.

70 ± 13% of the total products, representing a 5 fold increase in prostanoid formation compared to control. The greatest increase in prostanoid formation was of PGE_2 (10.2 × control formation); there was a smaller increase in the formation of PGD_2 (3.5 × control) and of TXB_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (2.5 × control) as shown in Figure 2. Because of differences in the individual increase in prostanoid formation by inflamed tissue the relative abundance of the prostanoids compared to control was changed such that $PGE_2 >>$, $PGD_2 >$, $TXB_2 >$ 6-keto-$PGF_{1\alpha} >$ $PGF_{2\alpha}$.

The results from the experiments in which the products were separated by t.l.c. in solvent system A cannot be compared directly to those in which the metabolites were separated in solvent system B because of the different extraction procedures used. However the relative change in [^{14}C]-AA metabolism between control and inflamed colon shows the same trends.

The major metabolites separated in solvent system A, as identified by their chromatographic mobility, were the prostaglandins and TXB_2, HHT and 11-, 12-, 15-HETE (Figure 3). The formation of these metabolites by control colonic tissue was low, although in inflamed colonic tissue the conversion to [^{14}C]-AA metabolites was increased significantly. The formation of the hydroxy acids was increased, HHT (5.1 × control) and 11-,12-,15-HETE (3.7 × control), as was the formation of the combined t.l.c. band for prostaglandin and TXB_2 (7.6 × control) as shown in Figure 3.

Table 1 Inhibition by indomethacin and BW755C of the metabolism of [^{14}C]-arachidonic acid by homogenates of inflamed guinea-pig colon

	% inhibition	
t.l.c. band	*Indomethacin* (6 μM)	*BW755C* (40 μM)
PGD_2	70 ± 10*	81 ± 10**
PGE_2	79 ± 8*	74 ± 6**
TXB_2	71 ± 10*	66 ± 6**
$PGF_{2\alpha}$	78 ± 11*	71 ± 6*
6-keto-$PGF_{1\alpha}$	76 ± 10*	71 ± 7*
HHT	70 ± 18*	41 ± 18
11-,12-,15-HETE	65 ± 13**	92 ± 6**
PG's + TXB_2	85 ± 13*	67 ± 30*

Results are expressed as % inhibition of [^{14}C]-arachidonic acid metabolism, by homogenates of inflamed guinea-pig colon, following 15 min preincubation with either indomethacin (6 μM) or BW755C (40 μM). Values are from 3–5 experiments for each compound. Statistical significance is shown as $*P < 0.05$; $**P < 0.01$.

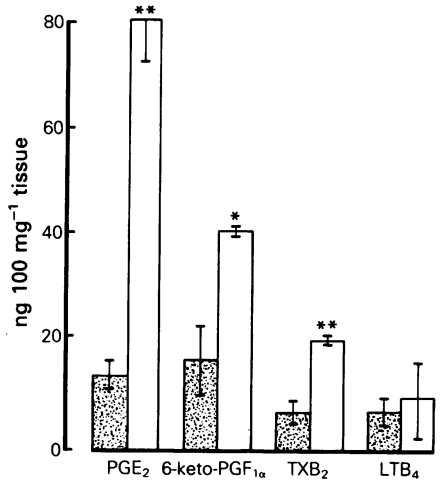

Figure 4 Formation from endogenous substrate of prostaglandin E_2 (PGE_2), 6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TXB_2) and leukotriene (LTB_4) by homogenates of control (stippled columns) and inflamed (open columns) guinea-pig colon, measured by specific radioimmunoassays. Results expressed as ng 100 mg^{-1} tissue are from 3 experiments (in duplicate); s.e.mean indicated by vertical lines. The level of statistical significance is shown as $*P<0.05$; $**P<0.01$.

The formation by the inflamed colonic mucosa of the [^{14}C]-AA metabolites separated in solvent system A and those separated in solvent system B was inhibited *in vitro* by the cyclo-oxygenase inhibitor indomethacin (6 μM). The metabolites were also inhibited *in vitro* by BW755C (40 μM), the combined cyclo-oxygenase and lipoxygenase inhibitor (Table 1).

There was no detectable formation from [^{14}C]-AA of LTB_4 or 5-HETE by either control or inflamed colon. When separated by t.l.c. in solvent system A the R_{aa} values (chromatrographic mobility of product with respect to arachidonic acid) for these metabolites were LTB_4 ($R_{aa} = 0.15$), 5-HETE ($R_{aa} = 0.44$) (Boughton-Smith *et al.*, 1983).

Radioimmunoassay

The homogenates of control guinea-pig colon formed, from endogenous arachidonic acid, immunoreactive PGE_2 and 6-keto-$PGF_{1\alpha}$ and in addition smaller amounts of immunoreactive TXB_2 and LTB_4. In inflamed colonic tissue the formation of the prostanoids, measured by radioimmunoassay, was significantly increased (Figure 4). As with the conversion of [^{14}C]-AA by inflamed colon, the greatest increase in endogenous metabolite formation was of PGE_2 (6.8 × control formation), with smaller increases in the formation of TXB_2 (3.8 × control) and of 6-keto-

$PGF_{1\alpha}$ (2.7 × control). The formation of immunoreactive LTB_4 was not increased by inflamed colonic mucosa. The relative abundance of the immunoreactive metabolites of arachidonic acid formed by inflamed tissue was $PGE_2 >> 6\text{-keto-}PGF_{1\alpha} > TXB_2 > LTB_4$.

The formation of immunoreactive prostanoids by inflamed colonic tissue was inhibited *in vitro* by indomethacin (6 μM); PGE_2 (53 ± 7% inhibition, $n = 3$, $P < 0.05$), 6-keto-$PGF_{1\alpha}$ (70 ± 9% inhibition) and TXB_2 (73 ± 13% inhibition). However, the formation of LTB_4 was unchanged, 8 ± 7 ng 100 mg^{-1} tissue for inflamed colon, compared to 9 ± 5 ng 100 mg^{-1} tissue with indomethacin. The formation of the prostanoids by inflamed tissue was also inhibited following incubation with BW755C (40 μM); 6-keto-$PGF_{1\alpha}$ (63 ± 9% inhibition, $n = 3$, $P < 0.05$), PGE_2 (49 ± 9% inhibition), TXB_2 (58 ± 20% inhibition), as was the formation of LTB_4 (79 ± 15% inhibition).

Discussion

In the present study, we have shown that there is an increase in the metabolism of arachidonic acid by homogenates of inflamed colon, taken from a guinea-pig model of colitis induced by the chemical hapten DNCB. Metabolism of [^{14}C]-AA by control colon was very low, although radiolabelled products which comigrated on t.l.c. with PGD_2, 6-keto-$PGF_{1\alpha}$, TXB_2, PGE_2 and $PGF_{2\alpha}$ were formed. In addition, small amounts of radiolabelled products co-migrating with HHT and 11-12-,15-HETE were also formed. The metabolism of [^{14}C]-AA was markedly increased in inflamed guinea-pig colon, the formation of all the major metabolites separated by t.l.c. being significantly increased. There was a similar marked increase in metabolism of endogenous arachidonic acid, the levels of immunoreactive $PGF_{2\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$ being significantly greater in homogenates of inflamed colon. By far the greatest increase in metabolite formation was of PGE_2, whether measured as the radiolabelled metabolite of [^{14}C]-AA (10 × control) or as a metabolite of endogenous arachidonic acid measured by specific RIA (7 × control formation).

In the present study we have used an intracolonic challenge of a 1% preparation of DNCB in order to obtain a reproducible colitis in sensitized animals. We found that the lower concentration of DNCB (0.25%) did not provide such a reproducible colitis, whereas a challenge concentration of 4% DNCB, similar to that used by others to assess the activity of anti-colitic drugs (Norris *et al.*, 1982b), had a direct inflammatory action on the colon of guinea-pigs not previously sensitized to DNCB. In a previous study in which a concentration of 0.25% DNCB was used to induce colitis in guinea-pigs (Norris *et al.*, 1982a), the control level of immunoreactive PGE_2 (18 ± 4 ng 100 mg^{-1} tissue) was similar to the present study (12 ± 2 ng 100 mg^{-1} tissue) and the increased formation of immunoreactive PGE_2 by inflamed colon (88 ± 33 ng 100 mg^{-1} tissue) was also very comparable to the present study (81 ± 8 ng 100 mg^{-1} tissue). An increase in PGE_2-like activity by microsomes prepared from inflamed colonic tissue, taken from a model of colitis in guinea-pigs, induced by feeding carrageenin, has also been reported, while supernatants of the same tissue were found to have a reduced capacity to metabolize $PGF_{2\alpha}$ (Hoult *et al.*, 1979). It is unlikely that in the present study, the prostaglandin metabolizing enzymes contribute to the changes in prostanoid levels observed, since these enzymes require the addition of co-factors to enable significant activity over the short incubation times employed. In numerous other types of inflammation, PGE_2 is considered to be the major cyclo-oxygenase metabolite present, often exceeding the concentration of other products 10 fold (Higgs & Vane, 1983). In the present study the formation of PGD_2, 6-keto-$PGF_{1\alpha}$,TXB_2 and $PGF_{2\alpha}$ were also increased. Whether the increased formation of these products is due to an increased metabolism of arachidonic acid by resident cells, the infiltration of inflammatory cells or platelets entrapped in the microcirculation of the colon is not known. However the increased formation of the prostanoids, particularly PGE_2, could lead to the oedema and hyperaemia seen in the inflamed guinea-pig colon, since these cyclo-oxygenase metabolites of arachidonic acid induce vasodilatation and thus enhance oedema formation (Wedmore & Williams, 1981; Higgs *et al.*, 1981).

In addition to prostanoids there was also an increase in the formation by inflamed guinea-pig colon of [^{14}C]-AA metabolites which co-migrate with HHT and 11-, 12-,15-HETE. The enzymic origin of the t.l.c. band corresponding to 11-,12-,15-HETE could not be confirmed as lipoxygenase, since its formation was inhibited by the cyclo-oxygenase inhibitor indomethacin, as well as by the combined cyclo-oxygenase lipoxygenase inhibitor, BW755C. Indomethacin has previously been reported to inhibit the formation of 11-HETE and 15-HETE by rat pleural leukocytes (Siegel *et al.*, 1979; Randall *et al.*, 1980) and 12-HETE formation by human platelets (Siegel *et al.*, 1979). Whether the effect of indomethacin reflects the formation of 11-HETE and 15-HETE via incomplete operation of the cyclo-oxygenase system (Randall *et al.*, 1980), or is a direct effect on lipoxygenase enzymes (Siegel *et al.*, 1979; 1980) is not however clear. In previous studies on human colon (Boughton-Smith *et al.*, 1983) a product co-migrating on t.l.c. with 11-,12-, 15-HETE was also found, and subsequent h.p.l.c. analysis of the band demonstrated that it was composed predominantly of 12- and 15-HETE. In contrast

to the present study using guinea-pig colon, the formation of the12- and 15-HETE formed by human colon was not inhibited by indomethacin but was inhibited by BW755C, thereby confirming the origin of these products from lipoxygenase enzymes. Clearly, further investigations are required into these species differences in susceptibility of lipoxygenase to inhibition by indomethacin. The formation of these arachidonic acid metabolites may be important since both 12-HETE and 15–HETE have chemotactic and chemokinetic properties and induce secretion of granule contents of polymorphonuclear leucocytes (Turner *et al.*, 1975; Borgeat *et al.*, 1976). Therefore, their increased synthesis by the inflamed guinea-pig colon could contribute to, and result from, the observed increased cellular infiltration.

Low levels of immunoreactive LTB_4 were detected in control colon but were not elevated in inflamed tissue. In addition, no product having the chromatographic mobility of LTB_4 was formed from [^{14}C]-AA by the homogenates of guinea-pig colon. In another study, in which chopped colonic tissue from guinea-pigs sensitized to ovalbumin was challenged with antigen *in vitro*, there was a considerable release of immunoreactive LTC_4 (Wolbling *et al.*, 1983). The formation of the LTC_4 from this model of immediate hypersensitivity is most probably from mast cells resident in the colon. The DNCB model of colitis in the guinea-pig is produced via a cell-mediated, delayed hypersensitivity response (Bicks *et al.*, 1965; 1967) and therefore it is unlikely that a release of LTC_4 from mast cells would occur. The low levels of LTB_4 formed in the present study by inflamed colonic tissue in which there is considerable polymorphonuclear leucocyte infiltration may be due to the tissue preparation and incubation conditions used. In a recent study on a whole cell preparation in which scrapings of colonic mucosa were taken from a model of acetic acid-induced colitis in rats, there was considerable conversion of [^{14}C]-AA to both 5-HETE and LTB_4 in the presence of the calcium ionophore, A23187 (Sharon & Stenson, 1985). Whether these products were formed during the short incubation (5 min) with [^{14}C]-AA or on subsequent homogenization is not clear.

In the inflamed guinea-pig colon, there is thus a marked increase in arachidonic acid metabolism. A similar increase in metabolism of arachidonic acid has previously been observed in inflamed colonic mucosa taken from patients with ulcerative colitis. Since many of the products formed from arachidonic acid have pro-inflammatory actions, their increased synthesis may be important in ulcerative colitis particularly during relapse of the disease. The model of colitis in the guinea-pig following challenge with 1% DNCB, which induces both macroscopic and histological signs of inflammation, offers a means of studying arachidonic acid metabolism in an immune inflammatory response in intestinal tissue.

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