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MODULATION OF OXIDATIVE STRESS IN THE GASTROINTESTINAL TRACT AND EFFECT ON RAT INTESTINAL MOTILITY

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Abstract—The amounts of different factors, which are involved in oxygen free radical production or in protection against oxygen radicals, were determined in different parts of the gastrointestinal tract (GI-tract). Glutathione and superoxide dismutase were present in lower amounts within the small intestine compared with the stomach and the large intestine. In the small intestine glutathione peroxidase and catalase both prevailed in the intestinal muscle compared to the mucosa, whereas in the large intestine both enzymes are equally distributed among the mucosa and muscle. Xanthine oxidase was mainly present in the small intestinal mucosa. Taken together, these results suggest that the large intestine is better provided with protective enzymic and non-enzymic factors against oxidative stress than the small intestine. The protective capacity of different intestinal preparations against lipid peroxidation in liver microsomes was assessed, and particularly the mucosal fractions from the small intestine showed a marked protection against lipid peroxidation, which is not easily explained with the presence of the enzymes measured in this study. Pretreatment of intestinal segments with hydrogen peroxide or cumene hydroperoxide resulted in a damaged contractile response of the longitudinal smooth muscle to methacholine in all parts of the GI-tract, expressed in a lower pD_2 value and a decreased maximal response. Pretreatment with these peroxides also decreased contractions after depolarization with K^+. The large intestine is more sensitive to hydrogen peroxide and cumene hydroperoxide than the small intestine, which parallels with the sensitivity to lipid peroxidation. The results obtained with hydrogen peroxide also correlate well with the catalase activity in the various segments of the intestine. In conclusion, oxidative stress in the GI-tract alters intestinal motility, especially in the large intestine. Probably this does not occur at the level of muscarinic receptors.

Several disease states or pathological situations in the gastrointestinal tract (GI-tract)† are characterized by the formation of oxygen derived free radicals, e.g. ischemia and subsequent reperfusion [1–4] and inflammation [5, 6]. Oxygen radicals can also be formed after intestinal intracellular drug metabolism [7]. In the case of ischemia and reperfusion the main cause of oxygen radical formation is xanthine oxidase which, combined with accumulated hypoxanthine, leads to substantial superoxide anion formation upon reperfusion [8–10]. One result of this oxygen radical damage is an increased vascular permeability [11, 12] which gives infiltration of neutrophils into the ischemic tissue. Neutrophils can also give rise to superoxide anion generation and contribute to the oxygen radical damage in intestinal ischemia [9]. Infiltration of neutrophils, leukocytes and macrophages is also a common feature in intestinal inflammation [5, 6, 13]. The intestinal mucosa is the most vulnerable region of the gastrointestinal wall in ischemia or inflammation [2, 4, 14]. However, also changes in the intestinal motility are described as a consequence of inflammation [15, 16], which implies that also the smooth muscle function can be disturbed by oxygen radicals.

The aim of this study is to establish whether oxygen radicals have an effect upon receptors or receptor effector systems and in this way affect motility. In our group indications have already been found that oxygen radicals can affect receptor systems and alter functionality of various tissues, e.g. heart [17] and airways [18].

In this study we investigated the vulnerability of the GI-tract to oxygen radical damage by establishing the amounts of different factors or enzymes which are involved in oxygen radical formation or in protection against such radicals. To this end the amounts of xanthine oxidase, glutathione, superoxide dismutase, glutathione peroxidase and catalase were measured in various parts of the GI-tract, to find out if variations could be observed within the GI-tract. Also attention was paid to the protective capacity of different fractions from the GI-tract against lipid peroxidation induced in liver microsomes. Finally, the sensitivity of the contractility of the smooth muscle for oxygen radicals was studied in functional experiments, in order to find out if receptor mechanisms were influenced or if the damage was less specific. In these experiments two reactive oxygen species generating systems were used, namely hydrogen peroxide and cumene

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† Abbreviations used: GI-tract gastrointestinal tract; DTNB, 5,5′-thiobis(2-nitrobenzoic acid); TNB, 5-thio-2-nitrobenzoic acid; GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GSH-px, glutathione peroxidase; TBA, thiobarbituric acid; MDA, malondialdehyde; DMSO, dimethylsulfoxide; CuOOH, cumene hydroperoxide; MeCh, methacholine.

hydroperoxide.

MATERIALS AND METHODS

Chemicals and drugs

Oxidized and reduced glutathione were obtained from Boeringer Mannheim GmbH (Mannheim, F.R.G.). Glutathione reductase (type III, from baker's yeast), xanthine oxidase (grade I, from buttermilk), cytochrome *c*, NADPH, xanthine, *N*-ethyl-maleimide, 5,5′-thiobis(2-nitrobenzoic acid) (DTNB), *o*-phthaldehyde, thiobarbituric acid (TBA), butylated hydroxytoluene, cumene hydroperoxide and methacholine were purchased from Sigma Chemical Co. (St. Louis, MO). 2-Vinylpyridine was obtained from Janssen (Beersse, Belgium), and was distilled prior to use. All other chemicals were from Merck (Darmstadt, F.R.G.) or from Baker (Deventer, The Netherlands).

Tissue preparation

Male albino Wistar rats weighing 200–220 g (Harlan C.P.B., Zeist, The Netherlands) were killed by decapitation, and the whole gastrointestinal tract was removed and placed in a physiological salt solution at 0°. After rinsing the intestine from its contents the GI-tract was divided into the stomach, the small intestine, the cecum and the colon. The small intestine was further divided into three parts, namely the duodenum, the jejunum and the ileum. The colon was also divided into three parts, a proximal, a transversal and a distal part. In order to distinguish between the mucosa and the muscle of these parts, the mucosa was scraped off with a scalpel knife. All fractions were homogenized in 50 mM phosphate buffer (pH = 7.4), containing 0.1 mM EDTA (Polytron blender and Potter Elvehjem Teflon-glass).

Determination of reduced and oxidized glutathione (GSH and GSSG) was performed in homogenates, all enzyme assays were performed in the supernatant obtained after centrifugation of the homogenates for 30 min at 90,000 *g*.

Enzyme measurements

Xanthine oxidase was determined according to Krenitsky *et al.* [19]. Measurements took place in 100 mM sodium phosphate buffer, pH 7.4, containing 0.1 mM EDTA. Molecular oxygen was used as electron acceptor, and xanthine (at 55 μM) as substrate. Activities were expressed as nmole uric acid formation per min per mg protein.

Glutathione. Homogenates were treated with tricloroacetic acid to precipitate proteins, and neutralized afterwards with NaOH, GSH and GSSG were determined enzymatically according to Griffith [20] and non-enzymatically as described by Hissin and Hilf [21].

Superoxide dismutase (SOD). The activity of SOD was determined by the method of McCord and Fridovich. The amount of SOD which inhibited the formation of reduced cytochrome *c* by 50% was defined as 1 Unit [22].

Glutathione peroxidase (GSH-px). The activity of GSH-px was measured according to Wendel [23]. Assay mixtures contained 0.21 mM NADPH, 1 mM GSH, 3 mM cumene hydroperoxide or 1 mM hydrogen peroxide as substrate, and 2 Units of glutathione reductase in 10 mM sodium phosphate (pH = 7.0) with 0.1 mM EDTA. One Unit of GSH-px activity was defined as that amount of enzyme which leads to a decrease of one μmole of NADPH per minute.

Catalase was assessed according to Aebi [24]. Enzyme activity was expressed as the rate constant of the reaction with H_2O_2 (sec^{-1}).

Protein determination. Protein was measured according to Bradford using the Biorad assay with bovine serum albumin as a standard.

Statistics. Statistical differences between different fractions were determined using Student's *t*-test.

Lipid peroxidation experiments

Rat liver microsomes, prepared according to Haenen and Bast [25], were incubated with 1 mM H_2O_2 or 1 mM cumene hydroperoxide in combination with 10 μM Fe^{2+} in 50 mM phosphate buffer (pH 7.4). Lipid peroxidation was measured as the formation of thiobarbituric acid (TBA) reactive material at various times after the start of the reaction [25]. Cytosolic fractions from the GI-tract were added to the incubations to study the protective capacity of these fractions.

Functional experiments

After rinsing the intestine from its contents, longitudinal segments of ±1 cm long from different parts of the GI-tract were mounted with 0.5 g of tension in 20 ml organ baths in Krebs–Ringer medium (composition: 118 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.3 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 6.1 mM glucose) oxygenated with 95% O_2/5% CO_2, and kept at 37° (pH = 7.4).

Contraction of the intestinal segments was induced in two ways, namely by stimulation of muscarinic receptors with methacholine or by depolarization with K^+. Following equilibration for 30 min, contractions were measured isotonically after cumulative additions of contractile agents to the organ baths. After 15 min washing, H_2O_2 or cumene hydroperoxide was added. Pretreatment with H_2O_2 was for 30 min and in the case of cumene hydroperoxide for 15 min. After this treatment the preparations were washed three times with fresh buffer for 10 min. Subsequently another concentration–response curve was obtained.

Mannitol (10 mM) or dimethyl sulfoxide (DMSO, 10 mM) was added together with H_2O_2 as a hydroxyl radical scavenger. Calculation of pD_2 values and statistics were performed using ALLFIT [26].

RESULTS

Preparation of homogenates

The result of the scraping procedure is shown in Fig. 1. From this figure it can be seen that this procedure led to a removal of the mucus and the tips of the intestinal villi. This fraction was called mucosa. The remaining "muscle" layer still contains the lower parts of the villi, and can be regarded as enriched muscle tissue. This fraction was further defined as muscle.

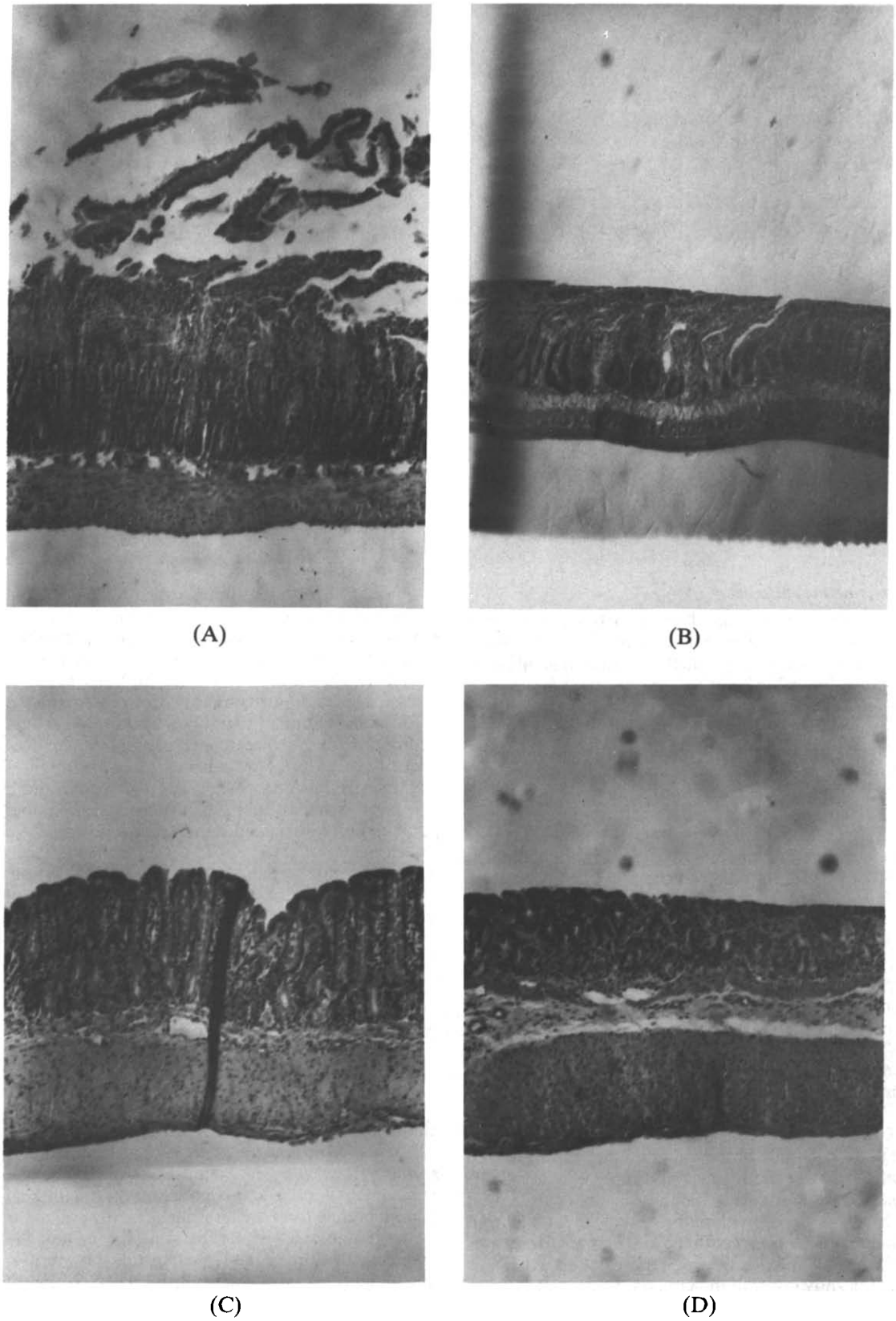

Fig. 1. Cross sections from the intestine of the rat. Photographs are taken from coupes of 7 μm treated with Haemaluin-eosine. Panel A and B represent the small intestine before (A) and after (B) scraping the mucosa. The same is shown for the large intestine (C and D). Scraping of the mucosa leads to a removal of the mucus and the tips of the intestinal villi ($\times$60).

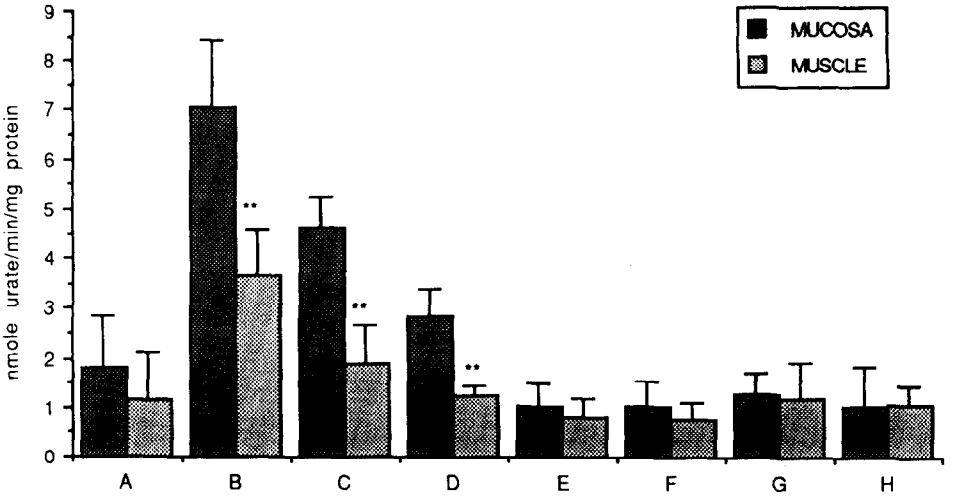

Fig. 2. Xanthine oxidase activity in the GI-tract. Mean values ± SD from 4–5 determinations are represented. Both in the mucosa and the muscle higher amounts of xanthine oxidase are found in the small intestine compared with the large intestine ($P < 0.05$). ** $P < 0.005$ compared to the corresponding mucosal fraction. Denomination: A = stomach, B = duodenum, C = jejunum, D = ileum, E = cecum, F–H = colon (proximal to distal).

Enzyme measurements

Figure 2 shows that high activities of xanthine oxidase activity are found in the mucosa of the small intestine. The activity is higher in the small intestine than in the colon, and within the small intestine it decreases from duodenum to ileum. Protease inhibitors, which prevent conversion of xanthine dehydrogenase to xanthine oxidase during homogenation, have not been used in this assay. This implies that the xanthine oxidase levels found in this study reflect both xanthine dehydrogenase and xanthine oxidase.

For GSH and SOD a similar gradient is seen. Relatively high amounts are found in the stomach. In the small intestine both the GSH and SOD contents are lower. Increasing amounts of both factors are observed going from duodenum to the distal colon. In the muscle a less marked gradient is seen (Figs 3 and 4). GSSG levels are much lower in each segment than the GSH levels. The results are presented in Fig. 3. Both the enzymatic and the non-enzymatic method roughly give the same picture, although in the stomach and the cecum higher GSSG amounts were found using the non-enzymatic method (data not shown).

In the small intestine, both glutathione peroxidase (GSH-px) and catalase are predominant in the muscle fractions. In the colon no differences can be seen between the mucosa and muscle as for GSH-px and catalase. Also for these factors the highest activity is found within the stomach. Going from duodenum to distal colon the GSH-px level increases within the muscle, whereas the catalase activity is significantly lower in the large intestine compared to the small intestine. In the mucosa however, the amount of catalase increases from duodenum to the colon. The results are presented in Figs 5 and 6.

Lipid peroxidation experiments

In phosphate buffer, no lipid peroxidation was observed in liver microsomes with 10 μM Fe^{2+} and 1 mM hydrogen peroxide after 30 min. Addition of mucosal cytosolic fractions (4 mg protein) from the small intestine led to an increase in lipid peroxidation, as shown in Fig. 7. This increase was also found in incubations without hydrogen peroxide.

The combination of 10 μM Fe^{2+} and 1 mM cumene hydroperoxide led to rapid lipid peroxidation in liver microsomes, which was maximal after five minutes and then remained constant for the next 30 min. Effects of different fractions of the GI-tract on lipid peroxidation by iron and cumene hydroperoxide are shown in Fig. 8. Mucosal fractions from the small intestine protect against lipid peroxidation, and the protection increases when higher amounts of cytosol from the mucosa are added. Mucosal fractions from the large intestine and muscular cytosolic fractions did not protect against lipid peroxidation. Only the cytosol from the duodenal muscle protects against lipid peroxidation to the same extent as the mucosal fraction.

Functional experiments

Methacholine contraction. Segments from the small intestine showed a marked spontaneous motility with an irregular frequency and amplitude. Segments of the colon also displayed a spontaneous motility, however with a much lower frequency. Methacholine induced a contraction of longitudinal smooth muscle from the intestine. The pD_2 value for methacholine was roughly the same throughout the small intestine but in the large intestine a lower pD_2 was found for methacholine (Table 1). Addition of H_2O_2 resulted in a transient contraction, which lasted for about one minute. This contraction was followed by a slowly developing relaxation, which reversed after about 15 min. Upon washing this rebound effect after the relaxation was abolished. Cumene hydroperoxide also showed a relaxation after a shortlasting

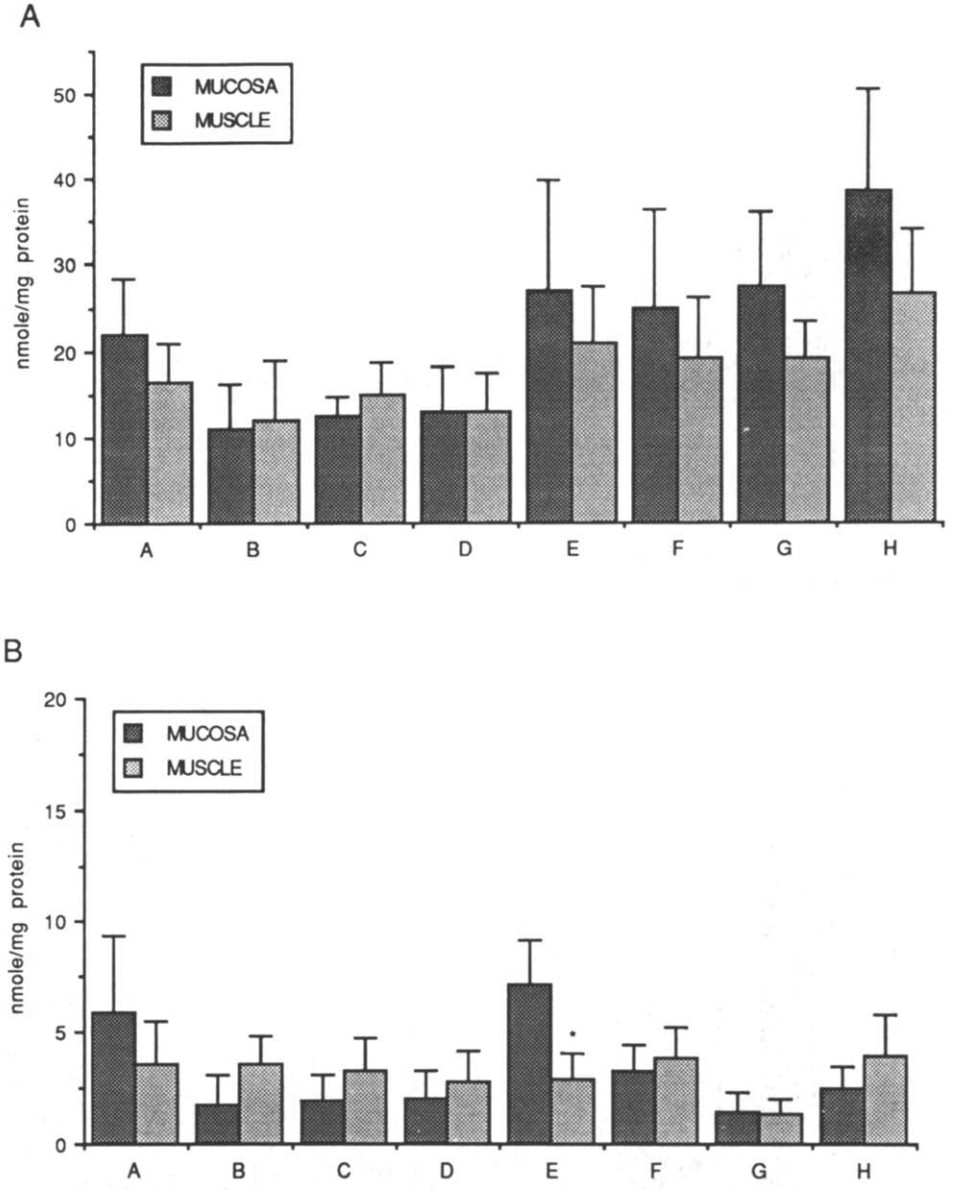

Fig. 3. Total glutathione (A) and GSSG (B) in the GI-tract. Both factors were expressed as GSH. Mean values ± SD from 5–7 determinations are shown. Significant higher amounts of total GSH are found in the distal colon compared with the small intestine ($P < 0.05$) both within the mucosa and the muscle. No differences were found in GSH levels in the mucosa compared with those in the muscle. A significant higher amount of GSSG was found in the cecum compared with the rest of the GI-tract. * $P < 0.05$ compared with the corresponding mucosal fraction. Denomination A–H as in Fig. 2.

contraction. Moreover the spontaneous motility was attenuated by these peroxides (not shown). After pretreatment with H_2O_2 (1 mM for 30 min) or cumene hydroperoxide (0.5 mM for 15 min) the methacholine response diminished. The dose–response-curve for methacholine shifted to the right, and also the maximal contraction decreased as shown in Fig. 9 and Table 1. These results show further that the damage by H_2O_2 or cumene hydroperoxide is larger in the colon than in the small intestine. Within the small intestine the jejunum was damaged most by pretreatment with cumene hydroperoxide. The damage by H_2O_2 was roughly the same throughout the small intestine.

Depolarization with K^+. In order to establish whether the diminished contraction after H_2O_2 or cumene hydroperoxide pretreatment is caused by an alteration of the receptor system or by a less specific effect, the effects of H_2O_2 or cumene hydroperoxide on contraction after K^+ depolarization was studied. For this purpose the K^+ concentration in the organ bath was elevated by cumulative addition of KCl. In the small intestine the maximal contraction after depolarization with K^+ was lower than after receptor

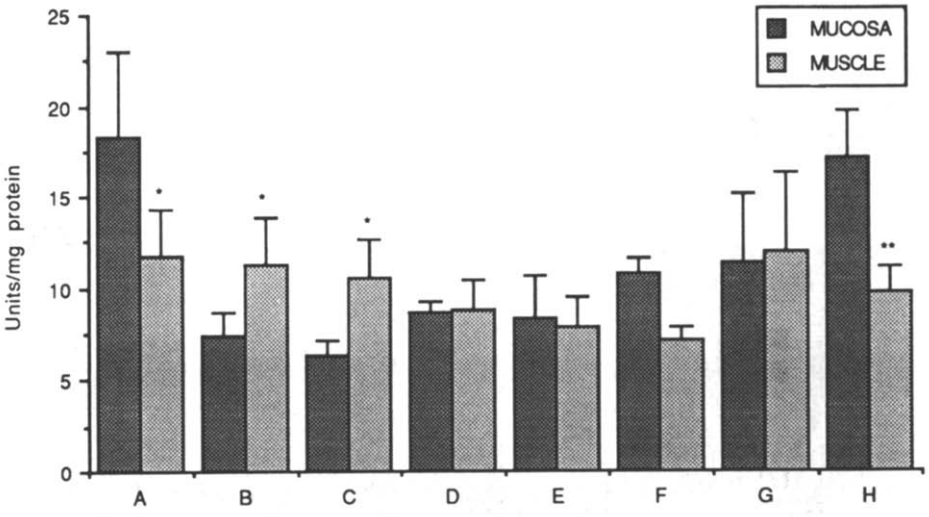

Fig. 4. SOD activity within the GI-tract. Mean values ± SD from 5–7 determinations are presented. In the mucosa SOD levels in the colon are higher than in the small intestine ($P < 0.05$). In the muscle no such differences are observed. * $P < 0.05$ compared to the mucosa from the same section. Denomination A–H as in Fig. 2.

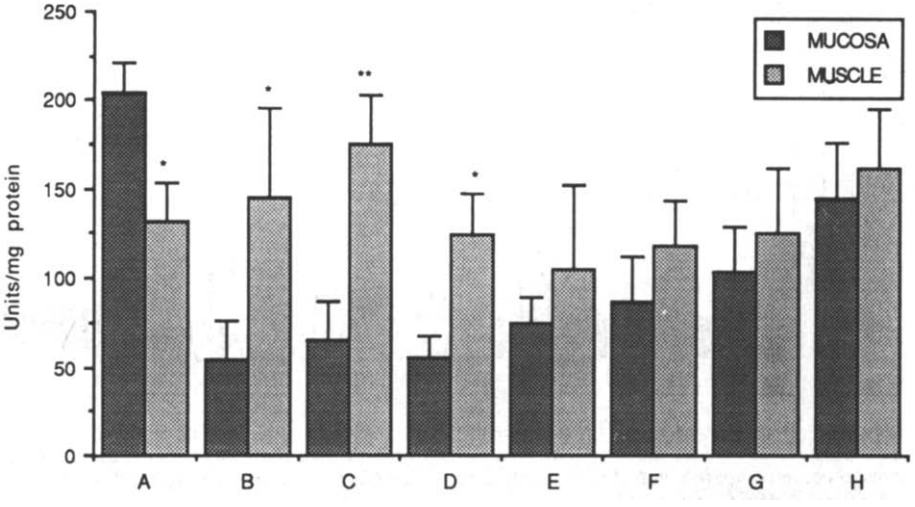

Fig. 5. GSH-px activity in the GI-tract. Mean values ± SD of 5–8 determinations with 3 mM cumene hydroperoxide as substrate are given. When hydrogen peroxide was used as substrate, similar activities were observed, suggesting that the presented data reflect the amount of the Se-dependent GSH-peroxidase. Within the mucosa a significantly higher GSH-px activity is found in the distal colon than in the small intestine. No such differences are found in the muscle. * $P < 0.05$ and ** $P < 0.005$ compared with the mucosa of the same section. Denomination A–H as in Fig. 2.

stimulation, namely about 80% (Fig. 10). The contraction via depolarization with K^+ decreased after pretreatment with H_2O_2 or cumene hydroperoxide. Also in this case the damage was larger in the colon than in the small intestine (Fig. 10).

In the presence of mannitol or DMSO (both at a concentration of 10 mM) no significant protection was observed against H_2O_2 pretreatment (not shown).

DISCUSSION

Within the small intestine xanthine oxidase is mainly present in the mucosa, whereas in the colon the enzyme is equally distributed among the mucosa and the muscle. The xanthine oxidase activity decreases from duodenum to ileum as found by Parks *et al.* [10]. Xanthine oxidase levels were lower in the large intestine which has also been found by

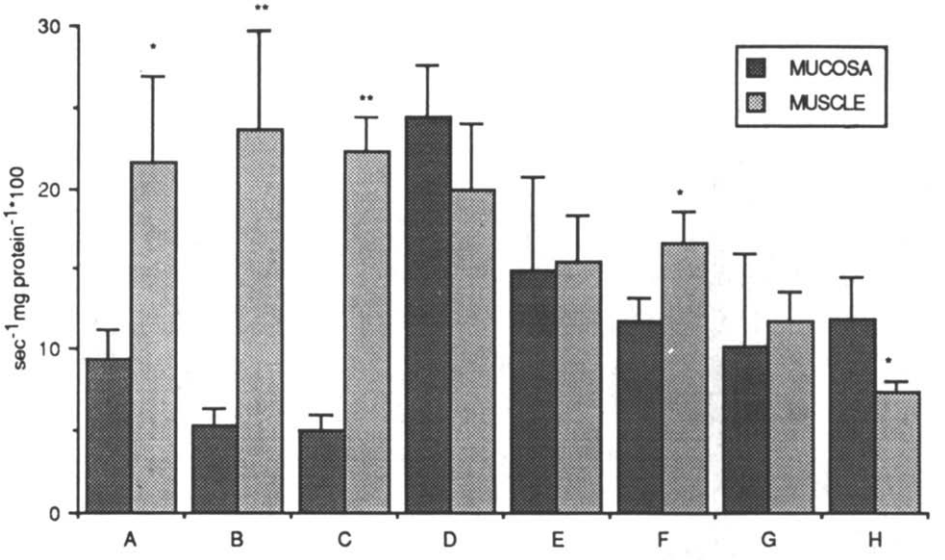

Fig. 6. Activity of catalase in the GI-tract. Mean values ± SD of 4–8 determinations are presented. Catalase activity was expressed as the rate constant of the first-order reaction with H_2O_2. In the muscle fraction from the distal part of the colon the catalase activity was significantly lower than in the small intestinal muscle. The mucosa from the colon however contains more catalase than the mucosa from the duodenum and jejunum ($P < 0.05$). * $P < 0.05$ and ** $P < 0.005$ compared with the mucosa of the same section. Denomination A–H as in Fig. 2.

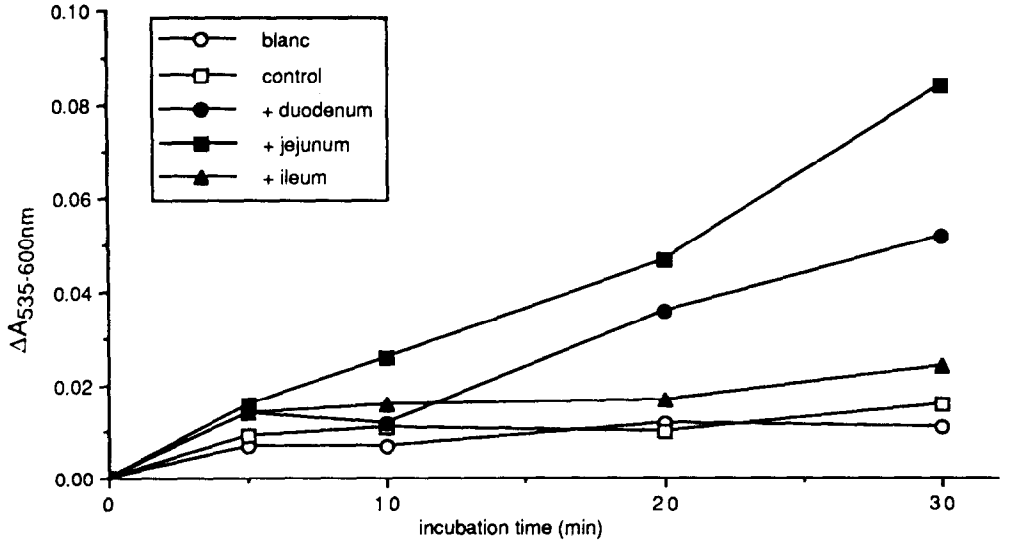

Fig. 7. Malondialdehyde (MDA) formation in rat liver microsomes after incubation (blanc) and after incubation with 1 mM H_2O_2 and 10 μM Fe^{2+} (control). Addition of mucosal cytosol fractions, containing 4 mg of protein from different parts of the small intestine led to an increased MDA formation. Data points represent the means of determinations in duplicate.

Ramboer [27]. This finding may explain why the small intestine and especially the mucosa is very vulnerable to ischemic damage. Xanthine oxidase, which is localized predominantly in the mucosa, is an important factor in oxidant damage [9].

No differences were observed in GSH amounts between the mucosa and muscle of the GI-tract. Relatively high amounts were established in the stomach and the large intestinal fractions, while the small intestine contained lower amounts. Literature data so far show contradictory results. In the mucosa, a similar gradient like that observed in this study has

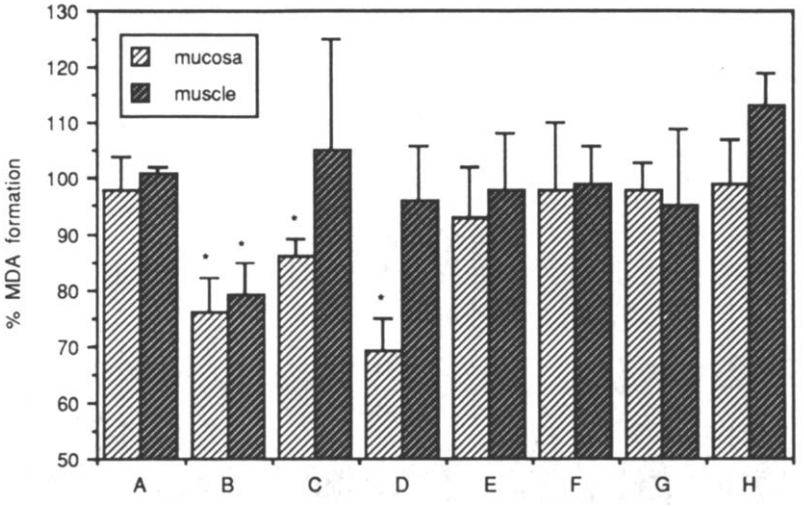

Fig. 8. Effects of cytosol fractions from the GI-tract (containing 3 mg of protein) on MDA formation induced by 1 mM cumene hydroperoxide and 10 μM Fe^{2+} in rat liver microsomes (incubation for 10 min). Mean values ± SD of 2–4 determinations are given. Only the cytosol fractions from the small intestine mucosa and the duodenal muscle significantly diminished formation of TBA-reactive material ($P < 0.05$). Denomination A–H as in Fig. 2.

Table 1. Effects of pretreatment with H_2O_2 or cumene hydroperoxide (CuOOH) on the pD_2 of methacholine (A) and the maximal contraction by methacholine (B) in longitudinal intestinal smooth muscle preparations from the rat. The pD_2 values after pretreatment were significantly different from control values in each case ($P < 0.05$). Only after pretreatment with H_2O_2 the maximal contraction was significantly diminished

A	Control	H_2O_2 (1 mM)	CuOOH (0.5 mM)
Duodenum	6.10 ± 0.12	5.24 ± 0.15	5.15 ± 0.22
Jejunum	6.20 ± 0.13	5.10 ± 0.31	5.15 ± 0.27
Ileum	6.39 ± 0.20	5.63 ± 0.36	5.71 ± 0.16
Proximal colon	5.93 ± 0.67	3.95 ± 0.64	—
Transversal colon	6.04 ± 0.20	4.94 ± 0.18	—
Distal colon	5.94 ± 0.33	4.39 ± 0.75	—
B	**Control**	**H_2O_2 (1 mM)**	**CuOOH (0.5 mM)**
Duodenum	100	64.6 ± 6.5	95.7 ± 9.0
Jejunum	100	65.1 ± 17.3	78.6 ± 13.7
Ileum	100	67.4 ± 11.3	121.3 ± 14.4

been described by Siegers *et al.* [28], whereas Chen *et al.* documented a higher glutathione level in the small intestine compared with the large intestine [29]. GSSG levels did not vary much within the GI-tract, and no large deviations are found in the GSH/GSSG ratio, which suggests that the oxidant/anti-oxidant status was roughly constant throughout the GI-tract.

Relatively high SOD levels were established in the mucosa of the stomach and the distal colon, while the SOD activity was significantly lower in the mucosa from the small intestine. These findings are in contrast with the observations by Loven *et al.*, who demonstrated higher SOD activities in the mucosa from the small intestine compared with the large intestinal mucosa [30]. A less marked gradient in SOD activity was seen in the muscular fractions from the GI-tract. Also variations were observed for GSH-px activity in the GI-tract. Again the highest activity was measured in the stomach. From the duodenum to the distal colon the activity increased slightly, which affirms data from Siegers *et al.* [28]. Only in the small intestine a significant difference could be noticed between GSH-px levels in the mucosa and muscle.

The longitudinal gradient for catalase was roughly the same as that for GSH-px. The main difference is seen in the muscle fractions where a higher catalase activity was demonstrated in the small intestine compared with the colon.

Roughly we can conclude that the colon is better equipped with GSH, GSH-px and SOD compared

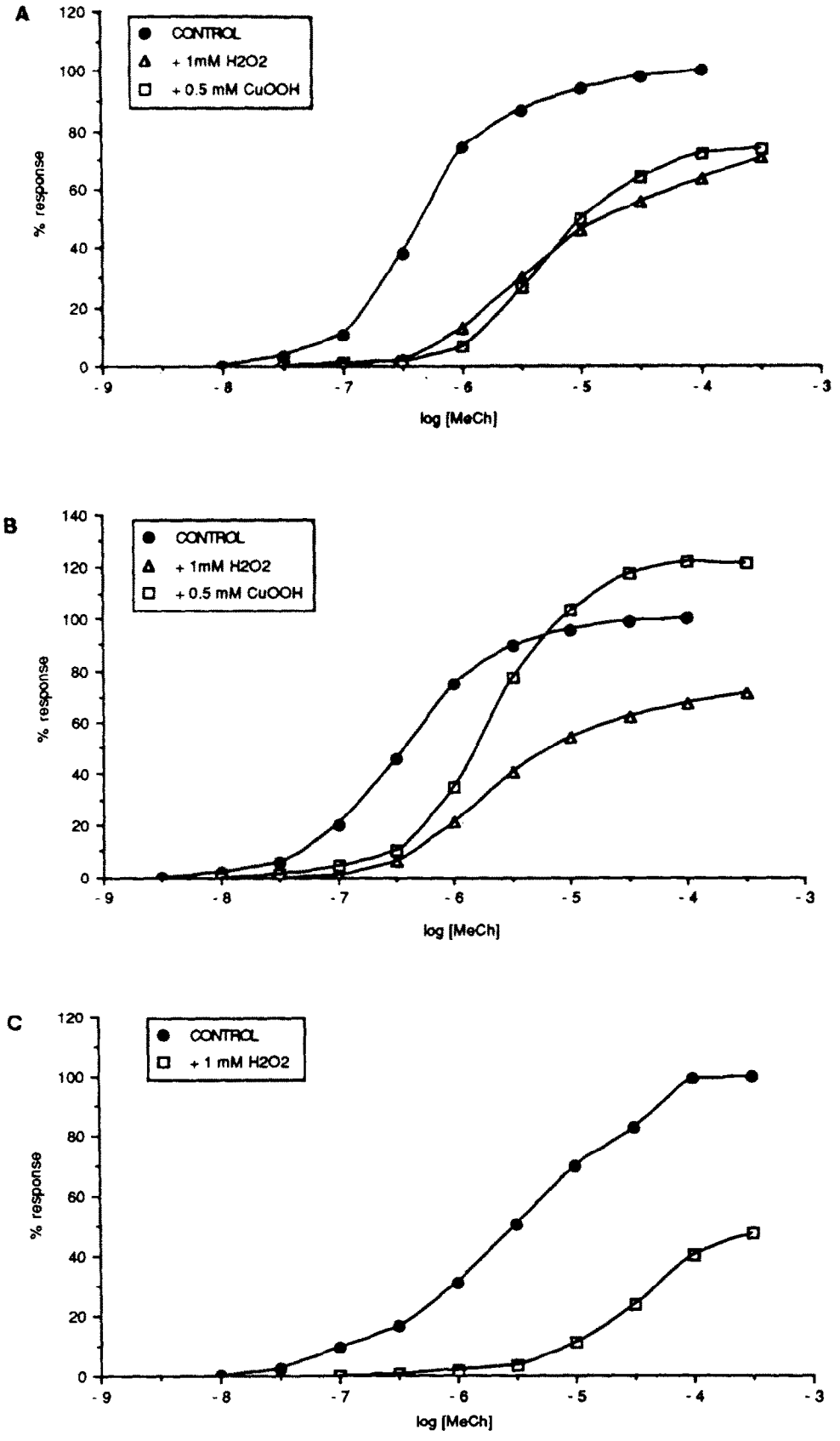

Fig. 9. Concentration–response-curves of methacholine on jejunum (A), ileum (B) and proximal colon (C) of the rat, before (CONTROL) and after pretreatment with H_2O_2 (1 mM) and CuOOH (0.5 mM).

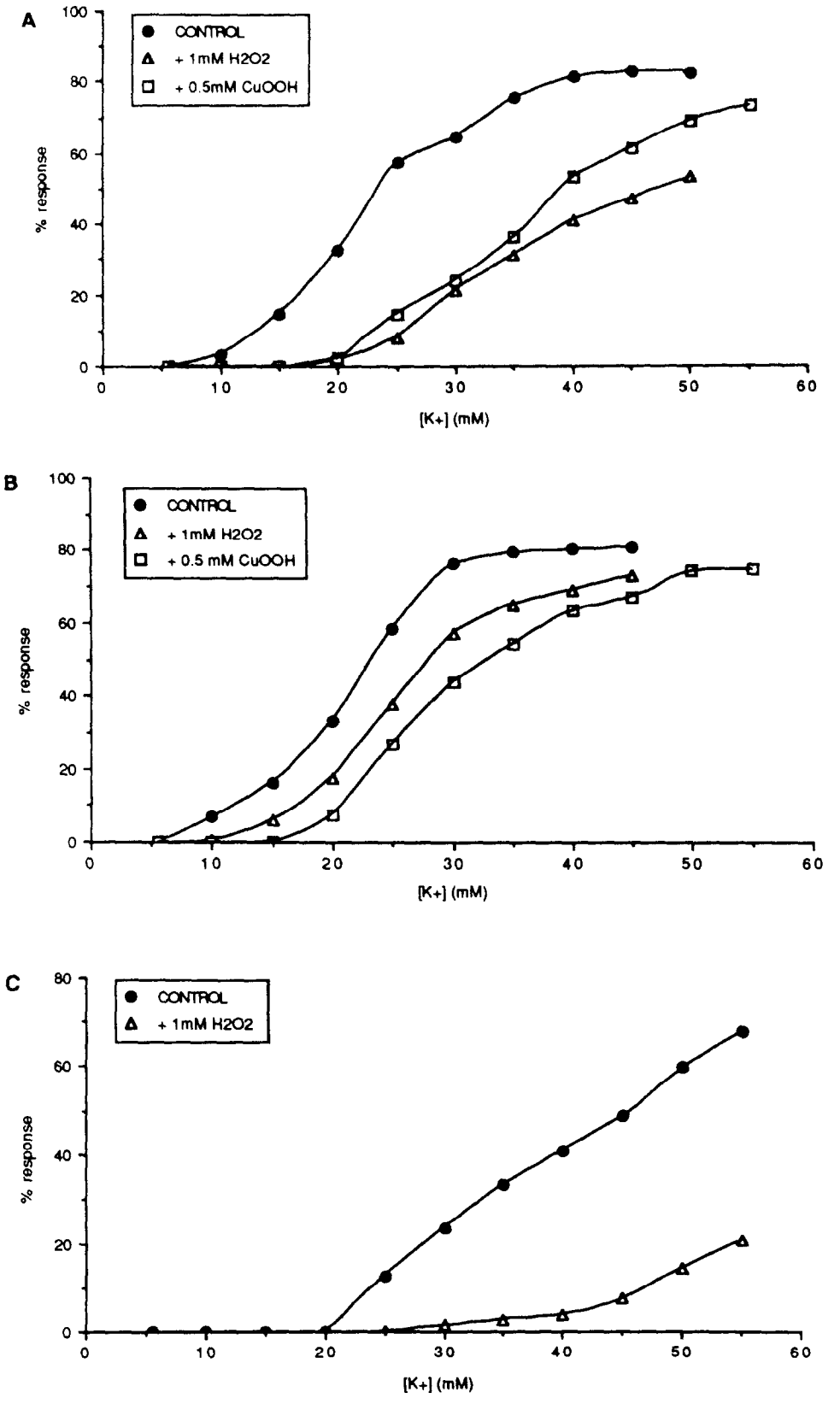

Fig. 10. Contraction of longitudinal segments of rat jejunum (A), ileum (B) and proximal colon (C) of the rat after depolarization with K^+, before (CONTROL) and after pretreatment with H_2O_2 (1 mM) and CuOOH (0.5 mM).

with the small intestine. For catalase the opposite seems to be the case. It is also striking that within the small intestine SOD, GSH-px and catalase levels are higher in the muscle than the mucosa. In this regard it should be recognized that these muscle fractions also contained the lower parts of the villi, and can therefore not be seen as pure muscle preparations. Taken together, these enzyme measurements suggest that the small intestine is most vulnerable to oxygen radical damage. GSH, SOD levels are significantly lower in the small intestine as compared with the large intestine. Moreover xanthine oxidase, involved in production of oxygen radicals and suggested to be of paramount importance in development of ischemic damage [9], prevailed in the small intestine. Further results did not corroborate this suggestion (vide infra).

After incubation of rat liver microsomes with H_2O_2 and Fe^{2+}, no lipid peroxidation could be measured as TBA reactive material. Addition of mucosal fractions from the small intestine led to a formation of TBA reactive material which increased slowly. This might be due to the presence of reductants like ascorbic acid which, together with Fe^{2+}, can lead to lipid peroxidation. Lipid peroxidation induced in rat liver microsomes by cumene hydroperoxide and Fe^{2+} was only inhibited by mucosal fractions from the small intestine. These findings can not be attributed to the presence of the enzymes measured in this study. Perhaps other factors are involved in the protection against lipid peroxidation, like the lipid-soluble factor identified by Diplock *et al.* [31]. Moreover, the mucosa from the small intestine is covered with a substantial amount of mucus, a mixture of glycoprotein polypeptides which has also antioxidant properties [32, 33] and is believed to play an important role in the protection of the intestinal wall against oxygen radical damage. Perhaps the mucus is responsible for the protection against lipid peroxidation in these studies. However the protection induced by the muscle fraction from the duodenum cannot be attributed to the presence of mucus, so at least in this case other factors are involved.

We further studied the effects of reactive oxygen species on the longitudinal smooth muscle function in functional experiments. H_2O_2 and cumene hydroperoxide affected the basal tone of the intestinal preparations. First a shortlasting contraction was observed, followed by a slow relaxation, which in the case of H_2O_2 was followed by a slow contraction. Contraction of smooth muscle by hydrogen peroxide has also been reported in airways [34]. This contraction was hypothesized to be caused by prostaglandins. In vascular smooth muscle a relaxation is induced by peroxides. This phenomenon was explained by a stimulation of guanylate cyclase by these peroxides [35]. This can also account for the relaxation found in our study. Also the spontaneous motility was diminished by these peroxides. The mechanism of this process is unknown and under further investigation. Pretreatment of intestinal preparations with H_2O_2 or cumene hydroperoxide resulted in a decreased methacholine response which was expressed in a lower pD_2 for methacholine and in some cases a decrease in the maximal response. The damage by these peroxides was larger in the colon which is in accordance with the results from the lipid peroxidation studies in which less protection by colon cytosolic fractions on formation of TBA reactive material was found. Especially the effects observed with cumene hydroperoxide correlate very well with the lipid peroxidation studies, in which the largest protection was found in the mucosa from the duodenum and the ileum, and a slightly less marked protection in the jejunum. This can be explained by the assumption that damage by cumene hydroperoxide is focussed on membranes because of its lipophilicity. The results obtained with hydrogen peroxide can also be explained by the different amounts of catalase present in different segments of the intestine. Damage after H_2O_2 can also be expected in the cytosol, because it easily crosses the plasma membrane. Mannitol or DMSO (both at a concentration of 10 mM) failed to affect the altered response after H_2O_2 pretreatment, so the damage by H_2O_2 is not likely due to hydroxyl radicals. Cumene hydroperoxide and H_2O_2 seemed to have similar harmful effects on contractions via depolarization with K^+ compared with the effects on methacholine contraction, which implies that the muscarinic receptor or the receptor system is not specifically damaged by 1 mM H_2O_2 or 0.5 mM cumene hydroperoxide. Probably the membrane function or the contractile apparatus is damaged by these pretreatments.

In conclusion these studies show that the colon is most vulnerable to treatment with H_2O_2 or cumene hydroperoxide, which can be explained by the amounts of catalase present and also correlates with the protective capacity against lipid peroxidation. This could imply that the motility is disturbed to the strongest extent in the colon during processes in which oxygen radicals and also H_2O_2 are formed.

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