INDOMETHACIN AND PROSTAGLANDIN EFFECTS ON ABSORPTION, RETENTION AND URINARY EXCRETION OF ^{51}CHROMIUM

By

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CHAPTER I

RESEARCH PROBLEM

Introduction

Chromium is an essential trace mineral required for carbohydrate and lipid metabolism in rats as well as in humans. Chromium deficiency can result in elevated blood cholesterol in addition to glucose intolerance (Mertz, 1993).

In the United States, 50-200 μg/day of chromium is recommended as the estimated safe and adequate intake for adults (NRC, 1989). In the late 1970s estimated mean chromium intake in the United States ranged from 25-89 μg/day (Kumpulainen et al., 1979). However, lower values have since been recorded using improved analytical techniques.

According to a study by Anderson and Kozlovsky (1985), when self-selected diets were analyzed for chromium content, most of the diets contained less than 50 μg/day. Bunker et al. (1984) also reported similar results from their study.

In 1966, Glinsmann and Mertz showed that in some cases of diabetes mellitus, chromium supplementation of 150 to 200 μg/day taken for at least three months improved glucose utilization. Contrary to that, Sherman et al. did not find improvement in the utilization of glucose with chromium supplementation in individuals with diabetes (Sherman et al., 1968).

Chromium in foods is generally decreased with processing (Wolf et al., 1974). Since chromium is needed for the metabolism of carbohydrate, foods low in chromium may deplete chromium from tissue stores and cause it to be excreted in the urine (Schroeder, 1968). Thus consumption of highly processed foods may result in increased excretion of chromium in urine as well as in depletion of body chromium stores (Mertz et al., 1974). The elderly are particularly at risk of deficiency because of their increased consumption of processed foods as well as their altered metabolism due to aging (Exton-Smith, 1972).

Glucose tolerance declines with aging (Jackson et al., 1982). Schroeder et al. (1962) showed that in humans, tissue chromium levels decreased as they grew older. Confirmation of this work is needed using newer analytical methods. According to Offenbacher (1992), chromium metabolism is altered in the elderly with decreased retention and increased urinary losses. Offenbacher and Pi-Sunyer (1980) suggested that some of the glucose intolerance in the elderly may be the result of chromium deficiency. Mertz (1993) concluded that severe chromium deficiency may contribute to diabetes and atherosclerosis in the elderly.

In the United States, the elderly are at risk for drug-nutrient interactions because of their excessive use of over-the-counter (OTC) as well as prescribed medications (Roe, 1986). Spicer et al. (1992) reported that ^{51}Cr absorption was significantly reduced in rats when dosed with over-the-

counter antacid or sucralfate, a medication used for ulcer therapy. There is a concern regarding chromium status with use of over-the-counter drugs.

Davis et al. (1995) studied the effects of five over-the-counter drugs [Tums (calcium carbonate); Maalox (aluminum hydroxide and magnesium hydroxide); vitamin C; aspirin; Bufferin; or water] on ^{51}chromium utilization by rats. They found that the aspirin group had increased absorption, tissue retention and urinary excretion of ^{51}chromium. The Maalox group had the lowest chromium absorption, lowest retention and lowest urinary excretion while the group dosed with Tums was the next lowest among the six groups. This study showed that some OTC drugs, particularly antacids, reduced chromium status of rats.

Drugs can affect nutritional status of the elderly especially when the dietary intake is marginal (Roe, 1985). In elderly patients, mineral depletion is the most common example of drug induced nutritional deficiencies (Roe, 1986). Unfortunately, data on drug-chromium interactions in humans are not available.

The nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin and indomethacin (which was used in the present study) are commonly used for rheumatoid and osteoarthritis. NSAIDs produce gastric ulcers in 20% of patients, who use them extensively for arthritis (Uribe, 1993). These analgesics can cause gastrointestinal (GI) ulcers as well as bleeding by inhibiting endogenous prostaglandin synthesis in

humans (Johansson et al., 1980) and in rats (Takeuchi et al., 1986). Thus long-term intake of NSAIDs can cause iron deficiency anemia by inducing blood loss from the gastrointestinal tract.

Gastrointestinal prostaglandins reduce and in some cases even prevent GI damage caused by NSAIDs (Robert, 1979). Prostaglandins of the E and I types have been found in gastric as well as intestinal mucosa (Konturek & Pawlik, 1986; Miller, 1983). There are a few studies which suggest that gastrointestinal PGs have a role in absorption of minerals such as zinc (Song and Adham, 1978) and calcium (Johansson et al., 1980).

The present study was designed to determine the effects of gastrointestinal prostaglandins on chromium absorption, tissue distribution and urinary excretion. Because chromium absorption in the body is very low and accurate measurement of chromium in biological fluids is difficult, ^{51}chromium was used in this experiment. Use of a radioactive isotope of chromium allows the sensitive evaluation needed to determine the effects of various drugs on chromium absorption.

Definitions

Indomethacin - a nonsteroidal anti-inflammatory drug which inhibits prostaglandin synthesis. Used clinically for the treatment of arthritis.

Misoprostol - an analogue of PGE_1. Used clinically for the treatment of ulcers (Cytotec®).

Vehicle - material used to administer the drug (In the present study, 1% Tween 80 in 0.9% saline).

16, 16-Dimethylprostaglandin E_2 ($DMPGE_2$) - an analogue of PGE_2.

Prostacyclin (PGI_2) - a naturally occurring prostaglandin.

Purpose and Objectives

The purpose of this study was to determine the effects of prostaglandins on chromium absorption, tissue distribution and urinary excretion. The following objectives were developed for this study:

1. To determine the effect of indomethacin, a blocker of prostaglandin synthesis, on ^{51}Cr absorption, retention and urinary excretion.
2. To determine the effects of misoprostol(PGE_1 analogue), 16, 16-Dimethylprostaglandin E_2 (16, 16-$DMPGE_2$), or prostacyclin (PGI_2) on ^{51}Cr absorption, retention and urinary excretion in adult male rats.

Hypotheses

The following hypotheses were developed for this study:

1. There will be no significant effect of intraperitoneally injected indomethacin, a blocker of prostaglandin synthesis, on ^{51}Cr absorption, retention or urinary excretion.
2. There will be no significant effects of intubated doses of misoprostol, 16, 16-Dimethylprostaglandin E_2, or prostacyclin on ^{51}Cr absorption, retention or urinary excretion.

Assumption

It is assumed that intraperitoneally injected indomethacin (5 mg/kg body wt), a prostaglandin inhibitor, has completely blocked the synthesis of endogenous prostaglandins.

Limitations

1. The bioavailability of ^{51}Cr from $CrCl_3$ may be different from dietary chromium.
2. Because of the use of animal models in this experiment, the results cannot be extrapolated directly to humans.

CHAPTER II

REVIEW OF LITERATURE

Chromium

In 1959, chromium was found to be an essential trace mineral required for the utilization of glucose in rats (Schwarz and Mertz, 1959). When laboratory rats were fed a chromium deficient diet, they developed a diabetes-like condition. The condition was reversed when chromium was reintroduced (Schroeder, 1966). In addition to impaired glucose tolerance, severe chromium deficiency also caused stunted growth, high mortality in young animals, shortened life span and increased incidence of aortic atherosclerosis in laboratory animals (Hambidge, 1978).

Jeejeebhoy et al. in 1977 found that trivalent chromium was necessary for carbohydrate metabolism in humans, as well as in animals, when diabetes-like symptoms were reversed with the administration of chromium in a 30-year old patient receiving total parenteral nutrition. Many of the above mentioned chromium deficiency symptoms also are common in humans (Jeejeebhoy, 1977).

In the United States, 50-200 μg/day of chromium is recommended as the estimated safe and adequate intake for adults (NRC, 1989). Anderson and Kozlovsky (1985), reported that when self-selected diets were analyzed for chromium content, most of the diets contained less than 50 μg/day.

Chromium in foods generally is decreased with processing (Wolf et al., 1974). The elderly are particularly at risk of deficiency because of their increased consumption of processed food as well as their altered metabolism due to aging.

Hopkins et al. (1968) showed improvement in glucose tolerance in children with protein-calorie malnutrition with the introduction of chromium. Gurson and Saner (1971) supported this finding.

There is controversy about improved glucose utilization with chromium supplementation in some cases of diabetes. According to Glinsmann and Mertz (1966), in some cases of diabetes mellitus including juvenile and maturity onset, chromium supplementation improved glucose utilization. But, according to Sherman et al., individuals with diabetes did not benefit from chromium supplementation of 50 μg of trivalent chromium three times daily taken for 16-weeks (Sherman et al., 1968).

Levine and coworkers (1968) investigated whether elderly subjects with abnormal glucose tolerance benefitted from chromium supplementation. They suggested that chromium supplementation improved glucose tolerance in some of the elderly subjects. However Doisy and colleagues (1971) did not find significant difference in the absorption of chromium between young and elderly subjects and the question of age effects on chromium utilization has not been resolved.

Doisy et al. (1968) were early investigators of the absorption of chromium in humans. They reported that when $CrCl_3$ was used, the absorption of chromium was about 1%. In 1986, Offenbacher and coworkers (1986) reported that the net absorption of chromium in subjects in a metabolic unit was 1.8%.

Absorption of chromium mainly depends on the form of chromium. Trivalent chromium is not as well absorbed as is hexavalent chromium, but trivalent chromium is more prevalent in foods (Mertz et al., 1974). Absorption of chromium is greatest in the jejunum (Chen et al., 1973). Chromium is also absorbed in the ileum and duodenum, the other two sections of the small intestine (Chen et al., 1973).

Absorption of chromium is influenced by various factors. Chen et al. (1973) studied the effects of chelating agents such as oxalate and phytate on chromium. Oxalate significantly increased chromium absorption. They suggested that oxalate may chelate with chromium and prevent it from undergoing olation. Formation of soluble complexes in the small intestine may improve the absorption of chromium. Phytate significantly decreased chromium absorption. Chen and coworkers suggested, that by binding chromium and forming insoluble complexes, phytate may have reduced the availability of chromium (Chen et al., 1973).

Hahn and Evans (1975) suggested that minerals such as chromium and zinc may share a common absorption pathway in the intestine. In zinc deficient rats, absorption and

intestinal content of the chromium increased. Also, chromium blocked the absorption of zinc and reduced intestinal zinc content. When zinc was reintroduced orally, enhancement of chromium absorption was stopped in those rats.

Chromium absorption, like the absorption of other minerals including iron, depends on prior chromium intake. In a study reported by Anderson and Kozlovsky (1985), chromium absorption in humans was inversely related to dietary chromium intake. At a dietary chromium intake of 10 μg, absorption was approximately 2%; as intake increased to 40 μg, chromium absorption decreased to 0.5%.

Absorbed chromium is excreted mainly via the kidneys (Mertz, 1969). Very little is lost in hair, perspiration or bile. Urinary chromium reflects recent chromium intake and is considered a good indicator of the amount of chromium absorbed (Anderson and Kozlovsky, 1985).

Prostaglandins

Prostaglandins (PGs) are a group of 20-carbon oxygenated fatty acids that are present in most mammalian cells and tissues (Robert, 1979). Prostaglandins were discovered in the early 1930's in human seminal fluid, where they were found in high concentration (Miller, 1983).

Gastrointestinal (GI) mucosal cells also have a relatively large quantity of PGs (Robert, 1979). Prostaglandins of E and I types have been found in gastric as well as intestinal mucosa and, following stimulation, are secreted into the

lumen (Konturek and Pawlik, 1986; Miller, 1983). They affect a number of gastric as well as intestinal functions (Robert, 1979). They show vasodilation activity, specifically in gastric mucosa. Both PGE_2 and PGI_2 are very potent gastroprotective prostaglandins (Bennett, 1989).

The mechanism by which PGs protect GI mucosa is still not fully understood. One of the functions of PGs, cyto-protection, involves protection of the GI tract against damage caused by various ulcer causing and necrotizing agents (Lacy, 1985). The dose at which PGs exhibit cytoprotective property is called the cytoprotective dose. Although many prostaglandins have cytoprotective properties, their potencies vary (Robert, 1979). For a potent prostaglandin, the cytoprotective dose is much lower than that of the less potent prostaglandin.

Certain PGs, such as PGs of E type, which possess cytoprotective properties even when given in very low doses, can protect the GI tract against damage (Miller, 1983). The cytoprotective property exhibited by PGs is different from the antisecretory property (Robert, 1979). The dose at which prostaglandins inhibit gastric acid secretion, as a reaction to the substances which produce gastric damage, is called the antisecretory dose (Robert et al., 1979). According to Robert (1979), some PGs showed cytoprotective properties with even as little as 1/100th of the antisecretory dose.

Mucus secretion is one of the other important mechanisms by which PGs protect GI mucosa. Some studies report that PGs

and their analogues such as 16, 16-Dimethylprostaglandin E_2 (16, 16-$DMPGE_2$) stimulate the release of mucus into gastric juice in animals (Bolton and Cohen, 1978; Tao and Wilson, 1984).

Several other mechanisms have been proposed for gastrointestinal mucosal defense. Stimulation of mucus and alkaline secretion, stimulation of mucosal growth, stabilization of lysosomal enzymes, and inhibition of gastric acid secretion are other possible actions (Konturek and Pawlik, 1986; Miller, 1983). Another theory is that prostaglandins such as the A, E and I types may protect against mucosal damage by increasing mucosal blood flow (Miller, 1983). But, some researchers have suggested that a cytoprotective prostaglandin such as 16, 16-$DMPGE_2$ does not increase gastric mucosal blood flow even in a cytoprotective dose (Jacobson, 1985)

Synthesis of PGs from membrane phospholipids can be stimulated by various factors such as neural or hormonal stimulation, neurotransmitters or hyperosmolar solutions (Konturek and Pawlik, 1986). Under physiological conditions, mechanical strain such as stretching a blood vessel or contraction of the intestine can increase PG formation. Tissue injuries may be another cause of PG formation (Konturek and Pawlik, 1986).

Nonsteroidal antiinflammatory drugs (NSAIDs) are potent inhibitors of prostaglandins. They can produce gastric damage including ulcers in animals and humans. But

supplemental prostaglandins even at very low doses (nonantisecretory doses) are useful in preventing or reducing NSAID induced mucosal damage in animals and humans (Wilson, 1986). Arthritic patients on long-term treatment with NSAIDs are often supplemented by prostaglandins or their analogues.

Indomethacin

According to Graham et al., 20% of patients who use NSAIDs regularly develop gastroduodenal ulcers (Graham et al., 1988). Indomethacin is a NSAID which inhibits the synthesis of prostaglandins. By blocking the synthesis of prostaglandins, NSAIDs induce gastrointestinal mucosal damage and thereby cause gastrointestinal ulcers in experimental animals (Whittle, 1976) and humans (Johansson et al., 1980).

Indomethacin, a very potent NSAID, produces intestinal lesions at 7.5 mg/kg body wt in rats (Robert, 1979). Takeuchi et al. (1986) found that 5 mg/kg indomethacin reduced PGs by 80-90% in gastric mucosa 30 minutes after administration without the mucosal damage.

Because PG's protect the GI mucosa, ulcer patients may benefit from the administration of PG's prior to NSAIDs (Yik et al., 1982). Johansson et al. (1980) reported that 1 mg of PGE_2 taken orally three times daily with indomethacin treatment reduced GI bleeding in patients with rheumatic diseases.

Van Kolfschoten et al. (1983) reported that PGE_2 can protect the stomach even when a high concentration of acid is

present. They also showed that there was a small but not significant increase in bicarbonate secretion, which suggests that bicarbonate secretion may not be the important factor in mucosal protection. Van Kolfschoten and coworkers therefore suggested that there may be a mechanism to protect the gastric mucosa other than inhibition of gastric acid secretion.

Using two different doses of various NSAIDs in humans, including aspirin, ibuprofen, indomethacin and naproxen, Lanza et al. (1979) investigated whether the effects on the gastric mucosa were dose dependent. They reported that severe gastric injury was caused by aspirin, by both doses of indomethacin and by the higher dose of naproxen. Damage caused by 100 and 150 mg/day of indomethacin appeared to be lower but not significantly different from damage caused by 3600 mg of aspirin. Lesser damage occurred with the lower dose of naproxen, and with both doses of ibuprofen and placebo.

When gastric mucosal tissue of rats was pretreated with local application of exogenous PGs or with mild irritants such as 20% ethanol or 5% NaCl, mild damage to the gastric mucosa occurred. But, this pretreatment protected gastric mucosa against subsequent application of necrotizing agents. The protection against mucosal damage may have been due to increases in the synthesis of endogenous prostaglandins. This is called adaptive cytoprotection (Konturek et al., 1982; Jacobson, 1986). In one of the studies, aspirin, given

either orally or subcutaneously, appeared to be cytoprotective in rats and prevented the intestinal lesions caused by indomethacin, a stronger NSAID than aspirin (Robert, 1979).

Misoprostol

Misoprostol is a synthetic analogue of prostaglandin E_1. Like most of the prostaglandins, misoprostol has both antisecretory and cytoprotective effects (Silverstein et al., 1986). Misoprostol showed cytoprotective effects against gastrointestinal injury caused by ethanol, hydrochloric acid, sodium hydroxide, hypertonic saline and taurocholate as well as against damage from NSAIDs like aspirin and indomethacin (Robert et al., 1979).

According to Dajani and Nissen, 50 μg q.i.d. (four times a day) misoprostol significantly inhibited gastric microbleeding caused by 975 mg q.i.d. aspirin in humans. Misoprostol also significantly reduced acid and chloride secretion at 50 μg q.i.d. (Dajani and Nissen, 1985).

Misoprostol is known for its effectiveness in preventing and, in some cases, even promoting healing of gastric as well as duodenal ulcers in humans (Agrawal and Dajani 1990; Brand et al., 1985 and Graham et al., 1988). Jaszewski and coworkers (1992) reported that 200 μg of misoprostol, four times daily, significantly increased the rate of healing of NSAID induced gastric ulcers in humans.

Bauer et al. (1986) compared three different doses (50, 100, and 200 μg/kg body wt) of misoprostol with cimetidine and sucralfate (50, 100 and 200 mg/kg) for mucosal protective properties in rats. They studied these drugs in five different ulcer-promoting models (aspirin, indomethacin, stress, sodium taurocholate and ethanol). Sucralfate did not show consistent protective effects in the five ulcer models. Cimetidine showed positive effects in some of those models, but not in all while misoprostol showed protective effects in all models. They reported that misoprostol, even in the low dose of 50 μg/kg, showed gastric mucosal protection in rats.

Prostaglandins may enhance gastric defense mechanisms. When given intravenously to animals, misoprostol is a very potent inhibitor of gastric acid secretion (Dajani et al., 1976). Other proposed defense mechanisms of misoprostol include prevention of gastric mucosal barrier disruption, stimulation of mucus secretion, stimulation of alkaline secretion, and enhancement of blood flow (Miller, 1983).

Bauer could not demonstrate that misoprostol significantly increased gastric mucosal blood flow (Bauer, 1985). However, other studies showed that there was an increase in mucosal blood flow which contributed to the cytoprotective property of misoprostol (Brand et al., 1985; Dajani and Nissen, 1985; Cheung, 1980; Larsen et al., 1981).

To evaluate the gastric mucosal protective properties of misoprostol, Silverstein et al. (1986) studied whether a clinical dose of misoprostol was sufficient to protect the

gastric mucosa of humans against peptic ulcer caused by aspirin. They reported that misoprostol did protect the gastric mucosa from the damaging effects of aspirin.

Cohen et al. (1983) reported that low doses of misoprostol were effective in humans in reducing GI bleeding which was caused by aspirin. In agreement with this finding, Hunt et al. (1983) reported that significant reductions in aspirin-induced gastric bleeding and in gastric acid secretion were observed when people were treated with a dose of 50 μg of misoprostol.

Various studies of misoprostol show that this synthetic analogue of PGE_1 is a drug with cytoprotective and antisecretory effects which can be used for treatment of aspirin induced mucosal damage. Also misoprostol did not interfere with the antirheumatic activity of NSAIDs (Agrawal and Dajani, 1990). This suggests that misoprostol may be therapeutically used for the treatment of NSAID-induced GI damage.

16, 16-Dimethyl prostaglandin E_2 ($DMPGE_2$)

A synthetic analogue of endogenous PGE_2, 16, 16-Dimethyl PGE_2 ($DMPGE_2$) is a potent antiulcer drug (Robert, 1979). This analogue of PGE_2 is effective in preventing indomethacin induced GI bleeding (Johansson et al., 1980).

Kauffman and coworkers suggested that $DMPGE_2$ has the ability to stimulate bicarbonate secretion (one of the mechanisms for protecting gastric mucosa) when applied

topically to canine mucosa (Kauffman et al., 1980). Kuo et al. reported that stimulation of bicarbonate secretion by 16, 16-DMPGE$_2$ in dogs is not a direct effect of the prostaglandin on mucosal cells (Kuo et al., 1983).

One proposed mechanism, that PGs protect mucosal damage by increasing mucosal blood flow, is controversial (Miller, 1983). According to Arakawa and coworkers (1989), although 16, 16-DMPGE$_2$ given at 5 μg/kg intragastrically, in rats prevented mucosal damage caused by 20 mg/kg indomethacin, it did not have any effect on the mucosal blood flow. Some researchers likewise reported that 16, 16-DMPGE$_2$ did not increase gastric mucosal blood flow even in a cytoprotective dose (Jacobson, 1985).

Various factors affect endogenous PG synthesis. According to Bode et al. (1988), alcohol affected PG synthesis in rats. They suggested that chronic ingestion of alcohol inhibited PG synthesis and thereby caused gastric mucosal damage in rats. According to Tarnawski et al. (1985), 16, 16-DMPGE$_2$ protected the gastric mucosa against ethanol-induced lesions.

Gilbert et al. (1984) reported that in healthy individuals pretreatment with prostaglandin prevented severe gastric mucosal injury after a single dose of aspirin. One milligram of prostaglandin E$_2$ given orally four times daily before aspirin ingestion prevented increased fecal blood loss in healthy individuals. Yik et al. (1982) observed a similar result with a lower dose of 0.5 mg of PGE$_2$ four times daily.

Kollberg and Slezak (1982) suggested that in addition to prevention of ulcers, PGE_2 can be used to speed up the healing process of duodenal ulcers. Patients treated with PGE_2, 0.5 mg 3 times daily and 1 mg at night, had significantly more healed ulcers than those in the placebo group (Kollberg and Slezak, 1982).

According to Miller et al. (1983), the oral antisecretory dose of $DMPGE_2$ for dogs is 50 μg/kg body wt. Thus, $DMPGE_2$ is an effective antiulcer drug which has cytoprotective and antisecretory properties.

Prostacyclin

Prostacyclin (PGI_2) is found in large quantities in the gastric mucosa. Prostaglandins of the I type as well as the E type are potent vasodilators (Konturek and Pawlik, 1986). PGI_2 also is a potent inhibitor of platelet aggregation (Kauffman et al., 1979). In a few studies, PGI_2 had cytoprotective as well as anti-ulcerogenic properties in rats (Balint & Varro, 1989; Mozsik et al., 1989).

Mozsik et al. (1991) reported that prostacyclin did not have cytoprotective and mucosal protective properties in surgically vagotomized rats indicating that an intact vagus nerve is required for the development of gastric cytoprotection. Prostacyclin (PGI_2) decreased gastric acid secretion in humans as well as animals (Isenberg, 1985).

Mozsik et al. (1989) studied the effect of PGI_2 on ethanol induced mucosal damage in rats. They reported that given

intragastrically 5 μg prostacyclin/kg body weight was 100% cytoprotective in rats.

PGI_2 was several times more potent than PGE_2 in inhibiting acid secretion in dogs when given intravenously. But when given intraarterially, PGE_2 was a more potent inhibitor than PGI_2 (Walus et al., 1980). Whittle et al. (1978) supported this finding. They also found that prostacyclin was a more potent gastric acid inhibitor than PGE_2 in rats.

During the development of gastric ulceration, the endogenous PGI_2 content decreased markedly depending on the degree of ulceration. This finding suggests that endogenous PGI_2 is one of the protective agents in gastric mucosa of animals as well as humans (Balint and Varro, 1989). Thus PGI_2, like most other prostaglandins, has cytoprotective and antiulcerogenic properties.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Forty-eight male Sprague Dawley rats (Sasco, Inc., Omaha, NE) were maintained on AIN-93G semipurified diet (Reeves et al., 1993) with no dietary variables for an average of 3 weeks (Table 1). The animals weighed 188±3 grams at the end of the diet period. The animals were deprived of food for 12 hours prior to the experiment but they had free access to distilled drinking water during that period. The animals were randomly assigned to 8 groups in a 2x4 factorial experiment.

Treatment Protocol

In this experiment each rat was first injected intraperitoneally with a dose of indomethacin or placebo. Thirty minutes later rats were intubated and dosed with one of four treatments (Figure 1). Thus twenty-four rats were injected intraperitoneally with 5 mg indomethacin/kg body wt suspended in 0.5% Tween 80 in 0.9% saline while the other 24 rats were injected with an equivalent volume of 0.5% Tween 80 in 0.9% saline (placebo).

All drugs for intubation were solubilized in small volumes of 95% ethanol and suspended in 1% Tween 80 in 0.9% saline. All treatments contained less than 2.2% ethanol. Thirty

Table 1

Composition of the Diet

Component	g/kg
Corn starch	397.5
Casein	200 0
Dextrinized corn starch	132.0
Sucrose	100.0
Soybean oil	70.0
Fiber	50.0
Mineral mix (AIN-93G-MX)	35.0
Vitamin mix (AIN-93-VX)	10.0
L-Cystine	3.0
Choline bitartrate	2.5
Tert-butylhydroquinone	0.014

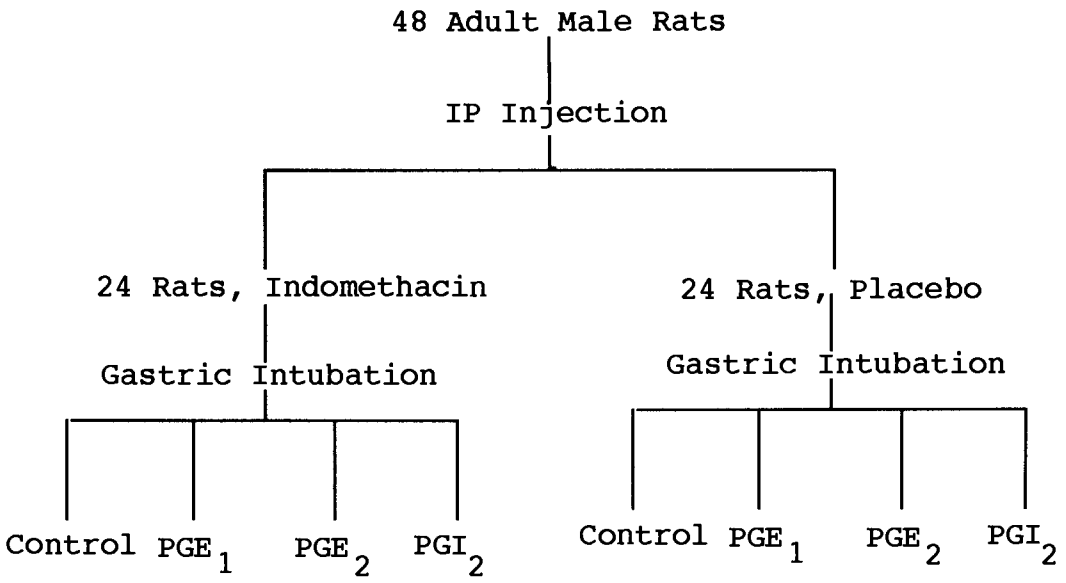

Figure 1. Experimental Design

minutes after the indomethacin injection, the animals were intubated and dosed with one of four different treatments. Treatments were 50 μg/kg body wt misoprostol (PGE_1 analogue), 7.5 μg/kg body wt of 16, 16-Dimethyl PGE_2 (16, 16-$DMPGE_2$, a PGE_2 analogue), 20 μg/kg body wt of prostacyclin (PGI_2) or control (the suspension used for other drugs). Immediately after intubation, the rats were given an oral dose of 100 μCi of $^{51}CrCl_3$ by micropipette.

Drugs

Drugs used were indomethacin (Sigma Chemical Co., St. Louis, MO), misoprostol (G.D. Searle & Co., Chicago, IL), 16, 16-$DMPGE_2$ (Sigma Chemical Co., St.Louis, MO) and prostacyclin (Sigma Chemical Co., St. Louis, MO). The $^{51}CrCl_3$ was obtained in 0.2 M HCl (New England Nuclear, Boston, MA). Indomethacin was suspended in 0.9% saline containing 0.5% Tween 80 (Sigma Chemical Co., St. Louis, MO) before injection. Misoprostol, 16, 16-$DMPGE_2$, and prostacyclin were solubilized in 95% ethanol and diluted with a suspension of 1% Tween 80 in 0.9% saline. Each drug was prepared just before use.

Drug Preparation

Indomethacin for intraperitoneal injection was prepared by adding 20 mg of indomethacin to 4.0 ml of 0.9% saline containing 0.5% Tween 80. The drug suspension was mixed with a vortex mixer for 15 seconds. The animals were then

injected with 5 mg/kg body weight of the indomethacin suspension. Preparation of the placebo was identical except no indomethacin was added.

The principal diluent for all intubated drugs was a suspension of 1% Tween 80 in 0.9% saline. Ethanol concentrations were kept below 2.2% for all drugs but varied somewhat due to differences in solubility. The control was prepared by adding 2.2% of 95% ethanol to the 1% Tween 80 suspension in 0.9% saline used for the other drugs.

Misoprostol was prepared by adding 20 µl of ethanol to 100 µg of ground tablet. This was followed by 4.0 ml of 1% Tween 80 in 0.9% saline which diluted the misoprostol to a final concentration of 25 µg/ml in 1% Tween 80 in 0.9% saline. The rats were given 50 µg/kg body wt of suspended misoprostol via gastric intubation.

To prepare the 16, 16-Dimethylprostagladin E_2 (16, 16 $DMPGE_2$), which was received as 1 mg in 100 µl of methyl acetate, 100 µl of 95% ethanol was added to make stock solution 1. One ml of 1% Tween 80 in 0.9% saline was added to 25 µl of stock solution 1 to make solution 2. Twenty-five microliters of solution 2 was further diluted with 2 ml of 1% Tween 80 in 0.9% saline producing the final concentration of 1.5 µg/ml. The rats were given 7.5 µg/kg body wt of suspended 16, 16-$DMPGE_2$ via gastric intubation.

Prostacyclin, which was given at 20 µg/kg body wt, was prepared by adding 200 µl of ethanol to 1 mg PGI_2 (stock solution 1). To 25 µl of stock solution 1, 275 µl of ethanol

and 12.2 ml of 1% Tween 80 in 0.9% saline were added producing the final concentration of 10 μg/ml.

Immediately after intubation, the rats were given an oral dose of 100 μCi of $CrCl_3$ (0.18 μg of chromium in 50 μl of 0.02 M HCl) by micropipette.

Experimental Procedure

After dosing, the animals were placed in individual metabolic cages for urine collection. Urine samples were collected at 2, 4 and 6 hours. Approximately 0.2 ml blood was collected from the tail at 0.25, 0.5, 1, 2 and 4 hours after ^{51}Cr dosing. At the 6th hour, the rats were anesthetized with ketamine (30 mg/kg body wt) and xylazine (2.2 mg/kg body wt). The animals were exsanguinated by cardiac puncture and blood samples were collected. Urine, blood and tissues including liver, kidney, spleen, heart, lung, testes, pancreas and bone were sampled and counted in the gamma counter to assess ^{51}Cr absorption, retention and urinary excretion.

Calculation of total ^{51}Cr in blood was done assuming blood to be 7% of body weight (Harkness and Wagner, 1983). Data are expressed as percent of intubated chromium dose. Samples of the dose were counted each day with tissue samples to allow correction for decay of the ^{51}Cr.

Statistical Methods

Data collected were analyzed using the Statistical Analysis System (SAS). Square root transformations were performed on the data to correct for non-homogeneity of variance. The general linear model (GLM) procedure in SAS was used for analysis of variance of the transformed tissue and urine data. A repeated measures analysis was performed on the transformed blood data. Differences among means were identified using the least significance difference test. Significance level was set at 0.05.

CHAPTER IV

INDOMETHACIN AND PROSTAGLANDIN EFFECTS ON ABSORPTION, RETENTION AND URINARY EXCRETION OF ^{51}CHROMIUM.

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ABSTRACT

Effects of prostaglandins on absorption, tissue distribution and urinary excretion of ^{51}Cr from $^{51}CrCl_3$ were investigated in a 2x4 factorial experiment. Forty-eight adult male rats were fasted overnight and injected intraperitoneally with indomethacin (5 mg/kg body wt) or placebo. Thirty minutes later rats were intubated and given one of four treatments: misoprostol (50 µg/kg body wt); a PGE_2 analogue, 16, 16-Dimethylprostaglandin E_2 (7.5 µg/kg body wt); prostacyclin (20 µg/kg body wt); or control (1% Tween 80 suspended in 0.9% saline containing 2.2% ethanol used for the other drugs). Immediately after intubation, rats were dosed with 100 µCi $^{51}CrCl_3$ by micropipette. Blood was collected from the tail at intervals after ^{51}Cr dosing. At the 6th hour, animals were exsanguinated by cardiac puncture. Indomethacin, a blocker of prostaglandin synthesis, significantly increased ($p<0.05$) ^{51}Cr in blood at all time periods tested except at 15 minutes. Likewise in tissues, indomethacin significantly increased ^{51}Cr retention. Urinary ^{51}Cr excretion at four and six hours was higher

($p<0.05$) in indomethacin-treated animals than in control animals. The PGE_2 analogue decreased ^{51}Cr absorption and tissue retention below control levels. Administration of a drug that blocks prostaglandin synthesis enhanced ^{51}Cr absorption while dosing with a PGE_2 analogue decreased ^{51}Cr absorption.

INTRODUCTION

Chromium is an essential trace mineral required for carbohydrate and lipid metabolism in rats as well as humans (Mertz, 1993). Chromium deficiency can cause elevated blood cholesterol in addition to glucose intolerance (Morris et al., 1992).

In the United States, 50-200 μg/day of chromium is recommended as the safe and adequate intake for adults (NRC, 1989). In the late 1970s estimated mean chromium intake in the United States ranged from 25-89 μg/day (Kumpulainen, 1979). However, lower values have since been obtained using improved analytical techniques. Chromium content of most self-selected diets was reported as less than 50 μg/day (Anderson and Kozlovsky, 1985; Bunker et al., 1984).

Chromium in foods generally decreases with processing (Wolf et al., 1974). Consumption of highly processed foods may cause increased excretion of chromium in urine as well as depletion of body chromium stores (Mertz et al., 1974). The elderly may be at particular risk of depletion because of decreased food intake due to decreased energy needs and

reduced access to a variety of foods (Nordstrom, 1982) as well as their altered metabolism due to aging (Exton-Smith, 1972).

Drug-nutrient interactions are another concern in the elderly. Because of the extensive use of over-the-counter (OTC) as well as prescribed medications, the elderly are particularly at risk for drug-nutrient interactions (Roe, 1986). Davis et al. (1995) showed that antacids reduced ^{51}Cr absorption in rats. Spicer et al. (1992) reported that ^{51}Cr absorption was significantly reduced in rats when dosed with over-the-counter antacid or with sucralfate, a medication used for ulcer therapy. In elderly patients, mineral depletion may be the most common example of drug induced nutritional deficiencies (Roe, 1986). Thus, there is a concern regarding chromium status with the use of the over-the-counter drugs.

On the other hand, Davis et al. (1995) showed that aspirin, a nonsteroidal antiinflammatory drug (NSAID), markedly enhanced absorption, tissue retention and urinary excretion of ^{51}Cr. Analgesics, including aspirin and indo-methacin, are used by the elderly for arthritis.

Indomethacin, another NSAID, inhibits prostaglandin synthesis and, thereby, induces gastrointestinal (GI) mucosal damage including GI ulcers in experimental animals (Whittle, 1976; Miller, 1983) and humans (Johansson et al., 1980). Prostaglandins protect the GI mucosa against NSAID-induced

mucosal damage (Agrawal et al., 1986; Balint and Varro, 1989; Brand et al., 1985).

The GI mucosa has a relatively large quantity of prostaglandins. There they affect a number of gastric as well as intestinal functions (Robert, 1979). The mechanism by which PGs protect GI mucosa is still not fully understood.

Song and Adham (1978) reported that pretreatment with indomethacin decreased zinc absorption and tissue distribution in rats by 60%. When zinc and prostaglandin were administered together, tissue distribution of zinc increased by 70%. They suggested that an increased level of PGs enhanced zinc absorption in rats. However, effects of gastrointestinal prostaglandins on chromium have not been studied.

One objective of the present study was to determine the effects of endogenous gastrointestinal prostaglandins on ^{51}Cr absorption, tissue distribution and urinary excretion. A second objective was to evaluate the effects on ^{51}Cr distribution of supplementing with various types of prostaglandins or analogues.

MATERIALS AND METHODS

Forty eight male Sprague Dawley rats (Sasco, Inc., Omaha, NE) were maintained on an AIN-93G semipurified diet (Reeves et al., 1993) with no dietary variables for an average of 3 weeks. Animals weighed 188±3gms at the end of the diet period. Food was withheld from the animals for 12 hours

prior to the experiment but they had free access to distilled drinking water during that period. The animals were randomly assigned to 8 groups in a 2x4 factorial design.

Twenty four rats were injected intraperitoneally with 5 mg/kg body wt indomethacin suspended in 0.5% Tween 80 in 0.9% saline while the other 24 rats were injected with the same volume of 0.5% Tween 80 suspension in 0.9% saline without indomethacin (placebo). Thirty minutes later the animals were intubated and dosed with one of four different treatments: Control (1% Tween 80 suspended in 2.2% ethanol and 0.9% saline used for the other drugs), 50 μg/kg body wt misoprostol (a PGE_1 analogue), 7.5 μg/kg body wt of 16, 16-Dimethylprostaglandin E_2 (a PGE_2 analogue) or 20 μg/kg body wt of prostacyclin (PGI_2). Immediately after intubation, the rats were given an oral dose of 100 μCi of $^{51}CrCl_3$ by micropipette.

Drug Preparation

Indomethacin for intraperitoneal injection dose was prepared by adding 20 mg of indomethacin to 4.0 ml 0.9% saline containing 0.5% Tween 80. Then the animals were injected with 5 mg/kg body weight of the indomethacin. Preparation of the vehicle (placebo) was identical except no indomethacin was added.

The principal diluent for all intubated drugs was a suspension of 1% Tween 80 in 0.9% saline. Ethanol concentrations were kept below 2.2% for all drugs but varied

somewhat due to differences in solubility. The control was prepared by adding 2.2% ethanol to the 1% Tween 80 suspension in 0.9% saline used for the other drugs.

Misoprostol was prepared by adding 20 µl of ethanol to 100 µg of ground tablet and diluting in 1% Tween 80 in 0.9% saline. Rats were given 50 µg/kg body wt of misoprostol by gastric intubation.

The 16, 16 $DMPGE_2$ was received as 1 mg in 100 µl of methyl acetate and was diluted with 100 µl of 95% ethanol. Immediately before gastric intubation, the PGE_2 was diluted to a final concentration of 1.5 µg/ml in 1% Tween 80 in 0.9% saline. Rats were intubated with 7.5 µg/kg body wt of PGE_2.

Prostacyclin was prepared by adding 200 µl of ethanol to 1 mg PGI_2. The PGI_2 was diluted to a final concentration of 10 µg/ml in 1% Tween 80 in 0.9% saline. Rats were intubated with 20 µg/kg body wt of PGI_2.

Immediately after intubation, the rats were given an oral dose of 100 µCi of $CrCl_3$ (0.18 µg of chromium in 50 µl of 0.02 M HCl) by micropipette.

Experimental Procedure

After dosing, the animals were placed in individual metabolic cages for urine collection. Urine samples were collected at 2, 4 and 6 hours. Approximately 0.2 ml blood was collected from the tail at intervals of 0.25, 0.5, 1, 2 and 4 hours after ^{51}Cr dosing. At the 6th hour, the rats were anesthetized with ketamine (30 mg/kg body wt) and

xylazine (2.2 mg/kg body wt). The animals were exsanguinated by cardiac puncture and blood samples were collected. Urine, blood and tissues including liver, kidney, spleen, heart, lung, testes, pancreas and bone were sampled and counted in the gamma counter to assess ^{51}Cr absorption, retention and urinary excretion.

Calculation of total ^{51}Cr in blood was done assuming blood was 7% of body weight (Harkness and Wagner, 1983). Data are expressed as percent of intubated chromium dose. Samples of the dose were counted each day with tissue samples to allow correction for decay of the ^{51}Cr.

Statistical Analyses

Data were analyzed as a 2x4 factorial experiment using the Statistical Analysis System (SAS). Square root transformations were performed on the data to correct for non-homogeneity of variance. The general linear model (GLM) procedure in SAS was used for analysis of variance of the transformed tissue and urine data. A repeated measures analysis was performed on the transformed blood data. Differences among means were identified using the least significance difference test.

RESULTS

Blood data were analyzed beginning at 30 minutes because of variable absorption at 15 minutes. No interactions were found between the drugs and indomethacin. Therefore, their

effects on the absorption, tissue distribution and urinary excretion of ^{51}Cr are discussed separately. Overall, animals pretreated with indomethacin, a blocker of prostaglandin synthesis, had significantly higher ($p<0.0003$) total ^{51}Cr in blood than control animals (Figure 2 and Table A2 in the Appendix and Figure A1 in the Appendix).

When comparing the effect of drugs on ^{51}Cr in blood (Figure 3 and Table A2 in the Appendix) the control group had higher ^{51}Cr absorption than the groups which received PGE_1 ($p<0.03$) and PGE_2 analogue ($p<0.0001$). The group dosed with PGE_2 analogue was lower ($p<0.03$) than all other treatment groups.

In tissues of indomethacin pretreated animals, retention of ^{51}Cr was greater ($p<0.03$) in all tissues except lung, compared to the control animals (Table 2). Most of the ^{51}Cr retained in tissues was in liver and kidney.

When drugs were compared, tissue retention of ^{51}Cr was reduced significantly ($p<0.05$) by PGE_2 analogue compared with all other groups in liver, kidney, spleen, testes and bone (Table 2). Tissue retention in control, PGE_1 and PGI_2 groups was not significantly different from each other. In heart, the PGE_2 group was significantly lower than the control and PGE_1 groups but not lower than PGI_2 group. In the lung and pancreas, ^{51}Cr retention was not significantly affected by the administered prostaglandins or analogues.

The total cumulative urinary excretion of ^{51}Cr was significantly higher ($p<0.05$) in indomethacin-treated animals than in control animals (Figure 4 and Table A4 in the

Appendix). Urinary excretion of ^{51}Cr at 4 hours was developing a trend. The significance level for the effect of drugs on urinary excretion of ^{51}Cr was 0.09. The group treated with PGE_2 analogue tended to be lower ($p<0.09$) than all others (Figure 5 and Table A4).

Significantly enhanced ^{51}Cr absorption, tissue retention and urinary excretion seen with indomethacin pretreatment appear to be mediated by the inhibition of gastrointestinal prostaglandins; supplementary PGE_2 analogue significantly decreased ^{51}Cr absorption and tissue retention below control levels.

DISCUSSION

The effects of prostaglandins on chromium metabolism have not been studied extensively. Similar to the present study, Davis et al. (1995) showed that by inhibiting PG synthesis, aspirin enhanced chromium absorption. Also, there are two other studies which suggest that PGs have a role in absorption of other minerals, such as zinc (Song and Adham, 1978) and calcium (Johansson et al., 1980)

Song and Adham (1978) studied the effects of PGs on zinc absorption in rats. They reported that by binding zinc, prostaglandins enhance transport of zinc across the intestinal mucosa. Pretreatment with indomethacin reduced the zinc content of the internal organs. But when indo -methacin and PG were given simultaneously, the zinc content of the internal organs was increased. They suggested that

increased levels of PG enhance zinc absorption. This result is contrary to the results of the present study, where indomethacin pretreatment significantly enhanced chromium absorption and tissue retention

Johansson et al. (1980) suggested that prostaglandins may have a role in calcium metabolism in humans. When indomethacin was used three times daily at 25 or 50 mg doses in the treatment of rheumatic diseases, they found a significant reduction in serum calcium. This suggests that prostaglandin may have a role in calcium homeostasis in humans.

For chromium, Davis et al. (1995) showed that absorption, tissue retention and urinary excretion of ^{51}chromium were significantly higher in animals dosed orally with aspirin than in control animals. They suggested that aspirin, a nonsteroidal antiinflammatory drug, inhibits endogenous prostaglandin synthesis and enhances chromium absorption.

In the present study, pretreatment of rats with 5 mg/kg body wt of indomethacin significantly increased chromium absorption, tissue retention and urinary excretion similar to the results reported by Davis and coworkers. Increased ^{51}Cr absorption and tissue retention seen in the present study appears to be due to prostaglandin inhibition.

Indomethacin is a nonsteroidal antiinflammatory drug which is commonly prescribed for rheumatoid arthritis. Indomethacin produces intestinal lesions at 7.5 mg/kg body wt in rats (Robert, 1979). According to Takeuchi et al. (1986) 5 mg/kg

body wt of indomethacin reduced PGs without damaging gastric mucosa.

Some studies have shown that a variety of PGs reduce GI side effects associated with NSAIDs including indomethacin treatment when taken together (Cohen et al. 1983; Johansson et al., 1980). This phenomenon has been called cytoprotection. Many prostaglandins have this property.

In the present study, administration of exogenous PGE_2 significantly decreased ^{51}Cr absorption, compared to control, which received no exogenous prostaglandins or analogues. Absorption of ^{51}Cr by animals dosed with PGI_2 and PGE_1 was between control and PGE_2.

NSAIDs including indomethacin and aspirin also work by inhibiting cyclooxygenase activity and in turn blocking prostaglandin synthesis (Kauffman, 1989). For example, NSAIDs block the production of PGs of the E and I type in the gastric mucosa.

Even though NSAIDs increase the absorption of chromium, they also cause gastric mucosal injury. The mechanism by which NSAIDs cause gastric mucosal injury is still not fully understood.

In summary, enhanced ^{51}Cr absorption seen with indomethacin pretreatment appears to be mediated by the inhibition of gastrointestinal prostaglandin synthesis. These results suggests that prostaglandins have a role in chromium metabolism.

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REFERENCES

Agrawal, N.M., Godiwala, T., Arimura, A. and Dajani, E.Z. (1986) Cytoprotection by a synthetic prostaglandin against ethanol-induced gastric mucosal damage: a double blind endoscopy study in human subjects. Gastrointest. Endosc. 32: 67-70.

Anderson, R.A. and Kozlovsky, A.S. (1985) Chromium intake, absorption, and excretion of subjects consuming self-selected diets. Am. J. Clin. Nutr. 41: 1177-1183.

Balint, G.A. and Varro, V. (1989) The role of endogenous and exogenous prostacyclin in the gastric mucosa under physiological and pathological circumstances. Acta Physiologica Hungarica 73: 193-197.

Brand, D.L., Roufail, W.M., Thomson, A.B.R. and Tapper, E.J. (1985) Misoprostol, a synthetic PGE_1 analog, in the treatment of duodenal ulcers. Dig. Dis. Sci. 30: 147S-158S.

Bunker, W., Lawson, M.S., Delves, H.T. and Clayton, B.E. (1984) The uptake and excretion of chromium by the elderly. Am. J. Clin. Nutr. 39: 797-802.

Cohen, M.M., Clark, L., Armstrong, L. and D'souza J. (1983) Reduced aspirin-induced fecal blood loss with simultaneous administration of misoprostol (PGE_1 methyl analogue). Gastroenterology 84: 1126 (abstract).

Davis, M.L., Seaborn, C.D. and Stoecker, B.J. (1995) Effects of over-the-counter drugs on chromium absorption in rats. Nutr. Res. 15: 201-215.

Exton-Smith, A.N. (1972) Physiological aspects of aging: relationship to nutrition. Am. J. Clin. Nutr. 25: 853-859.

Harkness, J.E. and Wagner, J.E. (1983) The rat. In: The Biology and Medicine of Rabbits and Rodents, 43-50. Philadelphia: Lea and Febiger.

Johansson, C., Kollberg, B., Nordemar, R., Samuelson, K. and Bergstrom, S. (1980) Protective effect of prostaglandin E_2 in the gastrointestinal tract during indomethacin treatment of rheumatic diseases. Gastroenterology 78: 479-483.

Kauffman, G. (1989) Aspirin-induced gastric mucosal injury: Lessons learned from animal models. Gastroenterology 96: 606-614.

Kumpulainen,J.T., Wolf, W.R., Veillon, C. and Mertz, W. (1979) Determination of chromium in selected United States diets. J. Agri. Food. Chem. 27: 490-494.

Mertz, W. (1993) Chromium in human nutrition: A review. J. Nutr. 123: 626-633.

Mertz, W., Toepfer, E.W., Roginski, E.E. and Polansky, M.M. (1974) Present knowledge of the role of chromium. Fed. Proc. 33: 2275-2280.

Miller T.A. (1983) Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. Am. J. Physiol. 245: G601-G623.

Morris, B.W., Blumsohn, A., MacNeil, S. and Gray, T.A. (1992) The trace element chromium-a role in glucose homeostasis. Am. J. Clin. Nutr. 55: 989-991.

National Research Council. (1989) Recommended dietary allowances. pp. 241-246, National Academy of Sciences, Washington, DC.

Nordstrom, J.W. (1982) Trace mineral nutrition in the elderly. Am. J. Clin. Nutr. 36: 788-795.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C., Jr. (1993) AIN-93 purified diets for laboratory rodents: Final report of the American Inst. of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123: 1939-1951.

Robert, A. (1979) Cytoprotection by prostaglandins. Gastroenterology 77: 761-767.

Roe, D.A., (1986) Drug nutrient interaction in the elderly. Geriatrics 41: 57-74.

Song, M.K. and Adham, N.F. (1978) Role of prostaglandin E_2 in zinc absorption in the rat. Am. J. Physiol. 234: E99-105.

Spicer, M.T., Stoecker, B.J., Orina, M. and Sangiah, S.(1992) Effects of antiulcer drugs on ^{51}Cr absorption, retention and urinary excretion in rats. Faseb Journal 6: A1590 (abstract).

Takeuchi, K., Ueki, S. and Okabe, S. (1986) Importance of gastric motility in the pathogenesis of indomethacin-induced gastric lesions in rats. Dig. Dis. Sci. 31: 1114-1122.

Whittle, B.J.R. (1976) Relationship between the prevention of rat gastric erosions and the inhibition of acid secretion by prostaglandins. Eur. J. Pharmacol. 40: 233-239.

Wolf, W., Mertz, W. and Masironi, R. (1974) Determination of chromium in refined sugars by oxygen plasma ashing flameless atomic absorption. J. Agric. Chem. 22: 1037-1042.

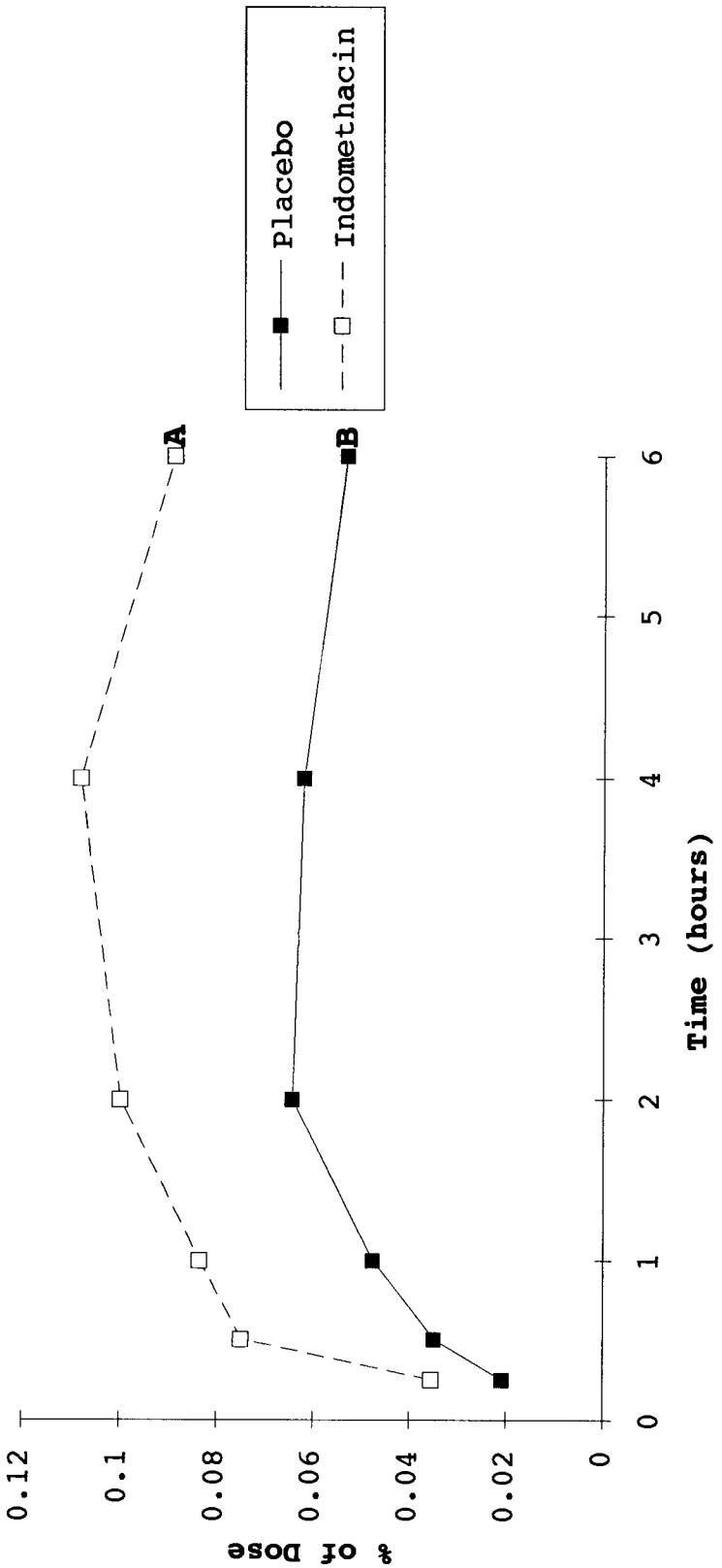

Figure 2. Effect of Indomethacin on Absorption of 51-Chromium in Blood

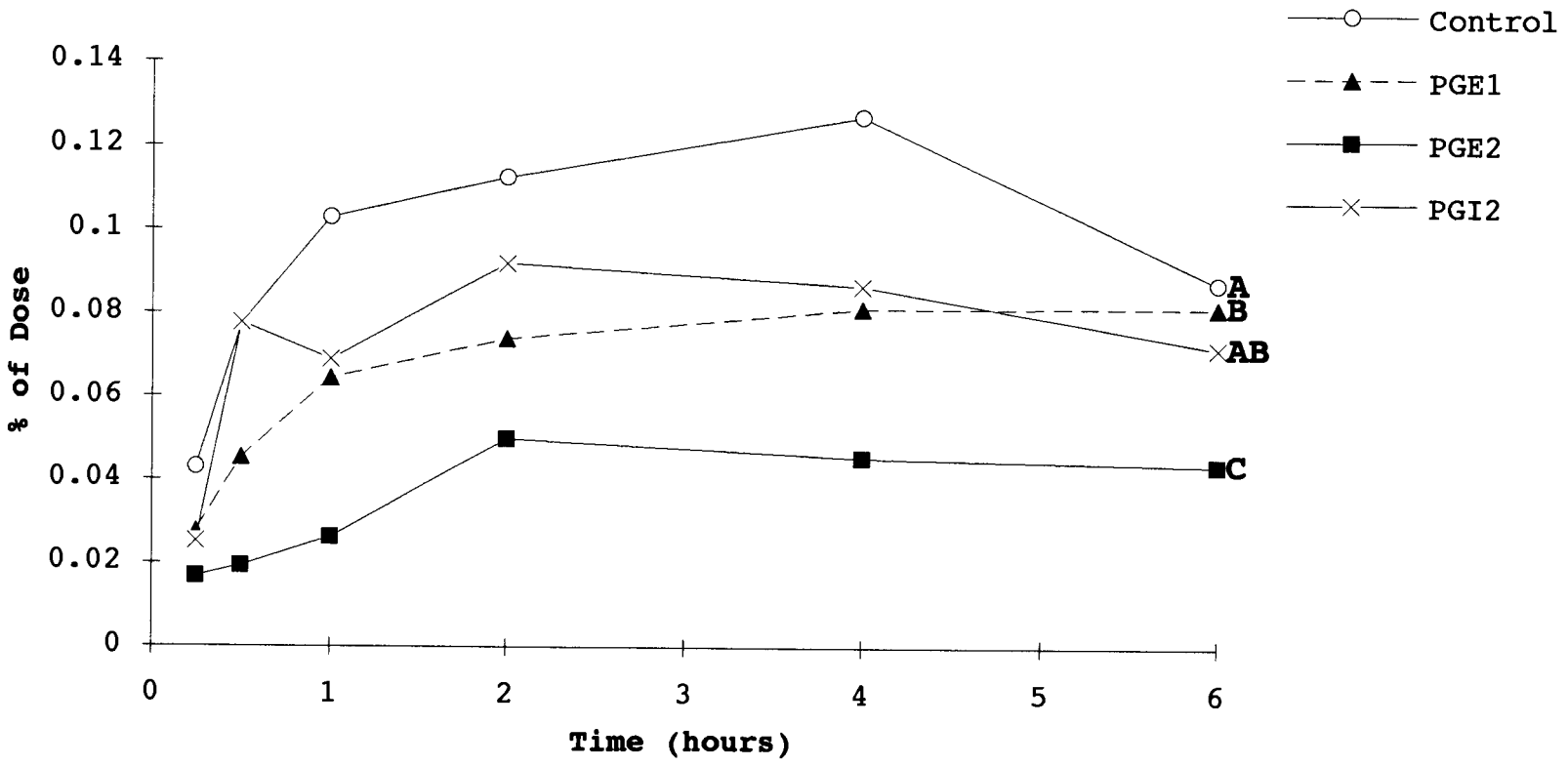

Figure 3. Effects of Prostacyclin and Prostaglandin Analogues on Absorption of 51-Chromium in Blood

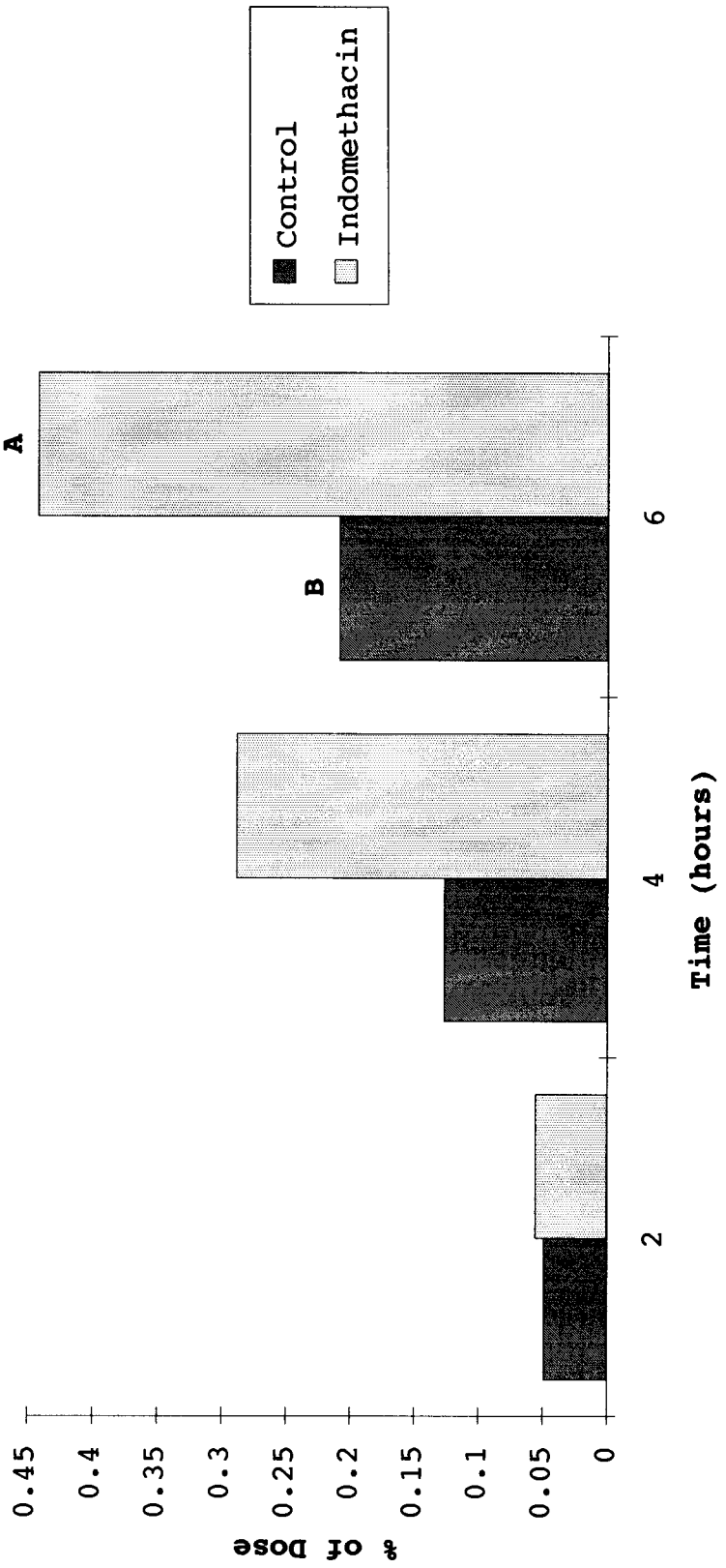

Figure 4. Effect of Indomethacin on Excretion of 51-Chromium in Urine

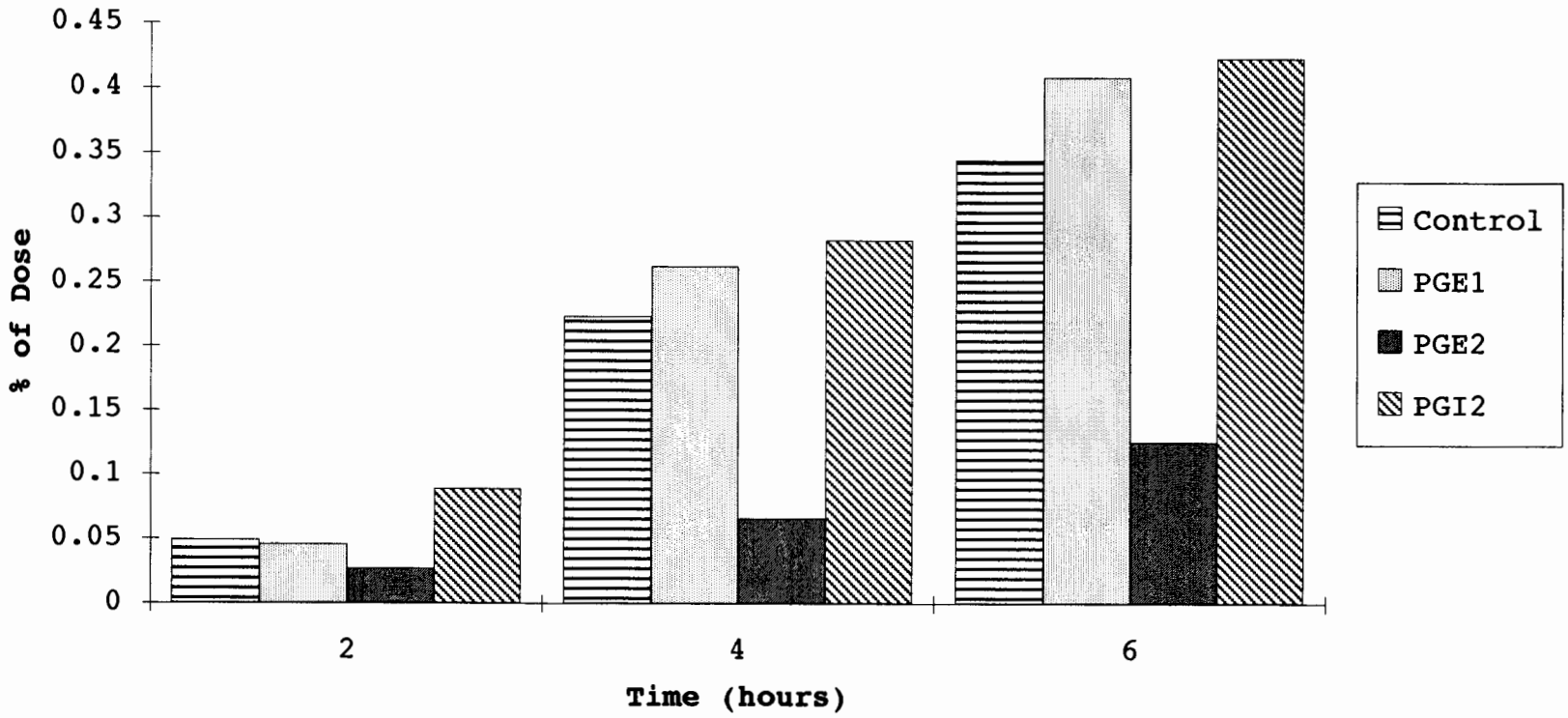

Figure 5. Effects of Prostacyclin and Prostaglandin Analogues on Absorption of 51-Chromium in Urine

Table 2.

Effects of Indomethacin and Various Prostaglandins on ^{51}Cr in Tissues 6 Hours After Dosing[1,2]

	Liver	Kidney	Heart	Lung	Testes	Bone	Spleen	Pancreas
	% of dose x 10^{-2}	% of dose x 10^{-2}	% of dose x 10^{-2}	% of dose x 10^{-2}	% of dose x 10^{-2}	% of dose/g x 10^{-2}	% of dose x 10^{-2}	% of dose x 10^{-2}
PG Inhibitor								
Placebo	1.09^{B}	1.24^{B}	0.09^{B}	0.17	0.20^{B}	0.08^{B}	0.045^{B}	0.018^{B}
Indomethacin	1.79^{A}	2.96^{A}	0.13^{A}	0.55	0.31^{A}	0.15^{A}	0.064^{A}	0.029^{A}
S.E.M.	± 0.16	± 0.37	± 0.01	± 0.22	± 0.03	± 0.01	± 0.005	± 0.003
DRUG								
Control	1.64^{a}	2.08^{a}	0.14^{a}	0.24	0.31^{a}	0.12^{a}	0.070^{a}	0.031
PGE_1	1.87^{a}	2.85^{a}	0.14^{a}	0.90	0.30^{a}	0.14^{a}	0.068^{a}	0.022
PGE_2	0.78^{b}	0.91^{b}	0.06^{b}	0.11	0.15^{b}	0.06^{b}	0.030^{b}	0.019
PGI_2	1.46^{a}	2.57^{a}	0.10^{ab}	0.19	0.26^{a}	0.13^{a}	0.050^{a}	0.022
S.E.M.	± 0.23	± 0.52	± 0.02	± 0.31	± 0.04	± 0.02	± 0.007	± 0.005
Source of variation								
PG inhibitor	0.0014	0.0003	0.0078	0.07	<0.006	0.0003	0.0064	<0.03
Drug	<0.004	<0.02	0.002	0.07	<0.02	0.0064	0.0007	0.29
PG inhibitor x Drug	0.41	0.52	0.91	0.76	0.49	0.41	0.84	0.78

[1]Mean ± pooled SEM. A square root transformation of data was done prior to statistical analysis.

[2]Means within a factor not sharing a common superscript are different ($p<0.05$).

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

Forty-eight adult male rats were fasted overnight and injected intraperitoneally with indomethacin or vehicle. Thirty minutes later, the rats were intubated with one of four treatments; a PGE_1 analogue, misoprostol; a PGE_2 analogue, 16, 16-Dimethyl PGE_2; PGI_2, prostacyclin or placebo. The intubated treatment was followed immediately by an oral dose of $^{51}CrCl_3$. Blood, urine and tissues were collected and counted in the gamma counter to assess ^{51}Cr absorption, retention and urinary excretion.

Indomethacin, a blocker of prostaglandin synthesis, significantly increased ($p<0.05$) ^{51}Cr in blood at all time periods tested except 15 minutes. In tissues, indomethacin significantly increased ^{51}Cr retention. Urinary ^{51}Cr excretion was higher ($p<0.05$) in indomethacin-treated animals than in control animals. Overall, blocking prostaglandin synthesis with indomethacin increased ^{51}Cr absorption.

Conclusions

In this experiment, indomethacin, a nonsteroidal antiinflammatory drug enhanced chromium absorption. Even though patients who use this drug may benefit by the enhancement seen in the present study, excessive use of

NSAIDs may cause GI damage and may ultimately cause ulcers. The increase in chromium absorption and tissue retention seen in the present study may be because of inhibiting PG synthesis. Supplementing with PGs or prostaglandin analogues to protect the GI mucosa from damage may be detrimental to chromium status.

Hypotheses

The following hypotheses were developed for this study:

1. There will be no significant effect of intraperitoneally injected indomethacin, a blocker of prostaglandin synthesis, on ^{51}Cr absorption, retention or urinary excretion.
2. There will be no significant effect of intubated doses of misoprostol, 16, 16-Dimethylprostaglandin E_2, or prostacyclin on ^{51}Cr absorption, retention or urinary excretion.

Hypothesis #1 was rejected because indomethacin significantly ($p<0.05$) increased chromium absorption. Retention of ^{51}Cr in tissues was higher ($p<0.05$) with indomethacin pretreatment. Urinary excretion of ^{51}Cr also increased significantly ($p<0.05$).

Hypothesis #2 was rejected because, 16, 16-DMPGE_2, an analogue of prostaglandin E_2, significantly reduced ^{51}Cr absorption and retention in tissues including liver, kidney, spleen, heart, testes and bone. Misoprostol, an analogue of prostaglandin E_1 significantly reduced ^{51}Cr absorption.

Recommendations

The following recommendations for future research were developed from this study.

1) Long term studies are needed to investigate the effects of extensive use of NSAIDs on the metabolism of chromium, since extended use of NSAIDs is quite common in patients with arthritis.
2) Experiments similar to the present experiment should be conducted with other analogues of prostaglandins used therapeutically to determine their interactions with chromium.
3) Studies should be done with a prostacyclin analogue, which would be more stable than prostacyclin. The prostacyclin used in the present study appeared to degrade after a few days. After the present study was completed, leftover prostacyclin was used for a different experiment and showed no effect. This would indicate that the prostacyclin may have degraded despite efforts always to keep it frozen or on ice.

Bibliography

Agrawal, N.M. and Dajani, E.Z. (1990) Options in the treatment and prevention of NSAID-induced gastroduodenal mucosal damage. J. Rheumatol. 17(Suppl 20): 7-11.

Agrawal, N.M., Godiwala, T., Arimura, A. and Dajani, E.Z. (1986) Cytoprotection by a synthetic prostaglandin against ethanol-induced gastric mucosal damage: a double blind endoscopy study in human subjects. Gastrointest. Endosc. 32: 67-70.

Anderson, R.A. and Kozlovsky, A.S. (1985) Chromium intake, absorption, and excretion of subjects consuming self-selected diets. Am. J. Clin. Nutr. 41: 1177-1183.

Arakawa, T., Nakamura, H., Chono, S., Satoh, H., Fukuda, T., Saeki, Y. and Kobayashi, K. (1989) Absence of effect of 16, 16-dimethyl prostaglandin E_2 on reduction of gastric mucosal blood flow caused by indomethacin in rats. Dig. Dis. Sci. 34: 1369-1373.

Balint, G.A. and Varro, V. (1989) The role of endogenous and exogenous prostacyclin in the gastric mucosa under physiological and pathological circumstances. Acta Physiologica Hungarica 73:193-197.

Bauer, R.F. (1985) Misoprostol preclinical pharmacology. Dig. Dis. Sci. 30: 118S-125S.

Bauer, R.F., Bianchi, R.G., Casler, J. and Goldstin, B. (1986) Comparative mucosal protective properties of misoprostol, cimetidine and sucralfate. Dig. Dis. Sci. 31: 81S-85S.

Bennett, A. (1989) Some new aspects of gastric mucosal protection damage. Acta Physiologica Hungarica 73: 179-183.

Bode, C., Ito, T., Rollenhagen, A. and Bode, J.C. (1988) Effect of acute and chronic alcohol feeding on prostaglandin E_2 biosynthesis in rat stomach. Dig. Dis. Sci. 33: 814-818.

Bolton, J.P. and Cohen, M.M. (1978) Stimulation of non-parietal cell secretion in canine heidenhain pouches by 16, 16-dimethylprostaglandin E_2. Digestion 17:291-299.

Brand, D.L., Roufail, W.M., Thomson, A.B.R. and Tapper, E.J. (1985) Misoprostol, a synthetic PGE_1 analog, in the treatment of duodenal ulcers. Dig. Dis. Sci. 30: 147S-158S.

Bunker, W., Lawson, M.S., Delves, H.T. and Clayton, B.E. (1984) The uptake and excretion of chromium by the elderly. Am. J. Clin. Nutr. 39: 797-802.

Chen, N.S.C., Tsai, A. and Dyer, I.A. (1973) Effect of chelating agents on chromium absorption in rats. J. Nutr. 103:1182-1186.

Cheung, L.Y. (1980) Topical effects of 16, 16-dimethyl prostaglandin E_2 on gastric blood flow in dogs. Am. J. Physiol. 238: G514-G519.

Cohen, M.M., Clark, L., Armstrong, L. and D'souza, J. (1983) Reduced aspirin-induced fecal blood loss with simultaneous administration of misoprostol (PGE_1 methyl analogue). Gastroenterology 84: 1126 (abstract).

Dajani, E.Z., Driskill, D.R., Bianchi, R.G., Collins, P.W. and Pappo R. (1976) SC-29333:a potent inhibitor of canine gastric secretion. Am. J. Dig. Dis. 21: 1049-1057.

Dajani E.Z and Nissen, C.H. (1985) Gastrointestinal cytoprotective effects of misoprostol. Dig. Dis. Sci. 30: 194S-200S.

Davis, M.L., Seaborn, C.D. and Stoecker, B.J. (1995) Effects of over-the-counter drugs on chromium absorption in rats. Nutr. Res. 15: 201-210.

Doisy, R.J., Streeten, D.H.P., Levine, R.A. and Chodos, R.B. (1968) Effects of metabolism of chromium in normals, elderly subjects and diabetics. In: Trace Substances in Environmental Health, II, pp. 75-82, Columbia, MO.

Doisy, R.J., Streeten, D.H.P., Souma, M.L., Kalafer, M.E., Rekant, S.I. and Dalakos, T.G. (1971) Metabolism of ^{51}chromium in human subjects-normal, elderly and diabetic subjects. In: Newer Trace Elements in Nutrition (Mertz, W. and Cornatzer, W.E., eds.), pp. 155-168, Marcel Dekker, Inc., New York, NY.

Exton-Smith, A.N. (1972) Physiological aspects of aging: relationship to nutrition. Am. J. Clin. Nutr. 25: 853-859.

Gilbert, D.A., Surawicz, C.M., Silverstein, F.E., Weinberg, C.R., Saunders, D.R., Field, A.D., Sanford, R.L., Bergman, D. and Washington, P. (1984) Prevention of acute aspirin-induced gastric mucosal injury 15-R-15 methyl prostaglandin E_2: An endoscopic study. Gastroenterology 86: 339-345.

Glinsmann, W.H. and Mertz, W. (1966) Effet of trivalent chromium on glucose tolerance. Metab. Clin. Exptl. 15: 510-520.

Graham, D.Y., Agrawal, N.M. and Roth, S.H. (1988) Prevention of NSAID-induced gastric ulcer with misoprostol: multicenter, double-blind, placebo-controlled trial. Lancet 2: 1277-1280

Gurson, C.T. and Saner, G. (1971) Effect of chromium on glucose utilization in marasmic protein-calorie malnutrition. Am. J. Clin. Nutr. 24: 1313-1319.

Hahn, C.J. and Evans, G.W. (1975) Absorption of trace metals in the zinc-deficient rat. Am. J. Physiol. 228: 1020-1023.

Hambidge, K.M.(1978) Zinc and chromium in human nutrition. J. Human Nutr. 32: 99-110.

Harkness, J.E. and Wagner, J.E. (1983) The rat. In: The Biology and Medicine of Rabbits and Rodents, 43-50. Philadelphia: Lea and Febiger.

Hopkins, L.L.Jr., Ransome-Kuti, O. and Majaj A.S. (1968) Improvement of impaired carbohydrate metabolism by chromium (III) in malnourished infants. Am. J. Clin. Nutr. 21: 203-211.

Hunt, J.N., Smith, J.L., Jiang, C.L. and Kessler, L. (1983) Effect of synthetic prostaglandin E_1 analog on aspirin-induced gastric bleeding and secretion. Dig. Dis. Sci. 28: 897-902.

Isenberg, J.I. (1985) Overview of clinical cytoprotection. Dig. Dis. Sci. 30: 81S-82S.

Jackson, R.A., Blix, P.M., Matthews, J.A., Hamling, J.B., Din, B.M., Brown, D.C., Belin, J., Rubenstein, A.H. and Nabarro, J.D.N. (1982) Influence of ageing on glucose homeostasis. J. Clin. Endocrinol. Metab. 55: 840-848.

Jacobson, E.D. (1985) Gastric blood flow and the gastric mucosal barrier. Dig. Dis. Sci. 30: 77S-80S.

Jacobson, E.D. (1986) Direct and adaptive cytoprotection. Dig. Dis. Sci. 31: 28S-31S.

Jaszewski, R., Graham, D.Y. and Stromatt, S.C. (1992) Treatment of nonsteroidal antiinflammatory drug-induced gastric ulcers with misoprostol. Dig. Dis. Sci. 37: 1820-1824.

Jeejeebhoy, K.N., Chu, R.C., Marliss, E.B., Greenberg, G.R. and Bruce-Robertson, A. (1977) Chromium deficiency, glucose tolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. Am. J. Clin. Nutr. 30: 531-538.

Johansson, C., Kollberg, B., Nordemar, R., Samuelson, K. and Bergstrom, S. (1980) Protective effect of prostaglandin E_2 in the gastrointestinal tract during indomethacin treatment of rheumatic diseases. Gastroenterology 78: 479-483.

Kauffman, G. (1989) Aspirin-induced gastric mucosal injury: Lessons learned from animal models. Gastroenterology 96: 606-614.

Kauffman, G.L., Reeve, J.J. and Grossman, M.I. (1980) Gastric bicarbonate secretion: Effect of topical and intravenous 16, 16-dimethyl prostaglandin E_2. Am. J. Physiol. 239: G44-G48.

Kauffman, G.L. and Whittle, B.J.R. (1982) Gastric vascular actions of prostanoids and the dual effect of arachidonic acid. Am. J. Physiol. 242: G582-587.

Kauffman, G.L., Whittle, B.J.R., Aures, D., Vane, J.R. and Grossman, M.I. (1979) Effects of prostacyclin and a stable analogue, 6β-PGI_1, on gastric acid secretion, mucosal blood flow and blood pressure in conscious dogs. Gastroenterology 77: 1301-1306.

Kollberg, B. and Slezak, P. (1982) The effect of prostaglandin E_2 on duodenal ulcer healing. Prostaglandins 24: 527-536.

Konturek, S.J., Brzozowski, T., Piastucki, I., Radecki, T., Dembinski, A. and Dembinska-Kiec, A. (1982) Role of locally generated prostaglandins in adaptive gastric cytoprotection. Dig. Dis. Sci. 27: 967-971.

Konturek, S.J. and Pawlik, W. (1986) Physiology and pharmacology of prostaglandins. Dig. Dis. Sci. 31: 6S-19S.

Kumpulainen, J.T., Wolf, W.R., Veillon, C. and Mertz, W. (1979) Determination of chromium in selected United States diets. J. Agri. Food. Chem. 27: 490-494.

Kuo, Y.J., Shanbour, L.L. and Miller, T.A. (1983) Effects of 16, 16-dimethyl prostaglandin E_2 on alkaline secretion in isolated canine gastric mucosa. Dig. Dis. Sci. 28: 1121-1126.

Lacy E.R. (1985) Prostaglandins and histological changes in the gastric mucosa. Dig. Dis. Sci. 30: 83S-94S.

Lanza, F.L., Royer, G.L., Nelson, R.S., Chen, T.T., Seckman, C.E. and Rack, M.F. (1979) The effects of ibuprofen, indomethacin, aspirin, naproxen and placebo on the gastric mucosa of normal volunteers. Dig. Dis. Sci. 24: 823-828.

Larsen, K.R., Jensen, N.F., David, E.K., Jensen, J.C. and Moody, F.G. (1981) The cytoprotective effects of 15-deoxy-16-alpha, betahydroxy-16-methyl PGE methyl ester (SC-29333) versus aspirin-shock gastric ulcerogesis in the dog. Prostaglandins 21(suppl): 119-124.

Levine, R.A., Streeten, D.H.P., and Doisy, R.J. (1968) Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects. Metabolism 17: 114-125.

Mertz, W. (1969) Chromium occurence and function in biological systems. Physiol. Rev. 49: 163-239.

Mertz, W. (1993) Chromium in human nutrition: A review. J. Nutr. 123: 626-633.

Mertz, W., Toepfer, E.W., Roginski, E.E. and Polansky, M.M. (1974) Present knowledge of the role of chromium. Fed. Proc. 33: 2275-2280.

Miller, T.A. (1983) Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. Am. J. Physiol. 245: G601-G623.

Miller, T.A., Kraemer, B.B., Henagan, J.M. and Foucar, C.E. (1983) Topical 16, 16-Dimethyl-prostaglandin E_2 effects on gastric morphology, hydrogen ion loss and bicarbonate secretion. Dig. Dis. Sci. 28: 641-648.

Morris, B.W., Blumsohn, A., MacNeil, S. and Gray, T.A. (1992) The trace element chromium-a role in glucose homeostasis. Am. J Clin. Nutr. 55: 989-991.

Mozsik, G., Garamszegi, M., Javor, T., Nagy, L., Suto, G., and Vincze, A. (1989) A pharmacological approach to cellular mechanisms of PGI_2-induced gastric cytoprotection on ethanol-induced gastric mucosal damage in rats. Acta Physiologica Hugarica 73: 207-211.

Mozsik, G., Kiraly, A., Garamszegi, M., Javor, T., Nagy, L., Suto, G., Toth, G. and Vincze, A. (1991) Failure of prostacyclin, β-carotene, atropine and cimetidine to

produce gastric cyto - and general mucosal protection in surgically vagotamized rats. Life Sci. 49: 1383-1389.

National Research Council. (1989) Recommended Dietary Allowances. pp. 241-246, National Academy of Sciences, Washington, DC.

Nordstrom, J.W. (1982) Trace mineral nutrition in the elderly. Am. J. Clin. Nutr. 36: 788-795.

Offenbacher, E.G. (1992) Chromium in elderly. Biol. Trace Elem. Res. 32: 123-131.

Offenbacher, E.G. and Pi-Sunyer, F.X. (1980) Beneficial effect of Cr-rich yeast on glucose tolerance and blood lipids in elderly subjects. Diabetes 29: 919-925.

Offenbacher, E.G., Spencer, H., Dowling, H.J. and Pi-Sunyer, F.X. (1986) Metabolic chromium balances in men. Am. J. Clin. Nutr. 44: 77-82.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C., Jr. (1993) AIN-93 purified diets for laboratory rodents: Final report of the American Inst. of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123: 1939-1951.

Robert, A. (1979) Cytoprotection by prostaglandins. Gastroenterology 77: 761-767.

Robert, A., Nezamis, J.E., Lancaster, C. and Hanchar, A.J. (1979) Cytoprotection by prostaglandins in rats: prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology 77: 433-443.

Roe, D.A. (1985) Therapeutic effects of drug nutrient interactions in the elderly. J. Am. Diet. Assn. 85: 174-178, 181.

Roe, D.A. (1986) Drug nutrient interaction in the elderly. Geriatrics 41: 57-74.

Schroeder, H.A. (1966) Chromium deficiency in rats: a syndrome simulating diabetes mellitus with retarded growth. J. Nutr. 88: 439-445.

Schroeder, H.A. (1968) The role of chromium deficiency in mammalian nutrition. Am. J. Clin. Nutr. 21: 230-244.

Schroeder, H.A., Balassa, J.J. and Tipton, I.H. (1962) Abnormal trace metals in man. J. Chron. Dis. 15: 941-964.

Schwarz, K. and Mertz, W. (1959) Chromium(III) and the glucose tolerance factor. Arch. Biochem. Biophys. 85: 292-295.

Sherman, L., Glennon, J.A., Brech, W.J., Klomberg, G.H. and Gordon, E.S. (1968) Failure of trivalent chromium to improve hyperglycemia in diabetes mellitus. Metabolism 17: 439-442.

Silverstein, F.E., Kimmey, M.B., Saunders, D.R. and Levine, D.S. (1986) Gastric protection by misoprostol against 1300 mg of aspirin :an endoscopic study. Dig. Dis. Sci. 31: 137S-141S.

Song, M.K. and Adham, N.F. (1978) Role of prostaglandin E_2 in zinc absorption in the rat. Am. J. Physiol. 234: E99-105.

Song, M.K. and Adham, N.F. (1985) Relationship between zinc and prostaglandin metabolisms in plasma and small intestine of rats. Am. J. Clin. Nutr. 41: 1201-1209.

Spicer, M.T., Stoecker, B.J., Orina, M., and Sangiah, S. (1992) Effects of antiulcer drugs on ^{51}Cr absorption, retention and urinary excretion in rats. FASEB J. 6: A1590 (abstract).

Takeuchi, K., Ueki, S. and Okabe, S. (1986) Importance of gastric motility in the pathogenesis of indomethacin-induced gastric lesions in rats. Dig. Dis. Sci. 31: 1114-1122.

Tao, P. and Wilson, D.E. (1984) Effects of prostaglandin E_2, 16, 16-Dimethylprostaglandin E_2 and a prostaglandin endoperoxide analogue (U-46619) on gastricsecretory volume [H^+] and mucus synthesis and secretion in the rat. Prostaglandins 28:353-366.

Tarnawski, A., Hollander, D., Stachura, J., Krause, W.J. and Gergely, H. (1985) Prostaglandin protection of the gastric mucosa against alcohol injury-A dynamic time related process. Gastroenterology 88: 334-352.

Uribe, A. (1993) Indomethacin inhibits cell proliferation in epithelium of the rat. Prostaglandins 45: 15-26.

Van Kolfschoten, A.A., Hagelen, F., Hillen, F.C., Jager, L.P., Zandberg, P. and Van Noordwijk, J. (1983) Protective effects of prostaglandins against ulcerogenic activity of indomethacin during different stages of erosion development in rat stomach. Dig. Dis. Sci. 28: 1127-1132.

Walus, K.M., Pawlik, W. and Konturek, S.J. (1980) Prostacyclin-induced gastric mucosal vasodilation and inhibition of acid secretion in the dog. Proc. Soc. Exp. Biol. Med. 163: 228-232.

Whittle, B.J.R. (1976) Relationship between the prevention of rat gastric erosions and the inhibition of acid secretion by prostaglandins. Eur. J. Pharmacol. 40: 233-239.

Whittle, B.J.R., Boughton-Smith, N.K., Moncada, S. and Vane, J.R. (1978) Actions of prostacyclin (PGI_2) and its product, 6-oxo-$PGF_{1\alpha}$ on the rat gastric mucosa in vivo and in vitro. Prostaglandins 15: 955-967.

Wilson, D.E. (1986) Therapeutic aspects of prostaglandins in the treatment of peptic ulcer disease. Dig. Dis. Sci. 31: 42S-46S.

Wolf, W., Mertz, W. and Masironi, R. (1974) Determination of chromium in refined sugars by oxygen plasma ashing flameless atomic absorption. J. Agric. Chem. 22: 1037-1042.

Yik, K.Y., Dreidger, A.A. and Watson, W.C. (1982) Prostaglandin E_2 tablets prevent aspirin-induced blood loss in man. Dig. Dis. Sci. 27: 972-974.

APPENDIX

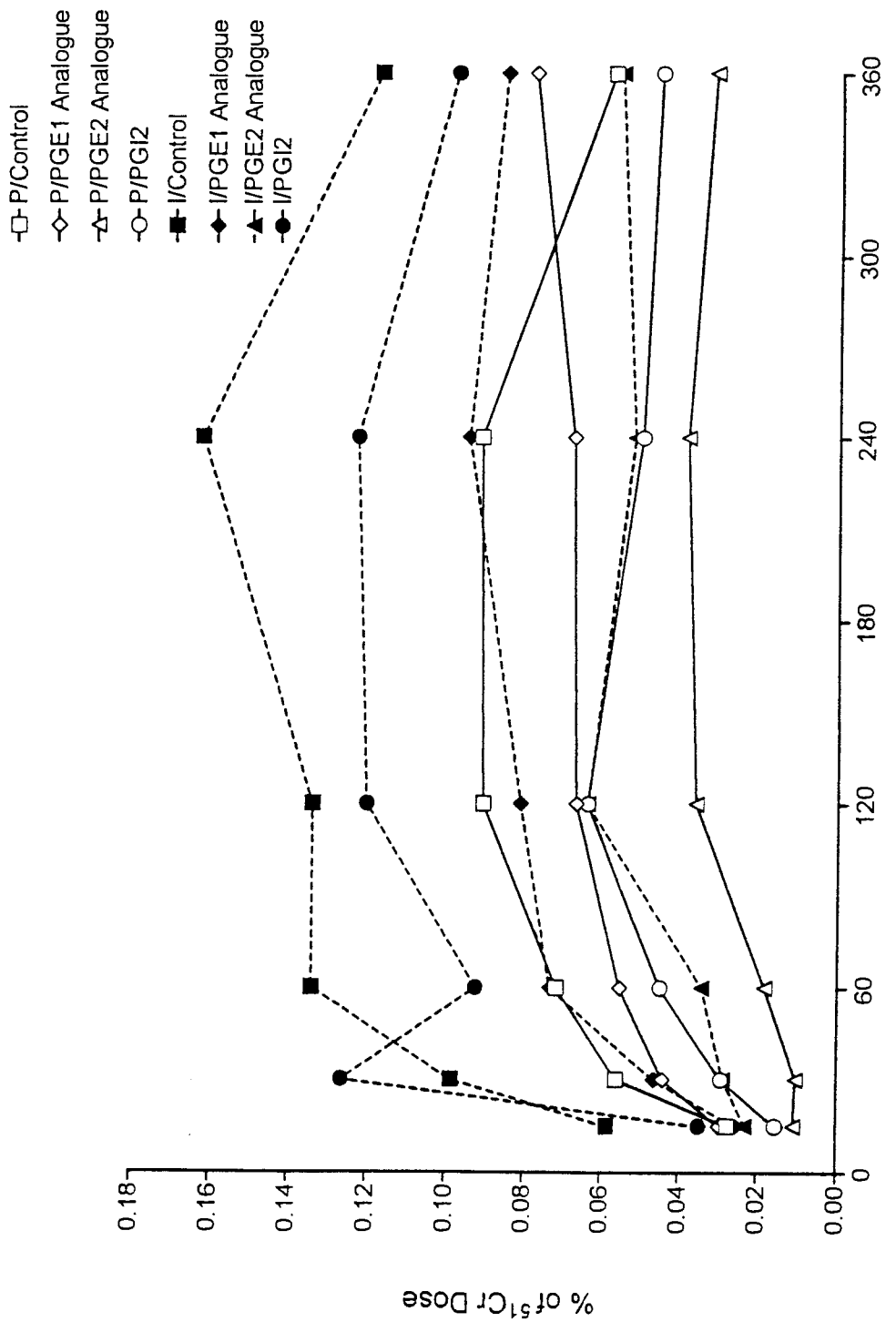

Figure A1. ^{51}Chromium in Blood

Table A1.
^{51}Chromium in Blood[1]

	0.25 hr	0.50 hr	1 hr	2 hr	4 hr	6 hr
Placebo, control	0.0275 ± 0.0113	0.0561 ± 0.0147	0.0717 ± 0.0211	0.0908 ± 0.0213	0.0915 ± 0.0273	0.0575 ± 0.0140
Placebo, PGE_1	0.0296 ± 0.0096	0.0442 ± 0.0179	0.0553 ± 0.0183	0.0668 ± 0.0176	0.0678 ± 0.0156	0.0778 ± 0.0158
Placebo, PGE_2	0.0107 ± 0.0034	0.0102 ± 0.0012	0.0182 ± 0.0025	0.0360 ± 0.0043	0.0386 ± 0.0083	0.0315 ± 0.0072
Placebo, PGI_2	0.0155 ± 0.0044	0.0292 ± 0.0069	0.0451 ± 0.0075	0.0639 ± 0.0076	0.0503 ± 0.0100	0.0454 ± 0.0090
Indomethacin, control	0.0584 ± 0.0125	0.0985 ± 0.0217	0.1340 ± 0.0239	0.1340 ± 0.0239	0.1624 ± 0.0171	0.1169 ± 0.0218
Indomethacin, PGE_1	0.0255 ± 0.0047	0.0464 ± 0.0068	0.0733 ± 0.0216	0.0811 ± 0.0106	0.0948 ± 0.0190	0.0851 ± 0.0166
Indomethacin, PGE_2	0.0229 ± 0.0053	0.0286 ± 0.0071	0.0342 ± 0.0118	0.0638 ± 0.0160	0.0522 ± 0.0064	0.0556 ± 0.0047
Indomethacin, PGI_2	0.0348 ± 0.0081	0.1263 ± 0.0524	0.0924 ± 0.0201	0.1202 ± 0.0174	0.1228 ± 0.0210	0.0977 ± 0.0150

[1]Mean ± SEM (n=6)

Table A2.
Effects of Indomethacin and Various Prostaglandins on ^{51}Cr in Blood[1,2]

Drug	15 min	30 min	1 hr	2 hrs	4 hrs	6 hrs
			% of dose x 10^{-2}			
PG Inhibitor						
Placebo	2.08^{B}	3.49^{B}	4.76^{B}	6.44^{B}	6.21^{B}	5.31^{B}
Indomethacin	3.54^{A}	7.49^{A}	8.35^{A}	9.98^{A}	10.8^{A}	8.88^{A}
S.E.M.	± 0.40	± 1.10	± 0.86	± 0.81	± 0.85	± 0.70
DRUG						
Control	4.29^{a}	7.73^{a}	10.29^{a}	11.24^{a}	12.70^{a}	8.72^{a}
PGE_1	2.76^{ab}	4.53^{ab}	6.43^{b}	7.39^{bc}	8.13^{b}	8.14^{a}
PGE_2	1.68^{b}	1.94^{b}	2.62^{c}	4.99^{c}	4.54^{c}	4.35^{b}
PGI_2	2.52^{b}	7.77^{a}	6.88^{ab}	9.20^{ab}	8.66^{b}	7.16^{ab}
S.E.M.	± 0.57	± 1.56	± 1.21	± 1.14	± 1.20	± 0.99
Source of Variation						
PG inhibitor	<0.02	0.01	0.0051	0.0034	0.0004	0.0008
Drug	<0.03	<0.03	0.0008	0.0031	0.0003	<0.02
PG inhibitor x Drug	0.20	0.16	0.48	0.62	0.21	0.24

[1]Mean ± pooled SEM. A square root transformation of data was done prior to statistical analysis.
[2]Means within a factor not sharing a common superscript are different ($p<0.05$).

Table A3.
^{51}Chromium in Urine[1]

	2 hr	4 hr	6 hr
		% of Dose	
Placebo, control	0.0614±0.0422	0.1813±0.0768	0.2499±0.1198
Placebo, PGE_1	0.0702±0.0151	0.1515±0.0357	0.3211±0.1086
Placebo, PGE_2	0.0234±0.0086	0.0707±0.0203	0.1125±0.0212
Placebo, PGI_2	0.0421±0.0076	0.1055±0.0245	0.1527±0.0362
Indomethacin, control	0.0374±0.0113	0.2646±0.0963	0.4391±0.1638
Indomethacin, PGE_1	0.0225±0.0099	0.3729±0.2019	0.4953±0.2412
Indomethacin, PGE_2	0.0312±0.0135	0.0604±0.0174	0.1382±0.0418
Indomethacin, PGI_2	0.1349±0.0726	0.4587±0.1407	0.6941±0.2258

[1]Mean ± SEM. (n=6)

Table A4.
Effects of Indomethacin and Various Prostaglandins on ^{51}Cr in Urine[1,2]

	Time		
	2 hr	4 hr	6 hr
	% of dose		
PG Inhibitor			
Control	0.049 ± 0.014	0.127 ± 0.37	0.209^{B} ± 0.069
Indomethacin	0.056 ± 0.014	0.289 ± 0.049	0.442^{A} ± 0.070
DRUG			
Control	0.049 ± 0.020	0.223 ± 0.068	0.344 ± 0.098
PGE_1	0.046 ± 0.020	0.262 ± 0.068	0.408 ± 0.098
PGE_2	0.027 ± 0.020	0.066 ± 0.068	0.125 ± 0.098
PGI_2	0.089 ± 0.021	0.282 ± 0.071	0.423 ± 0.102
Source of variation			
PG inhibitor	0.91	<0.06	0.03
Drug	0.20	<0.06	<0.09
PG inhibitor x Drug	0.14	0.27	0.29

[1]Mean ± SEM. A square root transformation of data was done prior to statistical analysis.
[2]Means within a factor not sharing a common superscript are different ($p<0.05$).

Table A5.
Effects of Indomethacin and Drugs on ^{51}Chromium in Tissues[1]

	Liver	Kidney	Heart	Lung	Testes	Bone	Spleen	Pancreas
				% of Dose				
Placebo, control	0.0121 ±0.0016	0.0114 ±0.0028	0.0011 ±0.0003	0.0018 ±0.0003	0.0022 ±0.0005	0.0010 ±0.0002	0.00056 ±0.00015	0.00028 ±0.00008
Placebo, PGE_1	0.0176 ±0.047	0.0214 ±0.0091	0.0012 ±0.0002	0.0030 ±0.0010	0.0029 ±0.0007	0.0010 ±0.0002	0.00062 ±0.00014	0.00013 ±0.00002
Placebo, PGE_2	0.0058 ±0.0013	0.0060 ±0.0012	0.0005 ±0.0001	0.0008 ±0.0002	0.0013 ±0.0003	0.0004 ±0.0001	0.00026 ±0.00006	0.00017 ±0.00008
Placebo, PGI_2	0.0082 ±0.0012	0.0110 ±0.0024	0.0007 ±0.0001	0.0012 ±0.0003	0.0017 ±0.0003	0.0007 ±0.0001	0.00036 ±0.00005	0.00015 ±0.00004
Indomethacin, ctrl.	0.0208 ±0.0037	0.0302 ±0.0083	0.0017 ±0.0003	0.0029 ±0.0006	0.0040 ±0.0008	0.0014 ±0.0003	0.00083 ±0.00014	0.00034 ±0.00008
Indomethacin, PGE_1	0.0199 ±0.0048	0.0355 ±0.0131	0.0016 ±0.0003	0.0150 ±0.0124	0.0032 ±0.0005	0.0018 ±0.0004	0.00074 ±0.00008	0.00031 ±0.00010
Indomethacin, PGE_2	0.0099 ±0.0011	0.0123 ±0.0022	0.0007 ±0.0001	0.0013 ±0.0001	0.0017 ±0.0002	0.0008 ±0.0002	0.00035 ±0.00004	0.00021 ±0.00007
Indomethacin, PGI_2	0.0211 ±0.0041	0.0403 ±0.0097	0.0012 ±0.0002	0.0027 ±0.0004	0.0035 ±0.0006	0.0019 ±0.0003	0.00064 ±0.00010	0.00029 ±0.00004

[1]Mean ± SEM (n=6)

VITA

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