THE DEFENSIVE EFFECTS OF RETINOIDS IN THE GASTROINTESTINAL TRACT (ANIMAL EXPERIMENTS AND HUMAN OBSERVATIONS)

PhD DISSERTATION

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ABBREVIATIONS

ADP: adenosyne diphosphate

AMP: adenosyne monophosphate

ATP: adenosyne triphosphate

adenocc.: adenocarcinoma

ATBC study: Alpha-Tocopherol, ß-Carotene Cancer Prevention study

BMI: body mass index

cAMP: cyclic adenosyne monophosphate

CARET: ß-Carotene and Retinol Efficacy Trial

cc.: carcinoma

CD: Crohn's disease

CDAI: Crohn's disease activity index

FAP: familiar adenomatous polyposis

GI: gastrointestinal

IBD: inflammatory bowel disease

IND: indomethacin

NSAIDs: non-steroidal antiinflammatory drugs

PGs: prostaglandins

PHS: Physician's Health Study

UC: ulcerative colitis

1. INTRODUCTION

1.1 Biochemistry of retinoids and carotenoids

Retinoids are a class of compounds consisting of four isoprenoid units bound in a head-to-tail manner. All retinoids may be formally derived from monocyclic parent compound containing five carbon-carbon double bounds and a functional group at the terminus of the acyclic portion (44) Vitamin A is a fat-soluble substance found in animal foods and dairy products. Vitamin A is available as preformed vitamin A, contained in liver, cod liver oil, butter, eggs, or as pro-vitamin A carotenoids, as found in dark green, red and yellow vegetables (7). These naturally occuring retinoids exist in the all-trans, 13-cis or 11-cis geometric configurations, with the great preponderance of the body's retinoids being present in the all-trans configuration (8). The alcohol all-trans retinol is the parent compound for all retinoids. Retinol is the precursor for synthesis of the biologically active retinadehyde and retinoic acid forms. Additionally, retinol is the precursor for the synthesis of retinyl esters (8). Within target tissues retinol is taken up by the cells from circulation and can either be oxidized to retinaldehyde, retinoic acid or esterified for storage (33). Retinaldehyde is not known to play another essential physiological role outside the vision, aside from serving as an intermediate in the enzimatic oxidation of retinol to retinoic acid (109). Retinoic acid is the biologically active retinoid form which is needed for mediating and maintaining cellular differentation (104).

1.2. Physiology of retinoids and carotenoids

All retinoids in the body originate in the diet either as provitamin A carotenoids or as preformed vitamin A (33). The dietary carotenoids and preformed retinoids undergo a series of metabolic conversions, extracellularly in the lumen of the intestine and intracellularly in the intestinal mucosa, which result in the preponderance of the absorbed dietary retinoid being converted to retinol (114). Pro-vitamin A carotenoids, such as ß-carotene, may be converted to

retinaldehyde through the cleavage by carotenoid-15,15'-dioxygenase or by an excentric cleavage pathway. Approximately 50 of over 600 carotenoids found in nature may be converted to vitamin A (114). The bioavailability of pro-vitamin A carotenoids is much less that of preformed vitamin A because of a variety of factors, including differences in efficacy of absorption and biochemical conversion (114). Although hundreds of carotenoids have been identified, only few have been found to exist in appreciable concentrations in human serum: lutein, zeaxanthin, α- and β-carotene, α- and β-cryptoxanthin (6).

Retinol is esterified in the intestinal mucosa, packaged as retinyl ester into chylomicra, and carried to the liver via the lymphatic circulation (8). A very small portion of the dietary retinoid is converted to retinoic acid (or comes in the diet as such) and enters the circulation through the portal system bound to serum albumin (116).

Approximately 90% of vitamin A in the body is stored in the liver as retinyl esters (37,43,138). The liver has the capacity to store enough vitamin A to last for several months, with longer storage capacity among adults than children (133). Retinol is released from the liver in combination with plasma retinol-binding protein (RBP) and transthyretin (TTR) (93,117). Retinol is poorly soluble in water and is carried in the blood sequestered inside the carrier proteins (124). Retinol seems to enter cells via specific receptors, although it is unclear whether all cells contain these receptors (117).

Vitamin A exerts its effects through retinoid receptors, which are found in the nucleus of cells. These receptors resemble steroid and thyreoid hormone receptors and support the idea that vitamin A acts much like a hormone (104). Retinol is converted into its active metabolite, all-trans-retinoic acid (ATRA) in the cells. Retinoic acid can control genes through specific receptors that belong to the superfamily of thyroid and steroid receptors (17,18). Retinoid acid receptors act as transcriptional activators for specific target genes. The retinoic acid receptor (RAR) is expressed as several isoforms referred to as RAR α-, β- and γ for which all-trans-

retinoic acid acts as a ligand), and retinoid X receptors (RXR) referred to as RXR α-, β- and γ for which 9-cis retinoic acid acts as a ligand (54). 9-cis retinoic acid seems to be functionally distinct from ATRA, and interconversion is may exist between the two isomers. Each RAR and RXR has a specific DNA-binding domain, a retinoic acid response element (RARE) by which these nuclear receptors may affect retinoic acid tranciptional activity. RAR and RXR receptors form heterodimers which bind to DNA and control gene activity. In addition, RXR receptors also can form heterodimers with vitamin D and thyroid hormone receptors (54). Numerous studies focus recently on the role of retinoid receptors in the process of carcinogenesis (4,57,63,115,132,139).

Retinoids and carotenoids have numerous biological functions (see detailed in discussion) such as regulating of growth, morphogenesis and differentiation of cells, they have a variety of effects on the cell membrane, they can influence the activity of different enzymes furthermore these compounds exert also immunomodulatory effects. Carotenoids are non-enzymatic antioxidants, therefore they are able to prevent genetic changes by preventing DNA damage caused by free radicals. They modulate membrane functions and stabilise initiated cells in promotional phase of carcinogenesis (114).

The role of retinoids in GI mucosal prevention has been intensively investigated in our department in the last two decades. Vitamin A and beta-carotene were shown to prevent the experimentally induced gastric mucosal lesions in animals (48), and these compounds were found to be effective in the treatment of patients with gastric peptic ulcer (97). Carotenoids have no inhibitory effects on gastric acid secretion neither in animals (48) nor in humans (42), however β-carotene was able to prevent the gastric mucosal damage in different experimental models such as ethanol (96 %) -, hydrochloric acid (0,6 M) - or indomethacin-induced mucosal damage (42,48,97) Furthermore the ulcer healing effect of vitamin A was proven in randomized, multicenter clinical studies (97). The gastroprotection induced by carotenoids does

not depend on the presence of vitamin A activity, β-ionone ring, number of unsaturated links or chemical structure of terminal part of molecules (48,74). Moreover, the analysis of gastric mucosal antioxidant mechanisms and free radical generation during β-carotene-induced gastric mucosal protection suggested that the scavenger character of β-carotene might partially explain its protective effect (129). The β-carotene-induced gastric gastroprotection was completely abolished by acute bilateral surgical vagotomy (130). Despite of these investigations, the exact mechanism of action is not fully understood yet.

Although carotenoids, such as ß-carotene, and vitamin A are often popularly regarded to be equivalent, there are large differences in biological functions of these two nutrients, especially regarding to antioxidant properties, since vitamin A is a less potent antioxidant that ß-carotene (114).

1.3. Retinoids and chemical carcinogenesis

A suboptimal diet might be related to approximately 30-60% of all cancer cases (98) the major part of which is possibly preventable by adequate dietary modifications (26). Chemopreventive potentials are conceivable for antioxidant micronutritients (9,11,12). Particularly intriguing are those with polyene structure, i.e. vitamin A-type retinoids and carotenoids such as ß-carotene (5,136,140).

Several animal and in vitro studies have now been reported related to the effects of antioxidants on cell proliferation, including GI epithelial cell proliferation. It has been observed 70 years before that the deficiency of vitamin A in animals provokes abnormal keratinizations, precancerous squamous metaplasia and cancers of various epithelia (137). The effectiveness of retinoids as inhibitors of chemical carcinogenesis of the epithelia of the digestive tract has been somewhat contradictious, although positive results have been obtained in studies of stomach, esophageal, liver and pancreatic carcinogenesis. The oral administration of retinyl ester prevented the occurence of papillomas and carcinomas in hamsters receiving polycyclic hydrocarbons (73).

Nitrosamine-induced esophageal carcinogenesis can also markedly be inhibited by administering retinyl-ester (72). Subsequent studies have shown that that synthetic retinoids such as 13-cis-retinoic acid also exert a protective effect against the induction of esophageal tumors with nitroso compounds, although etretinate was ineffective in in inhibiting esophageal carcinogenesis (73). Only few and contradictious data can be found concerning the effect of retinoids on chemically induced colon carcinogenesis. An antioxidant mixture containing ß-carotene and α-tocopherol reduced cell proliferation in the colon and rectum of mice (58). Organic and inorganic selenium with ß-carotene reduced colonic epithelial cell proliferation while concomitantly reducing the incidence and multiplicity of colon adenocarcinomas in rats given chemical carcinogens (92). Colorectal epithelial cell proliferation was reduced in rats given chemical carcinogens when given a diet low in fat and protein and high in vitamin E, selenium, vitamin A, and fiber (32). Synthetic retinoids are able to prevent azoxymethane-induced intestinal carcinogenesis in rats (53), although natural and synthetic retinoids have little effect on colon carcinogenesis iduced by aflatoxin and dimethylhydrazine (73). The few studies on liver carcinogenesis indicated that 13-cis retinoic acid was highly effective in reducing the incidence of liver tumors induced by 3-methyl-4-dimethylaminoazobenzene (19,73). Similar findings have been reported for spontaneous hepatomas in mice given various doses of retynil ester (73). Several synthetic retinoids inhibited the development of azaserine-induced pancreatic tumors in rats when admistered during the promotional phase of carcinogenesis (59). In colorectal carcinoma cell lines ascorbic acid enhanced the antiproliferative effect of vitamin A (51), emphasizing their interdependence.

1.4. Antioxidants on colorectal epithelial cell proliferation, polyp recurrence and carcinogenesis: clinical trials in patients

The effect of carotenoid supplementation on precancerous lesions and cancer incidence has been investigated in numerous clinical trials in the last two decades. Smaller trials in human

suggest that antioxidants can reduce colorectal epitethelial cell proliferation. Vitamin A, combined with ascorbic acid, α-tocopherol, furthermore selenium caused a significant reduction in the labeling index (LI) of the upper colonic crypts and the decrease in polyp recurrence in colorectal adenoma patients (14,15,16,23,65,95,103). Polyp formation could not be inhibited in larger full-scale trials (24,34,131) (details in discussion).

Prospective studies, including the particularly conclusive Basle study, Finnish cocort study, demonstrated that lower levels of retinol and carotene at entry were associated with a significantly increased relative risk for bronchus cancer and all other cancers (110,118,119) (details in discussion).

Few studies indicated a positive correlation between carotene intake and carcinogenesis. In the ß-Carotene and Retinol Efficacy Trial (CARET) (38,94) ß-carotene (30 mg) and vitamin A (25 000 IU) were supplemented for patients with high risk for lung cancer (cigarette smoking history or asbestos exposure), it was finished because of the results of the Alpha-Tocopherol, ß-Carotene Cancer Prevention Study (ATBC) (39,40).

There was an increase in cardiovascular disease and lung cancer mortality among those assigned the supplementation combination.

In the Physicians Health Study (PHS) (120) US male physicians were randomized to alternate daily 50 mg ß-carotene and 325 mg Aspirin, both active treatment, or both placebo. There was no significant effect of ß-carotene on total cancer incidence.

Numerous problems (1,10,41,126) have been pointed out with these three studies, see detailed in discussion.

1.5. Prevalance and importance of the checked GI diseases

Upper gastrointestinal (GI) ulceration from drugs and inflammatory bowel diseases (IBD) as well as GI malignancies are relative common GI pathologies which have an appreciable GI

mortality and morbidity. Furthermore, the role of environmental, genetic and nutritional factors in the aetiology of various cancers and other GI disorders emphasizes the importance of research in these GI conditions (101).

It has long been recognized that the relatively high incidence of GI ulceration arises from a variety of causes, among them stressful conditions, ulcerogenic drugs, infections, smoking and high intake of alcohol (91). While the incidence of peptic ulcer disease continues to be high, the international trend is, overall, towards a moderate decline (despite some fluctuations in reported statistics in some countries) except in individuals above 60 years of age where the incidence is increasing. Silent, or asymptomatic peptic ulcer disease which is evident in about 10% of patients is of particular importance because it often has a fatal outcome (101).

About 10% of the total Hungarian population suffer from peptic ulcer disease during the life span. It is true that the peak of disease incidence in patients (including males and females) appears after 50 years of age, however, the costs of therapy are extremely high, though we still do not know exactly how much (91).

The non-steroidal antiinflammatory drugs (NSAIDs) are widely used in medical treatment for decrease of inflammatory and pain processes in patients, however, these compounds cause often gastrointestinal mucosal damage (H^+ back diffusion, hemorrhage, bleeding) and different GI complains (dullness, epigastrial pain, vomitig) in a significant group of treated patients (78). The extent of NSAID consumption increased significantly in the Hungarian population suffering from different joint diseases. The number of patients who consumed NSAIDs treating their joint diseases is about 2-3 millions (20-30% of the nation) It is unambiguous that the number of patients suffering from NSAIDs-induced gastrointestinal mucosal damage increased in the last decade (91). This fact is enough for doing research in this field.

GI mucosal lesions induced by NSAIDs are considered to involve multiple pathogenic elements, such as deficiency of prostaglandins (PGs), gastric hypermotility, disturbances in

microcirculation, oxygen free radicals, neutrophil activation and direct damaging effect on the gastric mucosa (weak vs strong acid-induced physico-chemical properties, like salicylic acid) (78,125). However, these components closely interact with each other and it would be difficult to determine which pathogenic element is of prime importance in the ulcerogenic response to NSAIDs. Indeed, neutrophil activation is caused by alteration of arachidonic acid metabolism, such as PG deficiency; gastric hypermotility leads to microcirculatory disturbances, resulting enhancement of neutrophil adherence to the vascular endothelium; and the production of oxygen radicals is brought about by neutrophil-endothelium cell interaction as well as haemodynamic alterations due to gastric hypermotility (125).

Nowadays, the extents and ratio of cyclooxigenase 1 (Cox-1) and cyclooxigenase 2 (Cox-2) inhibition are dominantly in the international interesting (52,76). The extent of actions of NSAIDs on COX 1. and 2. inhibition can be separated from each other experimentally, but we don't have enough concrete data on the NSAIDs induced decrease of gastric mucosal blood flow (GMBF) in patients, however, it's sure that it's extent can not reach such level to be able to produce a real tissue hypoxia in the gastric mucosa (78).

Studies performed in animals can improve us to understand the mechanisms of GI mucosal damage and help in prevention. The presence of tissue hypoxia has been suggested as one of the main aetiological factors in the development of gastric mucosal injury (in acute ulcer phase and in other injuries in later phase) based on the basic physiological blood flow observations of Menguy et al (66-69).

A wide biochemical-pharmacological approach of ulcer disease has been applied in our Department in the last three decades (Mózsik et al.) (80,84,85,87,89) and this shift could be observed in international research.

A special experimental approach to this problem has been established by the simultaneously carried out measurements of the biochemical compounds of membrane-bound ATP-dependent

energy systems such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and cyclic adenosine monophosphate (cAMP) which gives an excellent biochemical „cross-section" of the target (stomach) organ together with lactate level (80,84,85,87,89).

The energy is stored in form of ATP in the gastric mucosa and the measurement of it's actual tissue level informs us about the dynamic equilibrium between the ATP resynthesis and breakdown. The gastric mucosal ATP can be splitted in direction of ADP (by membrane ATPase) or cyclic AMP (by adenylate cyclase) liberating a free energy source for the tissue functions, while ADP and cAMP transforms to AMP supplying further energy liberation. AMP is a common energy storing adenosine compound originating from both pathways of ATP-ADP and ATP-cAMP breakdowns (transformations). The liberated energy is used for the regulation of the different functions of the cells (80,84,85,87,89).

When the energy of different cellular functions originates from the gastric mucosal ATP breakdown, however, the results of activity of plasma membrane enzymes (membrane ATPase, adenylate cyclase) need to recognise in the changes of mucosal ADP, cAMP and AMP. Furthermore, different biochemical parameters can be calculated from the values of ATP, ADP, AMP: a) adenylate pool (ATP+ADP+AMP),

b) ratio of ATP/ADP and

c) „energy charge" {(ATP+0.5 ADP) / (ATP+ADP+AMP)} (82).

The value of energy charge is theoretically 1, when all of adenosine compounds are in phosphorylated form, and 0, when these compounds are in dephosphorylated form (2)

After the basic observations of Menguy et al. (66-69), experimental and clinical researchers accepted these results as a strong correlation existing between the decrease of gastric mucosal blood flow and the decrease of gastric mucosal ATP level was considered to be a consequence of tissue hypoxia in the gastric mucosa (without presence of any scientifically proved

arguments) (82). The biochemical measurement of the decrease of gastric mucosal ATP is not enough proof for the existence of tissue hypoxia alone, because the simultaneous elevation of tissue lactate is also basically necessary (82). The biochemical measurements of adenosine compounds offer an excellent biochemical research information on the extent of tissue oxygenation, when the observations are simultaneous from the same tissue sample. Furthermore, the existence of tissue hypoxia can be characterized well by the extremely low values of tissue ATP, "energy charge" and increased levels of tissue lactate (78,82).

It is very important to emphasize that the observations of Menguy et al. were carried out in rats with hemorrhagic shock (66-69).

Earlier Davenport proved that weak acids induce gastric H^+ backdiffusion in presence of a strong – like HCl – acid (20,21). Later Mozsik et al. demonstrated the decreased breakdown of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) in rats treated with sodium salicylate plus pylorus-ligation (81), while it's value increased only in pylorus-ligated rats (80). These and other observations proved the role of ATP dependent energy systems in the development of gastric hyperacid secretion in this experimental model.

Na-salicylate and indomethacin are both NSAIDs, however, their actions are significantly different, e .g. salicylate acts as week acid, meanwhile, indomethacin produces inhibition of prostaglandin synthesis (75,77,83). These previous data suggest a significant difference in the metabolism of the stomach, in time of development of the gastric mucosal damage produced by sodium salicylate and indomethacin (20,21). However, we have only few data on drugs-induced intracellular metabolic changes in the GI mucosa, therefore our aim was to investigate this matter on the membrane-bound ATP dependent energy systems (ATP-ADP, ATP-cAMP) and to study the changes in the feedback mechanisms between them.

The incidence of IBDs is distinctly on the rise in Europe and USA though few studies consider this fact partly to be the consequence of improved efficiency in their diagnosis (71). It is true that with a total number of approximately 80 illnesses per 100 000 inhabitants they are comparatively rare but these illnesses are not only a burden for the individual suffer but also put them under considerable psychological strain. The aetiology of IBDs still remains unclear. Although hereditary factors, microorganisms harmful to the mucosa and certain nutritional (eating) habits are assumed to be causal factors there is a little hard evidence to substantiate these assumptions (50,127). The familiar association of IBDs, reinforced by the demonstration of increased incidence of both Crohn's disease and ulcerative colitis in monozygotic twins, is quite compelling evidence for an inherited component in the aetiology of both these diseases (29,50). Genetic markers, such as HLA class II antigens and cytokine polymorphisms, have provided interesting data and may well prove additional factors in the predisposition to IBD. Of the HLA associations observed, that for the DR2 system is relatively strong. Within this system, however, there appear to be variations that may be due to ethnic origins (e.g. high frequency of DRB 1.1502 alleles in Japanese and Jewish patients with ulcerative colitis but not in white non-Jewish subjects) (50,111).

Crohn's disease is frequently complicated by various nutritional disturbances (17,55). Although it is important to correct these disturbances in treating of CD patients, the nutritional status of CD patients has been poorly documented, especially concerning vitamin status. Among various reports of vitamin A deficiency in different gastrointestinal and liver diseases (49,134), relatively few studies have investigated CD (30, 141). There has been inadequate documentation of the prevalence of vitamin A deficiency in patients with CD who are a population at a risk of protein malnutrition and fat malabsorption as a result of diseased or resected small bowel. No observations can be found in the literature describing the changes of all serum carotenoids in patients with IBD and GI cancer.

Colorectal adenocarcinoma is the second most common malignancy in Europe and the USA and leads to considerable mortality (13,56). The disease is rare in Asia and Africa, this difference is thought to be largely environmental rather than racial. 45% of all GI malignancies is colorectal compared with 25% stomach cancer, 15% pancreatic cancer, 11% esophageal cancer and 4% liver and bile duct cancer in West-European Countries (46). The prevalence of GI cancers is increasing in Hungary except of gastric cancer. The incidence of colorectal cancer increases with age, the average age at diagnosis is between 50 and 60 years (56).

Colorectal cancer has multifactiorial aetiology, numerous environmental and genetic aspects have been investigated. It is estimated that perhaps 35% (range 10-70%) of all cancer mortality in the United States could be attributable to dietary factors (99,136). Ecologic and migration studies indicate the importance of environmental factors in colon cancer (108). Diet appears to have a particularly strong association with occurence of this cancer, and thus it offers promise for intervention (99,136). The observation that higher colon cancer mortality rates occures in the countries where meat and fat was higher led to the hypothesis that these food items contributed to an individual's risk of developing colon cancer and was a stimulus for the current interest in dietary intake in most analytic studies of the etiology of colon cancer (100). **The oldest hypothesis** asserts that fat intake increases bile acid production, ultimately increasing the exposure of the bowel mucosa to the toxic, trophic and cancer promoting effects of bile acids. The capacity of colonic flora to transform bile acids into potential carcinogens has been found to be greater in populations with high rates of colon cancer and among meat-eating populations than in vegetarian populations (100). **A more recent hypothesis** is the cooked food hypothesis. High-fat diets content greater amounts of carcinogenis heterocyclic amines (from meat proteins) and promoters as a consequence of cooking at high temperature (cooking in fat produces higher temperature than cooking in water) (18). **A third and recent hypothesis** is that high consumption of meat, particularly red meat, may increase fecal concentrations of iron, which

catalyses oxidative reactions, leading to increased lipid peroxidation and oxidative DNA damage and to an increased risk of colorectal cancer (3). Furthermore the protective role of vegetable and fruit consumption, dietary fibers, calcium, vitamin D and the potential carcinogenic effect of succrose have been proved in the last decades (99).

The role of dietary components is well known in another types of GI malignancies also. Consumption of salted, pickled and smoked foods have been associated with increased risk of gastric cancer in case-control studies (31). The strong, consistent inverse association between consumption of fruits and vegetables clear. Relatively high intake of ß-carotene and vitamin C is consistently associated with reduced risk of gastric cancer, the Basle study found a negative correlation between serum carotene levels and risk of gastric cancer. Similar results were found in case of esophageal cancer (31).

2. AIMS

The aims of these thesis were: 1. To prove further intracellular effects of carotenoids on the membrane-bound ATP-dependent energy systems in a well identified animal experiment and 2. To measure serum levels of carotenoids (vitamin A, lutein, zeaxanthin, α-, β-carotene, α-, β-cryptoxanthin) with or without the presence of vitamin A activity in patients with different gastrointestinal acute and chronic inflammations, precancerous and malignant diseases.

2.1. The investigation of NSAID induced gastric mucosal damage in Na-salicylate and IND treated rats

2.1.1. Gastric mucosal damage (number and severity of mucosal lesions) and changes in gastric secretory responses

2.1.2. To check the energetic changes in gastric mucosa

2.1.3. The investigation of the protective effects of vitamin A and ß-carotene in IND-induced ulcer model

2.1.3.1. The effect of vitamin A and ß-carotene on number, severity of gastric mucosal lesions and gastric secretory responses

2.1.3.2. Vitamin A and ß-carotene induced changes in gastric mucosal energy systems

2.2. Changes of serum carotenoid levels in different GI diseases (human observations)

Few and contradictious data can be found in the literature related to the possible changes of serum levels of carotenoids in different human diseases (9,11,34,110, 117-119), therefore we checked these parameters in the following GI pathological conditions:

2.2.1. GI inflammatory diseases

2.2.1.1. Gastritis (with and without Helicobacter pylori colonisation)

2.2.1.2. Crohn's disease

2.2.1.3. Ulcerative colititis

2.2.2. Patients with hepatitis and cirhhosis (HCV and alcoholic)

2.2.2.1 Patients with alcoholic hepatitis

2.2.2.2 Patients with HCV hepatitis

2.2.2.3 Patients with alcoholic cirrhosis

2.2.3. Colorectal polyps (with different histological classification)

2.2.4. Malignant GI diseases

2.2.4.1. Esophagus carcinoma

2.2.4.2. Gastric adenocarcinoma

2.2.4.3. Pancreas adenocarcinoma

2.2.4.4. Hepatocellular carcinoma

2.2.4.5. Colorectal adenocarcinoma

3. MATERIALS AND METHODS

3.1. The animal experiments were carried out random on both sexes of CFY (Sprague-Dawley) strain rats (LATI, Gödöllõ, Hungary), weighing 180 to 210 body weight. The animals were fasted 24 hr before the experiments, but water was allowed ad libitum.

The animals were separated into different groups and went over different surgical procedures and pharmacological treatments. The observation periods were done at 4 h in all groups of animals, meanwhile the different surgical interventions and the intragastric and systemic treatments were done at 0 hr of the observations. The following experimental groups were created:

3.1.1.group A: sham-operation (laparatomy) was carried out (control group);

3.1.2. group B: pyloric ligation was carried out alone;

3.1.3. group C: pyloric ligation was done, the animals received 2 ml saline solution intragastrically (i.g.);

3.1.4. group D: the treatment of animals was the same that in group B, however they received i.g. 200 mg/kg sodium salicylate dissolved in 2 ml of 150 mmol/L HCl;

3.1.5. group E: the rats went over pyloric ligation (as in group B) and received 2 ml saline solution i.g, and furthermore were treated with 20 mg/kg indomethacin s.c. The animals received saline solution+indomethacin treatment immediately after surgery;

3.1.6. group F: animals received 20 mg/kg indomethacin (at the onset of observations) without any surgical intervention;

3.1.7. group H: the treatment of animals was the same as in group F and they received vitamin A or ß-carotene (in doses of 0.1-1.0-10 mg/kg) per os.

Animals in all groups received 2 ml saline solution subcutaneosly to protect them from dehydration. The animals were sacrified at 4 h after the surgical procedure and the pharmacological treatments, when the gastric secretory responses (volume and acid output) and

gastric mucosal lesions were noted. The biochemical observations were carried out from the homogenate of gastric mucosa at the end (4h) of the observations.

The tissue levels of ATP, ADP, AMP, and lactate were measured enzymatically (Boehringer Ingelheim, Germany), while tissue level of cAMP by RIA (Beckton-Dickinson, Orangeburg, USA). The protein content was assayed by the method of Lowry et al (60). The ratio of ATP/ADP, the values of adenylate pool (ATP+ADP+AMP) and the „energy charge" {(ATP+0.5 ADP) / (ATP+ADP+AMP)} were calculated (88).

The gastric secretory responses (volumes, acid output) were measured in the 4h pyloric ligated rats. The volume of gastric secretion was expressed in ml/100 g body weight / 4 h while the gastric acid secretion in μE/100 g body weight / 4 h. The number of acute haemorrhagic mucosal lesions was counted, while the severity was evaluated by a semiquantitative scoring system, described previously (88) in a blinded fashion without knowledge of previous treatment. A computerized planimetric method was also used in part of the study to confirm the evaluation of lesions and a close correlation ($r = 0.92$, $p<0.01$) was found between these two methods (118). The gastric mucosal lesions were expressed as number and severity (means+/- SEM). The parametric results were mathematically evaluated by Anova test, while semiquantitative results (ulcer severity) by Mann-Whitney's test.

3.2. Human observations: carotenoids in human serum samples were measured with high pressure liquid chromatography (HPLC) method (25). We measured the serum level of vitamin A, lutein, zeaxanthin, alfa- and beta-cryptoxanthin, alfa- and beta-carotene.

Preparation of samples for HPLC measurements : 2 ml of the serum sample was shaken with 2 ml of 96% ethanol for 2 min., following by an extracting with 3 ml of hexane. The mixture was centrifuged for 10 min.. As an internal standard canthaxantin was added to the removed homogenous organic phase. Then it was evaporated to dryness in vacuum, and the residue was

dissolved in the mixture of 300 µl methanol and 80 µl dichlormethane. 50 µl of this solution was injected. The chromatographic system consisted of a gradient former Model 250 B (Gynkotec, Germany), HPLC pump Model 300 B Glenco injector (Gynkotec,Germany), and a time programmable UV-vis detector Model 166-2. equipped with Gold chromatography software (Beckmann, USA). The column was 150x4.6 mm packed with Chromsil-C 0.186 mm not endcapped reversed phase packing. The eluent was 3% (v/v) water in methanol (A), methanol (B) and 40 % (v/v) dichlormethane in methanol. The flow rate was 1.5 ml/min.. The gradient program was 100% A 30 sec., to 100% B in 3 min., to 100% C in 4 min.(linear steps). The time program of wavelength was 325 nm for 3.5 min.(detecting vitamin A), then 450 nm (detecting other carotenoids). Quantification: The chromatograms were evaluated quantitatively by relating the peak areas of the individual compounds to that canthaxantin used as internal standard. The ratios of the molar extinctions of the authentic samples to that of canthaxantin were employed as correction factor of the detector signals.

The results were given in µmol/liter. The results of 47 healthy persons (24 males, age: 50±12 years, 20 females, age: 49±10 years) were used as control values in the statistical analysis. The results were expressed as means±SEM. Anova test was used for the statistical evaluation.

The serum levels of patients with the following GI diseases were checked:

3.2.1. Patients with GI inflammatory diseases:

3.2.1.1. 35 patients with gastritis (among them **24 with Helicobacter pylori colonisation**) (20 males, age: 37±15 years, 15 females 36±12 years);

3.2.1.2. 49 patients with an established diagnosis of CD (21 males, 28 females, their age ranged rom 17 to 51 years; means ± SD: 27.4±13.4 years) were included in this study. The duration between the onset of the symptoms and the measurement was 4.7±3.8 years. The site of involvement was the small bowel only in 20 patients, the large bowel only in 7

and both in 22. There were no patients with any previous history of intestinal resection All of the patients were free of any nutritional treatment, non of them was receiving vitamin A supplements. The Crohn's disease activity (CDAI) index was higher than 150 in 22 patients and lower in 28 patients (CDAI: Best et al, Gastroenterology 70:439-44,1970). The majority of them were out-patients and maintenance therapy was given them.

3.2.1.3. 35 patients years with ulcerative colitis (17 males, age: 35±15 years, 18 females age: 33±12). The majority of them were out-patients and maintenance therapy was given them.

3.2.2. Patients with hepatitis and cirrhosis

3.2.2.1. 8 patients with alcoholic hepatitis (8 males, age 38±10 years);

3.2.2.2. 75 patients with chronic C hepatitis (45 males, age 42±14 years, 32 females age: 38±12 years);

3.2.2.3. 25 patients with alcoholic liver cirrhosis (17 males, age 40±15 years, 8 females age: 38±12 years);

3.2.3. Patients with adenomatous colorectal polyps (59 patients, 35 males, age: 59±15 years, 24 females, age 62±13 years); 39 patients had tubular adenoma (10 with mild, 16 with moderate, 13 with severe dysplasia), 11 patients had villouse adenoma (4 with mild, 4 with moderate, 3 with severe dysplasia) and carcinoma was found in the removed polyp in 9 cases. The patients were separated into 4 groups depending on the grade of dysplasia.

3.2.4. 98 patients with different types of gastrointestinal malignancy were checked. The majority of them were in a good general condition, only some of them had metastasis. The patients were divided into smaller groups depending on the localisation of their disease.

3.2.4.1. 8 patients with esophagus carcinoma (cc. planocellulare) (8 males, age: 60±10 years);

3.2.4.2. 21 with gastric cancer (adenocc.) (16 males, age 64±12 years, 5 females age: 68±10 years);

3.2.4.3. 10 patients with pancreas adenocarcinoma (6 males, age 56±11 years, 4 females age: 63±9 years);

3.2.4.4. 15 with hepatocellular carcinoma (8 males, age 60±8 years, 4 females age: 57±13 years);

3.2.4.5. 44 patients with colon adenocarcinoma (26 males, age 66±16 years, 18 females age: 65±14 years);

The diagnosis was based on clinical, laboratory, endoscopic, radiographic and histological findings. The histopathological diagnosis was based on the World Health Organisation (WHO) classification (47), the histopathologists in the two medical centres were blinded to the serum results.

4. RESULTS

4.1. Results in animal experiments

4.1.1. Gastric secretory responses and development of gastric ulcers in 4 hr pylorus-ligated plus sodium salicylate or indomethacin treated rats

The results are presented in Table I. and Figs 1-4. The gastric secretory responses were unchanged in pylorus-ligated rats without vs. with intragastrically applied 2 ml saline solution (group B vs. group C). When the animals received sodium-salicylate (group D) intragastrically then the volume of gastric secretory volume increased ($p<0.001$) meanwhile the gastric acid output decreased significantly ($p<0.001$). No significant changes were found in gastric secretion in pylorus ligated plus indomethacin (group E) vs. only pylorus-ligated rats (group B), however, gastric ulceration was found in group E. The gastric secretory response did not modify the indomethacin induced gastric ulceration (group E vs. group F) with or without pyloric ligation.

Vitamin A and β-carotene did not influence the gastric secretory responses. After the co-treatment of IND+vitamin A or β-carotene (group H) the number and severity of IND-induced mucosal injury decreased dose dependently (Figs. 1-4.).

These results clearly indicate both sodium salicylate and indomethacin produce gastric mucosal injury in the same extent, however the changes in the gastric secretory responses (volume, acid output) are completely different. Furthermore the gastric secretory responses (volume and acid output) are the same in only pylorus-ligated (group B), pylorus-ligated plus intragastrically 2 ml saline solution treated (group C) rats than those in pylorus-ligated plus intragastrically saline plus indomethacin treated group (group E). These observations prove that the changes in the gastric secretory responses (volume, acid output) can be separated from the development of drug-induced mucosal damage (at least under these experimental circumstances).

Table I. Gastric secretory response and gastric mucosal damage in the studied experimental groups of animals (means +- SEM) (n=number of animals).

Groups of animals (n)	Gastric secretion (ml/100 g b.w./4h)	Acid output (uEq/100 g b.w./4 h)	Number and severity of gastric mucosal lesions	
A (n=20)	-	-	-	-
B (n=11)	1.9+-0.2	180+-20	-	-
C (n=11)	2.0+-2 NS	190+-20 NS	-	-
D (n=10)	3.1+-0.4^{+++}	110+-15^{+++}	14.2+-1^{+++}	27.2+-2.1^{+++}
E (n=11)	2.2+-0.1NS	140+-20 NS	17+-2^{+++}	20+-1^{+++}
F (n=20)	-	-	19+-1^{+++}	20+-1^{+++}

Abbreviations: Group A: sham-operated rats; group B: pyloric-ligated rats; Group C: pyloric-ligated rats+2 ml saline solution was given intragastrically; Group D: pyloric-ligated rats+200 mg/kg sodium salicylate dissolved in 2 ml of 150 mmol/l HCl; Group E: pyloric ligation+2 ml saline solution intragastrically+20 mg/kg indomethacin sc.; Group F: animals received 20 mg/kg indomethacin (at the onset of observations) without any surgical intervention;

P values were calculated between the Group C vs. D and Group C vs. F. Abbreviations: NS, not significant; +++: $P<0.01$

Figs. 1-4.: Number and seceerity of gastric mucosal lesions in animals treated with IND, IND+vitamin A or ß-carotene (means±SEM).

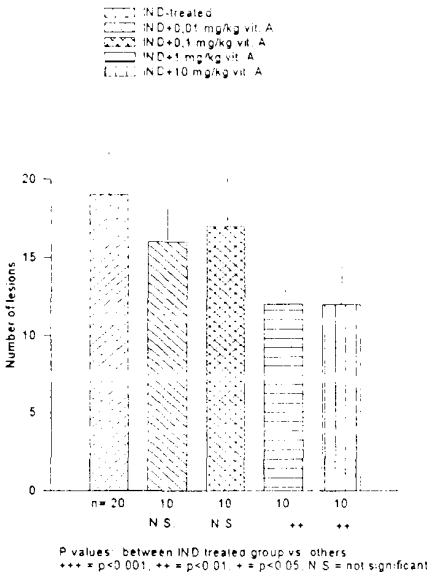

Fig. 1. Number of gastric mucosal lesions in animals treated with IND+vitamin A (means±SEM).

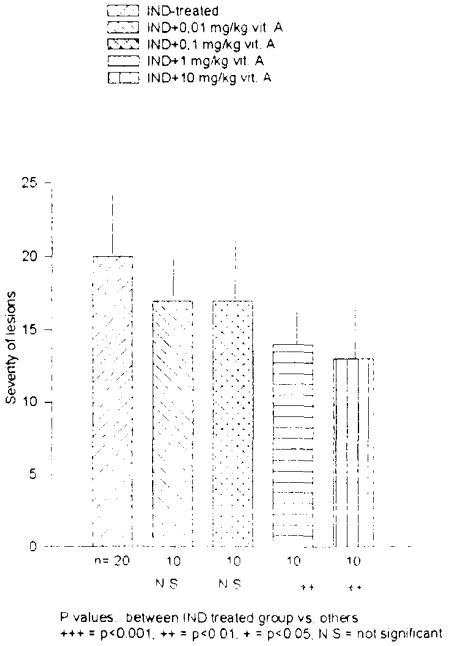

Fig. 2. Severity of gastric mucosal lesions in animals treated with IND+vitamin A (means±SEM).

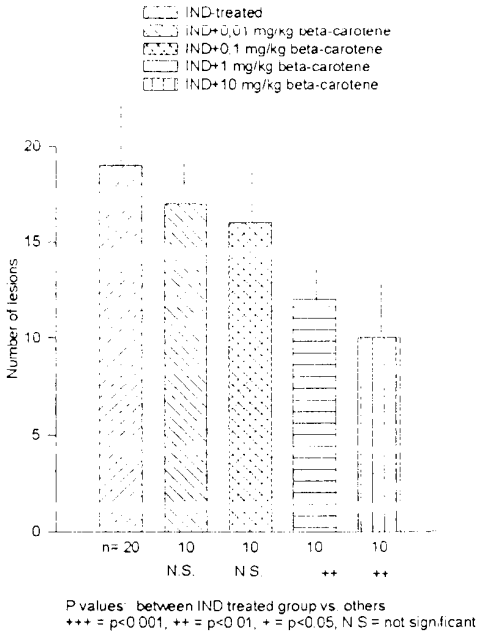

Fig. 3. Number of gastric mucosal lesions in animals treated with IND+ß-carotene (means±SEM).

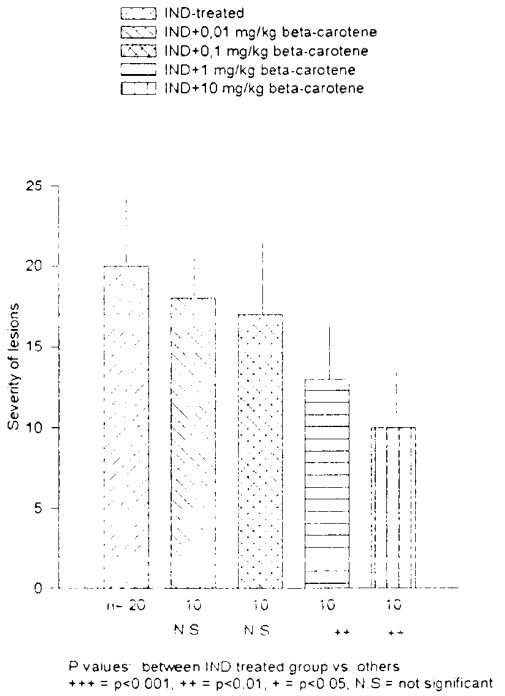

Fig. 4. Severity of gastric mucosal lesions in animals treated with IND+ß-carotene (means±SEM).

4.1.2 Sodium salicylate and indomethacin induced metabolic changes in the gastric mucosa in 4 h pylorus-ligated rats

The biochemical results in sodium salicylate and indomethacin-treated animals are presented in Table II and Figs. 5-18

The gastric mucosal ATP decreased significantly in the group B ($p<0.001$), C ($p<0.001$), D ($p<0.001$), E ($p<0.001$) and F ($p<0.001$). The gastric mucosal ADP increased in the groups B ($p<0.001$), C ($p<0.001$) and F ($p<0.001$), while it's value decreased in groups D ($p<0.001$) and E ($p<0.001$). The gastric mucosal AMP was decreased in all (B,C,D,E,F) groups. The gastric mucosal cAMP was also decreased in all (B,C,D,E,F) groups. The values of adenylate pool (ATP+ADP+AMP) was decreased in all (B,C,D,F) groups. The values of energy charge are between 0.4 and 0.5. The ratio of ATP/ADP decreased significantly in group B ($p<0.001$), C ($p<0.001$), E ($p<0.001$) and F ($p<0.001$), meanwhile it's value was increased significantly in group D (Table II.).

After the co-treatment of IND+vitamin A (0.01-0.1-1.0-10.0 mg/kg body weight) or beta-carotene (0.01-0.1-.1.0-10.0 mg/kg) (group H), the tissue level of ADP decreased dose-dependently, meanwhile the tissue level of ATP, ATP/ADP, cAMP, AMP increased significantly and dose-dependently, however the adenylate pool showed an increasing tendency by the administration of increasing dosage of vitamin A and beta -carotene. The value of energy charge did not change significantly by the application of vitamin A and beta-carotene (Figs. 5-18.)

Table II.

Changes in the gastric mucosal biochemical parameters in the groups mentioned in Table 1. The biochemical results were expressed as means +- SEM, n indicates the number of animals

Groups of animals Measured and calculated experimental parameters	A (n=20)	B (n=11)	C (n=11)	D (n=20)	E (n=11)	F (n=20)
ATP[x]	11.1+-0.4	2.3+-0.2^{+++}	3.6+-0.8^{+++}	6.8+-0.4^{++}	5.7+-0.3^{+++}	4.6+-0.3^{+++}
ADP[x]	13+-0.4	22+-1^{+++}	16.9+-0.6^{+++}	8+-0.6^{++}	11+-0.2^{+++}	17.8+-0.4^{+++}
AMP[x]	14.1+-0.5	4.8+-0.3^{+++}	9.1+-0.3^{+++}	7.3+-0.4^{++}	5.2+-0.2^{+++}	10.5+-0.3^{+++}
Ratio of ATP/ADP	0.85+-0.05	0.11+-0.01^{+++}	0.21+-0.01^{+++}	2.1+-0.0[illegible]$^{+++}$	0.52+-0.02^{+++}	0.29+-0.01^{+++}
„Adenylate pool"[+]	38.3+-1	29.1+-0.8^{+++}	29.6+-0.8^{+++}	32.1+-0.[illegible]$^{+++}$	22.+-0.7^{+++}	32.1+-0.6^{+++}
„energy charge"	0.46+-0.02	0.48+-0.02	0.4+-0.02^{+++}	0.49+-0.02^{+++}	0.51+-0.02	0.42+-0.01^{+++}
cAMP[xx]	3.35+-0.03	0.73+-0.01^{+++}	1.05+-0.02^{+++}	1.08+-0.01^{+++}	1.47+-0.01^{+++}	1.34+-0.02^{+++}

Abbreviations: Group A: sham-operated rats; group B: pyloric-ligated rats; Group C: pyloric-ligated rats+2 ml saline solution was given intragastrically; Group D: pyloric-ligated rats+200 mg/kg sodium salicylate dissolved in 2 ml of 150 mmol/l HCl; Group E: pyloric ligation+2 ml saline solution intragastrically+20 mg/kg indomethacin sc.; Group F: animals received 20 mg/kg indomethacin (at the onset of observations) without any surgical intervention;

[x]=nmol/mg gastric mucosal protein
[xx]=pmol/mg gastric mucosal protein

P values were calculated between the results obtained in group A vs. others obtained in the different groups.

Figs. 5-18. Changes in biochemical parameters in saline-, IND, IND+vitamin A or ß-carotene treated groups

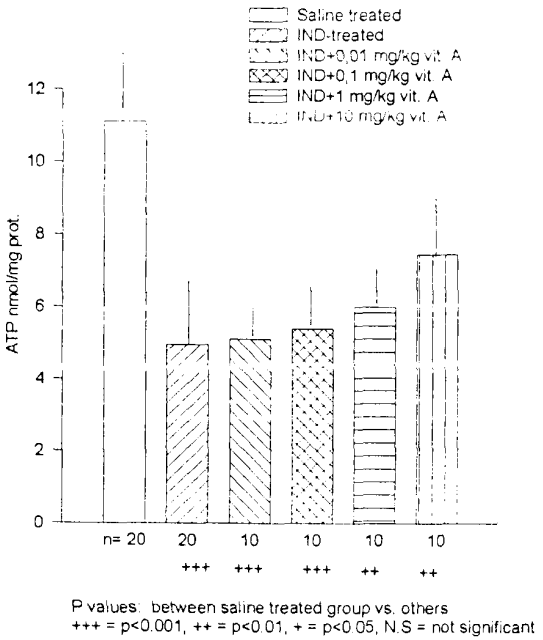

Fig. 5. Gastric mucosal ATP level in saline-, IND, IND+vitamin A treated groups (means±SEM, nmol/mg gastric mucosal protein)

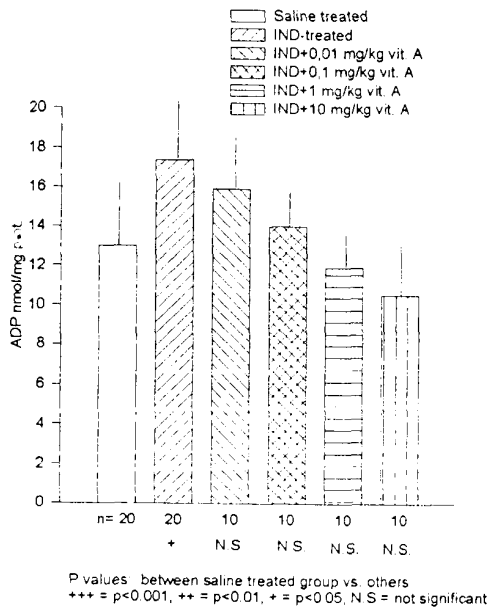

Fig. 6. Gastric mucosal ADP level in saline-, IND, IND+vitamin A treated groups (means±SEM, nmol/mg gastric mucosal protein)

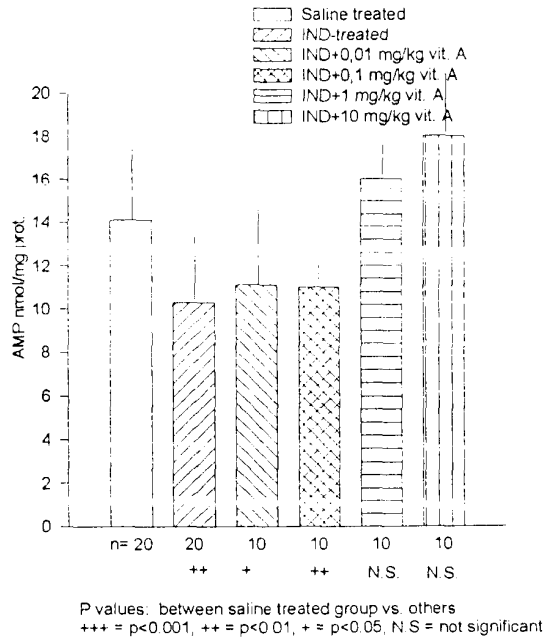

Fig. 7. Gastric mucosal AMP level in saline-, IND, IND+vitamin A treated groups (means±SEM, nmol/mg gastric mucosal protein)

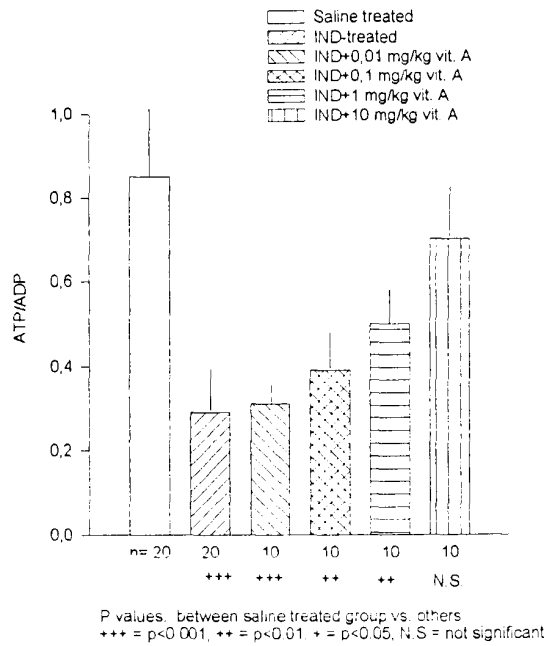

Fig. 8. Gastric mucosal ATP/ADP ratio in saline-, IND, IND+vitamin A treated groups (means±SEM)

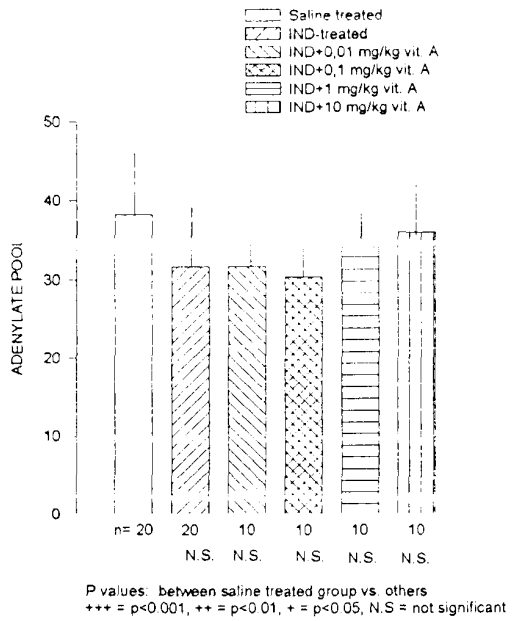

Fig. 9. Gastric mucosal adenylate pool in saline-, IND, IND+vitamin A treated groups (means±SEM)

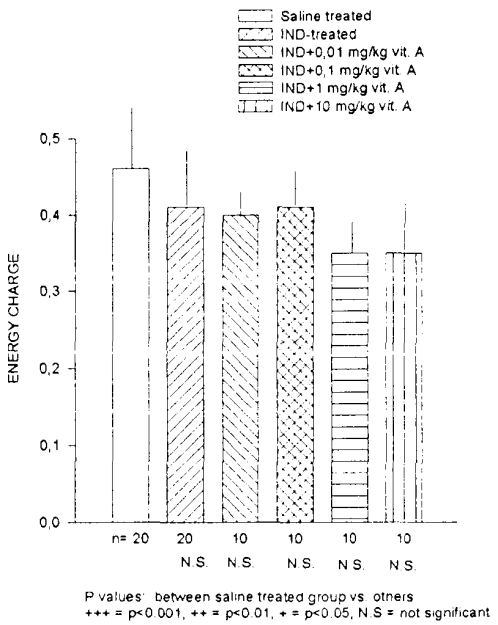

Fig. 10. Gastric mucosal energy charge in saline-, IND, IND+vitamin A treated groups (means±SEM)

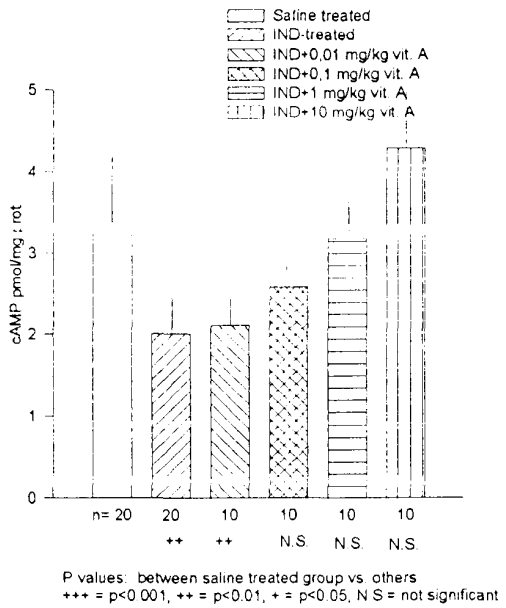

Fig. 11. Gastric mucosal cAMP level in saline-, IND, IND+vitamin A treated groups (means±SEM, pmol/mg gastric mucosal protein)

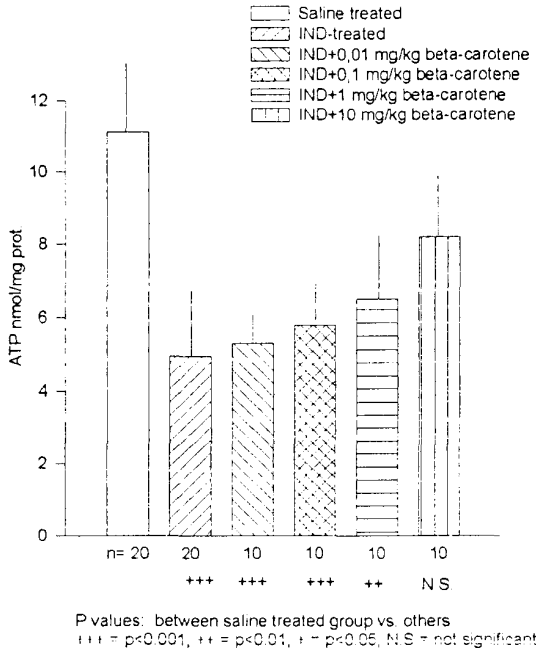

Fig. 12. Gastric mucosal ATP level in saline-, IND, IND+beta-carotene treated groups (means±SEM, nmol/mg gastric mucosal protein)

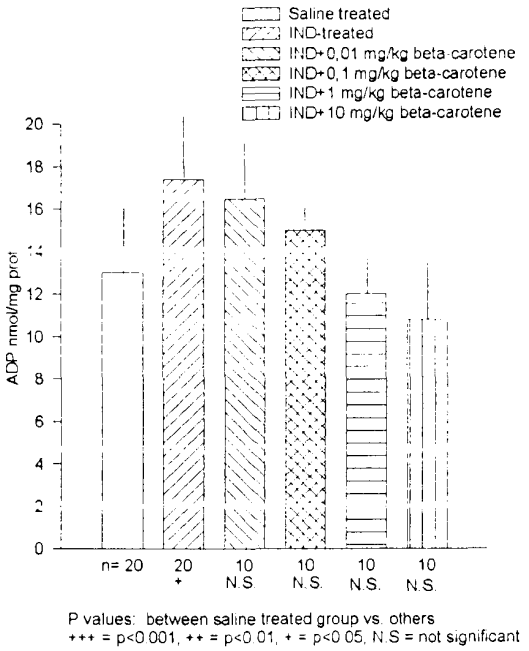

Fig. 13. Gastric mucosal ADP level in saline-, IND, IND+beta-carotene treated groups (means±SEM, nmol/mg gastric mucosal protein)

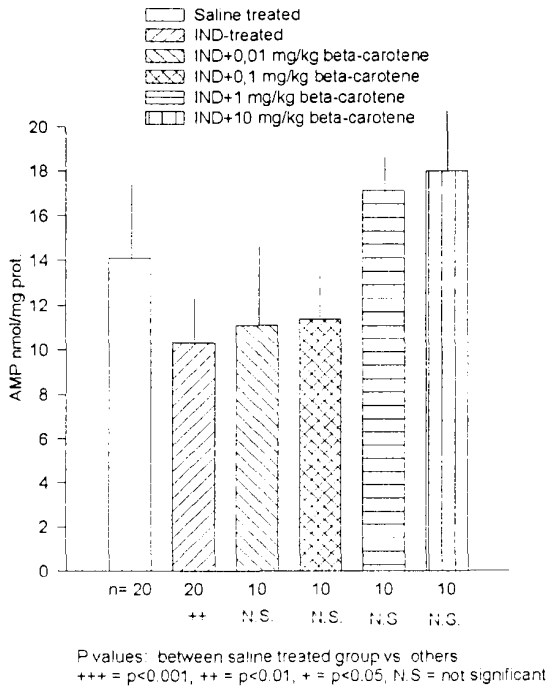

Fig. 14. Gastric mucosal AMP level in saline-, IND, IND+beta-carotene treated groups (means±SEM, nmol/mg gastric mucosal protein)

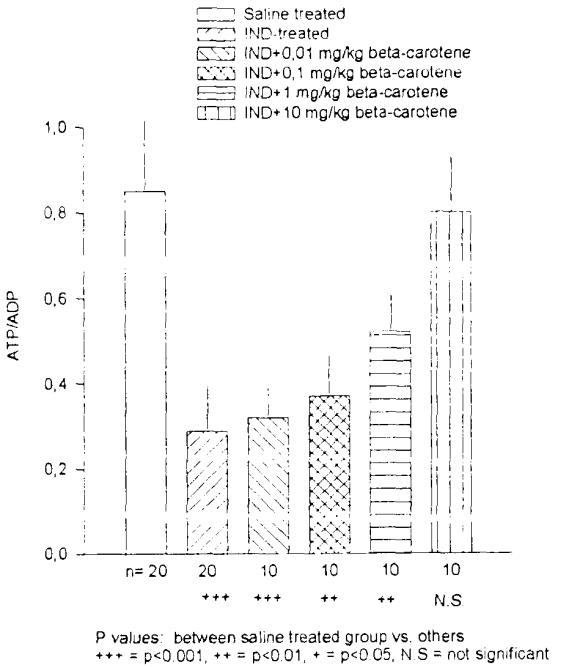

Fig. 15. Gastric mucosal ATP/ADP ratio in saline-, IND, IND+beta-carotene treated groups (means±SEM, nmol/mg gastric mucosal protein)

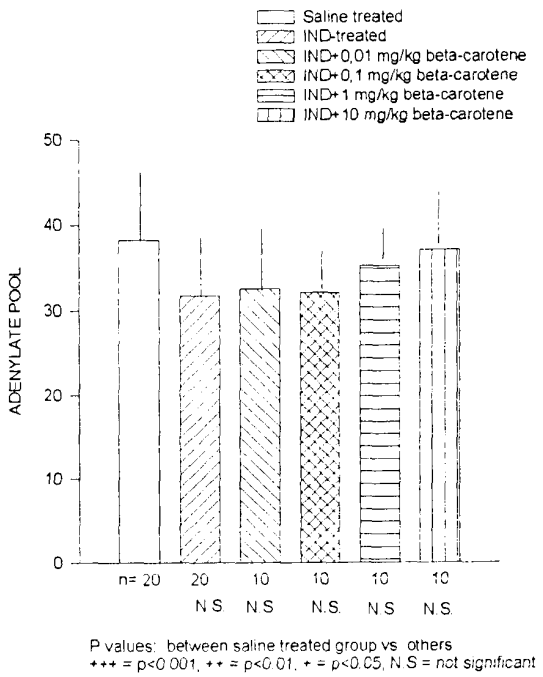

Fig. 16. Gastric mucosal adenylate pool in saline-, IND, IND+beta-carotene treated groups (means±SEM, nmol/mg gastric mucosal protein)

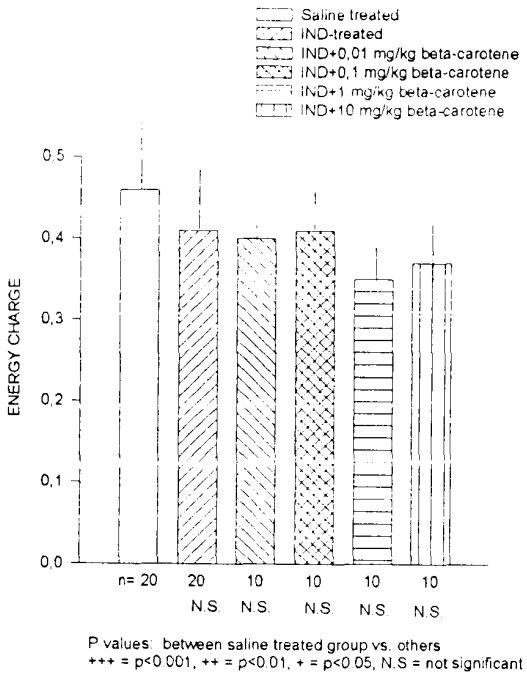

Fig. 17. Gastric mucosal energy charge in saline-, IND, IND+beta-carotene treated groups (means±SEM, nmol/mg gastric mucosal protein)

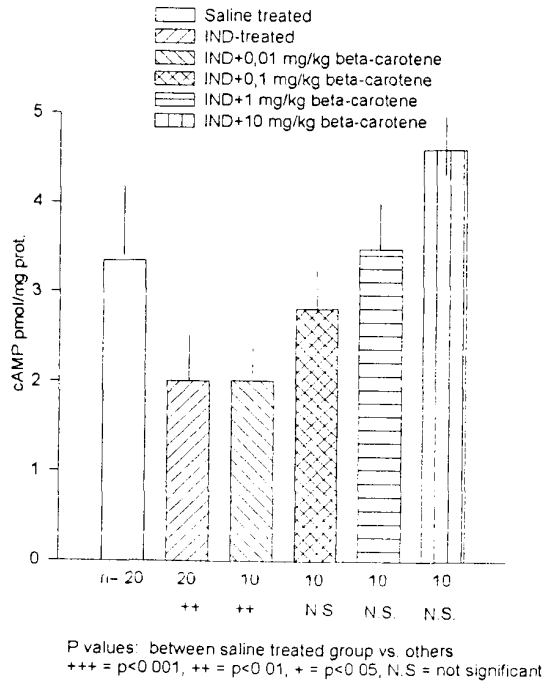

Fig. 18. Gastric mucosal cAMP level in saline-, IND, IND+beta-carotene treated groups (means±SEM, pmol/mg gastric mucosal protein)

4.2. Human observations

4.2.1. Patients with inflammatory GI diseases (Figs. 19-24).

Significant changes were not found between the results of healthy volunteers and patients suffering from gastritis with or without Helicobacter pylori colonisation.

Five of the seven measured carotenoids were depleted in CD patients (107), the serum levels of vitamin A, luthein, zeaxanthin, α- and β-carotene were significantly lower in patients than in the control group (zeaxanthin: $p<0.001$; vitamin A, lutein, α- and β-carotene: $p<0.01$).

The serum level of α- and β-cryptoxanthin showed a slight, but not significant decrease.

A strong correlation was found between vitamin A level and CDAI ($r=0.7$). There was no correlation between the duration of symptoms and carotenoid status. We did not find significant differences in the serum levels of carotenoids depending on the site of involvement.

The patients had lower mean serum albumin and cholesterol levels then the controls (not significant), but there was no correlation between these parameters and vitamin A level (Table IV.).

We found a significant decrease in the serum level of vitamin A ($p<0.001$) and β-carotene ($p<0.001$) in patients with ulcerative colitis (108), a moderate, but not significant decrease in the serum level of lutein and zeaxanthin, all of the other checked carotenoids were in the normal range. There was no correlation between the duration of symptoms and carotenoid status. We did not find significant differences in the serum levels of carotenoids depending on the site of involvement. Neither in patients with ulcerative colitis, nor in CD patients were found differences depending on gender.

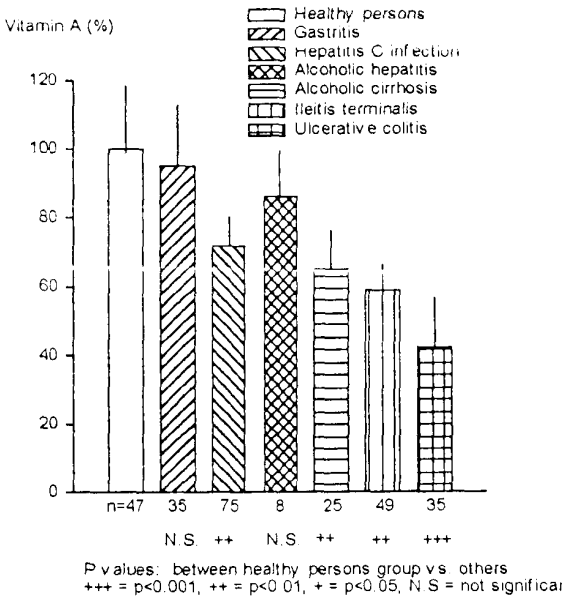

Fig. 19. Serum level of vitamin A in patients with gastritis, alcoholic and HCV hepatitis, alcoholic liver cirrhosis, Crohn's disease and ulcerative colitis (expressed in percent values of healthy subjects as means±SEM)

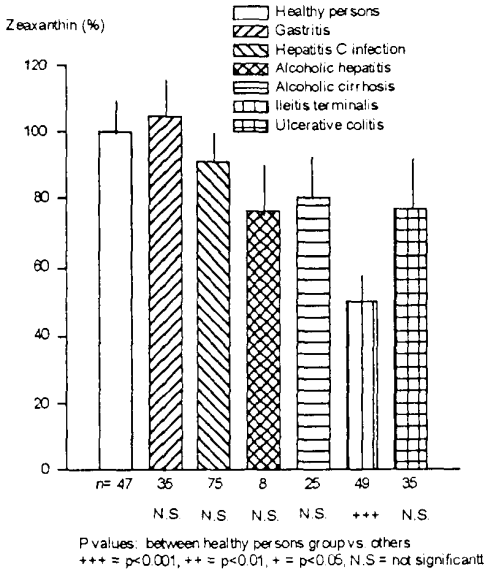

Fig. 20. Serum level of zeaxanthin in patients with gastritis, alcoholic and HCV hepatitis, alcoholic liver cirrhosis, Crohn's disease and ulcerative colitis (for further explanation see text on Fig. 19.)

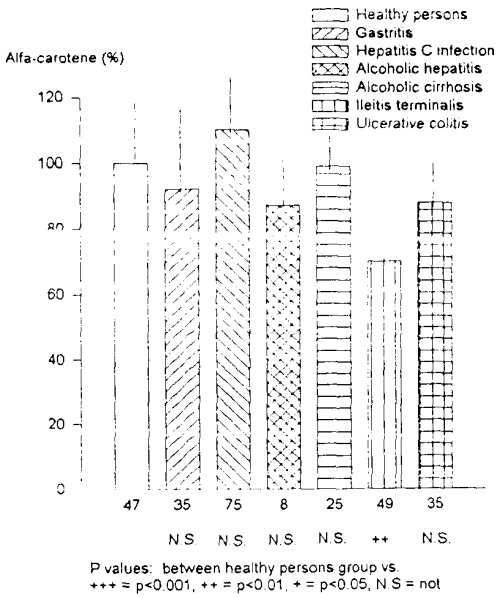

Fig. 21. Serum level of α-carotene in patients with gastritis, alcoholic and HCV hepatitis, alcoholic liver cirrhosis, Crohn's disease and ulcerative (for further explanation see text on Fig. 19.)

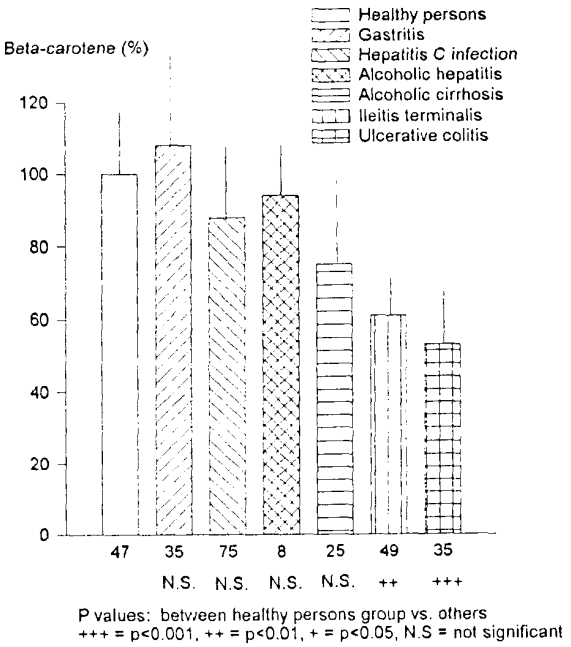

Fig. 22. Serum level of β-carotene in patients with gastritis, alcoholic and HCV hepatitis, alcoholic liver cirrhosis, Crohn's disease and ulcerative colitis (for further explanation see text on Fig. 19.)

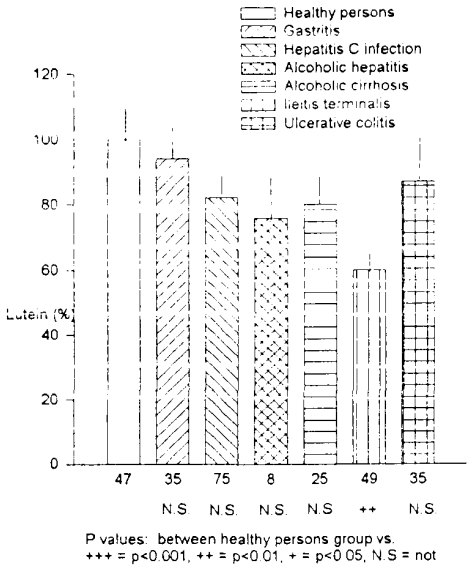

Fig. 23. Serum level of lutein in patients with gastritis, alcoholic and HCV hepatitis, alcoholic liver cirrhosis, Crohn's disease and ulcerative colitis
(for further explanation see text on Fig. 19.)

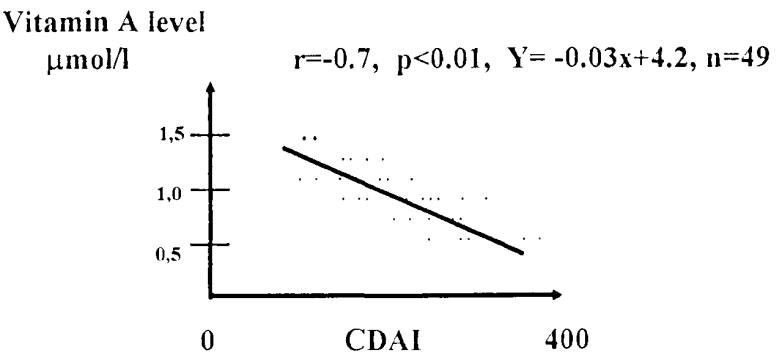

Fig. 24. Correlation between vitamin A level and CDAI in patients with Chrohn's disease

4.2.2. Patients with colorectal polyp (Figs. 26-30.)

The serum levels of vitamin A ($p<0.01$) and zeaxanthin ($p<0.01$) were significantly lower in patients with different histological types of colorectal polyp, than in the control group (106). The most significant decrease was found in patients with adenocarcinoma in the polyp.

The serum level of beta-cryptoxanthin and beta-carotene showed a slight, but not significant decrease.

The serum level of lutein and alfa-cryptoxanthin did not differ significantly from the control values.

Carotenoids are fat soluble therefore the absorption of these materials depends on fat absorption. In case of malnutrition or malabsorption the serum carotenoid levels will be also lower than the normal values. To preclude the possibility of malabsorption and malnutrition, the body mass index (BMI) of the patients, the serum level of cholesterol, albumin, total protein and haemoglobin were checked. Neither in patients with ulcerative colitis nor in patients with colorectal polyp differed these parameters significantly from that of controls (Table IV.), these parameters did not indicate malabsorption or malnutrition.

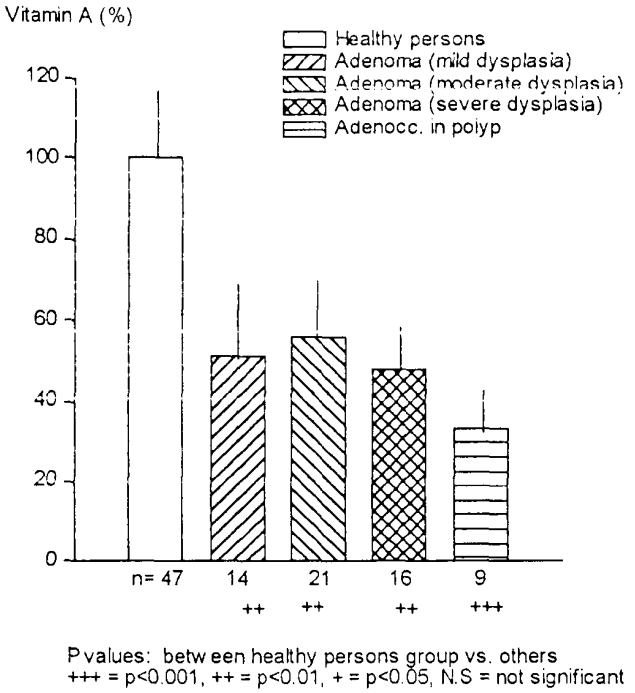

Fig. 26. Serum vitamin A levels in patients with different histologically graded colorectal polyps (for further explanation see text on Fig. 19.)

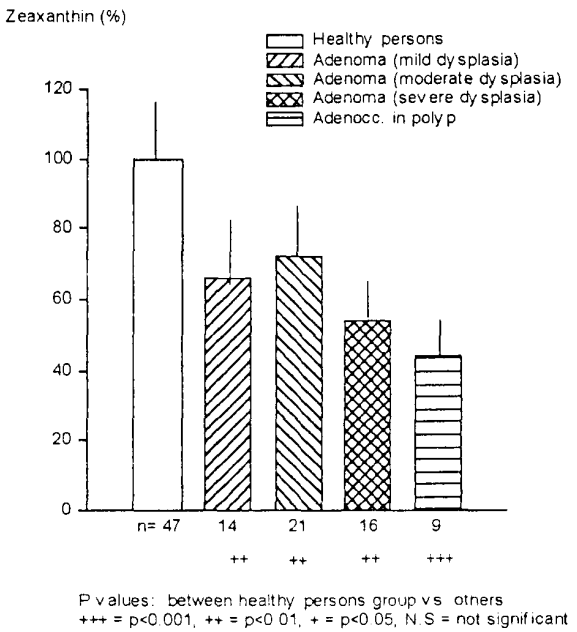

Fig. 27. Serum zeaxanthin levels in patients with different histologically graded colorectal polyps (for further explanation see text on Fig. 19.)

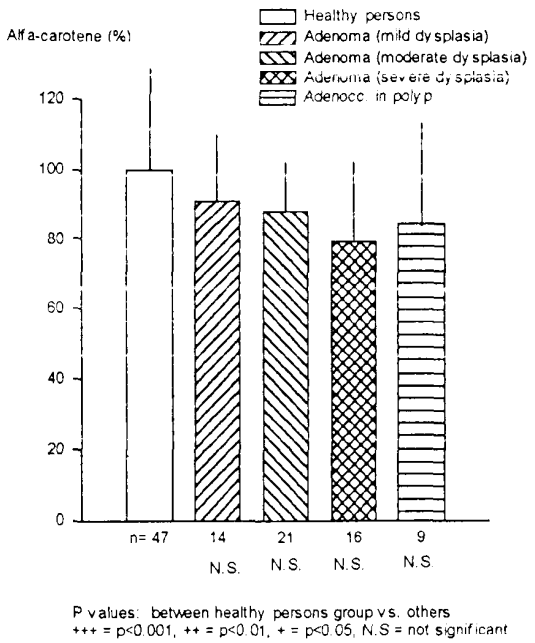

Fig. 28. Serum α-carotene levels in patients with different histologically graded colorectal polyps (for further explanation see text on Fig. 19.)

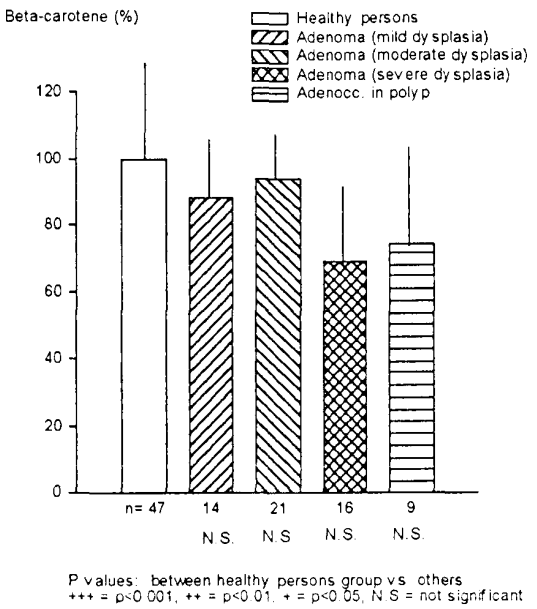

Fig. 29. Serum β-carotene levels in patients with different histologically graded colorectal polyps (for further explanation see text on Fig. 19.)

The serum level of β-cryptoxanthin and β-carotene showed a slight, but not significant decrease in patients with pancreas and colorectal adenocarcinoma, significant decrease was found in not one of the checked diseases. The serum level of lutein, α-carotene and β-cryptoxanthin did not differ considerably from the control values.

Significant differences were not found between the results of males and females, neither in the control group, nor in the patients with adenoma therefore we did not calculate the results of genders separated. The checked serum parameters did not indicate malabsorption or malnutrition (Table IV.).

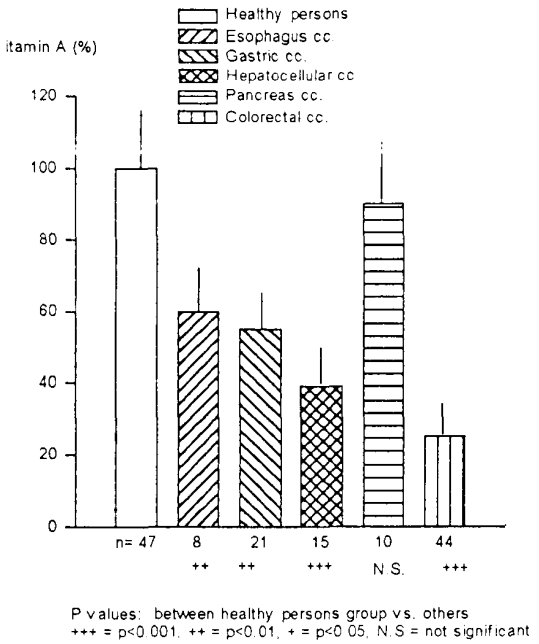

Fig. 31. Serum levels of vitamin A in different human malignant GI diseases (for further explanation see text on Fig. 19.)

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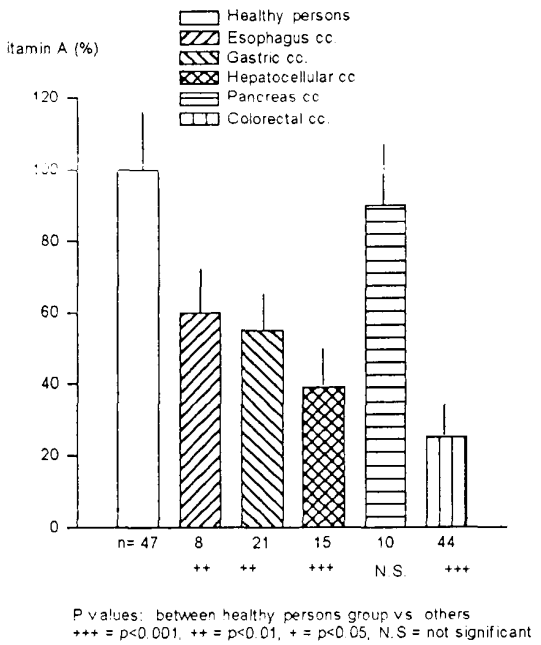


Fig. 31. Serum levels of vitamin A in different human malignant GI diseases (for further explanation see text on Fig. 19.)

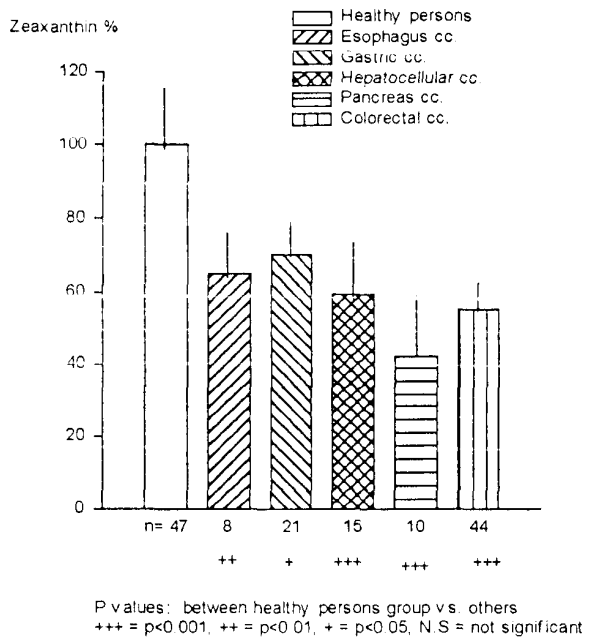


Fig. 32. Serum levels of zeaxanthin in different human malignant GI diseases (for further explanation see text on Fig. 19.)

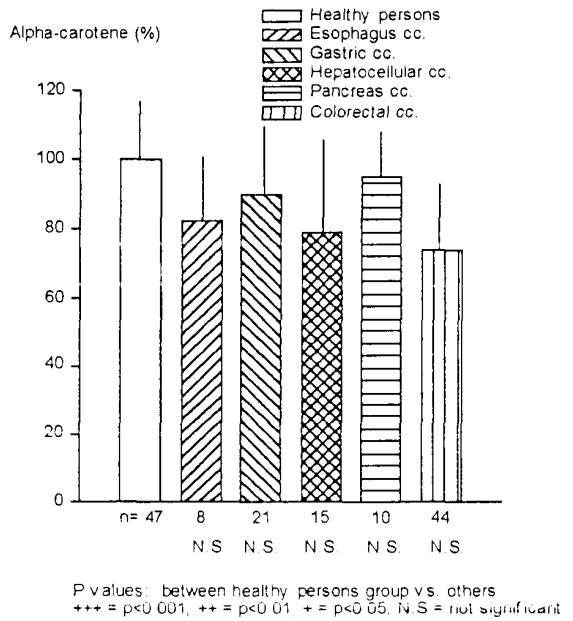


Fig. 33. Serum levels of α-carotene in different human malignant GI diseases (for further explanation see text on Fig. 19.)

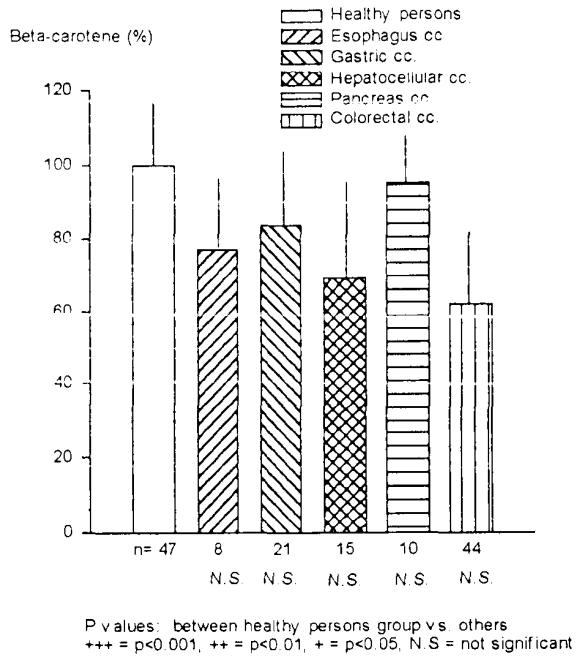


Fig. 34. Serum levels of β-carotene in different human malignant GI diseases (for further explanation see text on Fig. 19.)

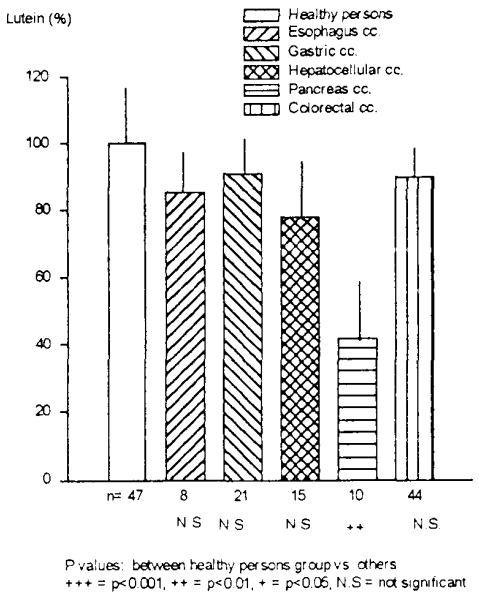


Fig. 35. Serum levels of lutein in different human malignant GI diseases (for further explanation see text on Fig. 19.)

Table III.

Changes of serum carotenoids in patients with different GI diseases (compared to the results of healthy subjects)

	VITAMIN A	ZEAXANTHIN	α-CAROTENE	β-CAROTENE	LUTEIN	α-CRYPTO-XANTHIN	β-CRYPTO-XANTHIN
GASTRITIS n=35	NS	NS	NS	NS	NS	NS	NS
C HEPATITIS n=75	↓↓	NS	NS	NS	NS	NS	NS
ALC. HEPATITIS n=8	NS	NS	NS	NS	NS	NS	NS
ALC. CIRRHOSIS n=25	↓↓	NS	NS	NS	NS	NS	NS
CROHN'S DISEASE n=49	↓↓	↓↓↓	↓↓	↓↓	↓↓	NS	NS
ULCERATIVE COLITIS n=35	↓↓↓	NS	NS	↓↓↓	NS	NS	NS
COLORECTAL POLYP n=59	↓↓	↓↓	NS	NS	NS	NS	NS
ADENOCC. IN POLYP n=9	↓↓↓	↓↓↓	NS	NS	NS	NS	NS
ESOPHAGUS CC. n=8	↓↓	↓↓	NS	NS	NS	NS	NS
GASTRIC CC. n=21	↓↓	↓	NS	NS	NS	NS	NS
HEPATOCELLULAR CC. n=15	↓↓↓	↓↓↓	NS	NS	NS	NS	NS
PANCREAS CC. n= 10	NS	↓↓↓	NS	NS	↓↓	NS	NS
COLORECTAL CC. n=41	↓↓↓	↓↓↓	NS	NS	NS	NS	NS

↓↓↓= p<0,001; ↓↓=p<0,01; ↓=p<0,05; NS= not significant

Table IV.: BMI, serum cholesterol, albumin, total protein and hemoglobin levels in different groups of patients (means±SD)

	BMI	SE CHOL. mmol/l	SE ALB. g/l	TOTAL PROT. g/l	HGB g/l
HEALTHY (n=47)	24±4.4	5.6±1.25	39.9±4.23	75.6±7.18	139±18.
GASTRITIS (n=35)	25.3±4.8	5.81±2,1	40.9±5.01	72.2±6.2	135 ±12
C HEPATITIS (n=75)	23.5±5.9	5.45±1.46	35.8±4.75	68.54±6.77	130±14.3
ALC. HEPATITIS (n=8)	26.7±4.3	5,85±1.52	36.9±4.53	74.82±7.84	129.±18
ALC. CIRRHOSIS (n=25)	28.1±5.6	3.6±0.85	28.9±4.23	70.18±8.65	121±16
CD (n=49)	22±3.8	4.64±0.71	32.9±4.25	71.15±9.56	122±10
ULCERATIVE COLITIS (n=35)	25.8±4.3	5.44±0.91	34.3±5.45	75.56±7.68	120±10
COLORECTAL POLYPOSIS (n=59)	26.9±6.8	5.84±1.21	37.8±3.23	72.18±6.23	135±7
ADENOCC.IN POLYP (n=9)	26.5±6.4	5.73±1.22	41.3±4.96	73.45±9.78	139±14
ESOPHAGEAL CC. (n=8)	24.3±7.1	6.01±1.12	36.31±6.21	74.13±8.74	131±13
GASTRIC CC. (n=21)	23.5±5.8	5.86±1.26	42.9±4.95	69.25±7.68	126±15
PANCREAS CC. (n=10)	22.2±4.2	5.62±0.89	39.9±5.58	67.65±5.84	114±18
HEPATOCELL. CC. (n=15)	25.8±6.1	5.95±1.32	33.6±5.05	65.15±8.81	120.96±19
COLORECTAL CC. (n=44)	24.9±5.1	6.01±1.32	34.9±4.53	71.45±8.88	125±20

5. DISCUSSION

Different experimental circumstances were used for the critical evaluation of physiological (volume of gastric secretion, acid output) and pathological (number and severity of gastric mucosal damage) changes in the membrane-bound ATP-dependent energy systems and the gastric mucosal damage produced ig. administration of sodium salicylate (in dose of 200 mg/kg dissolved in 150 mmol HCl) and by indomethacin (20 mg/kg sc. given).

The following main actions (mechanisms) were tested: a.) action of sc. administered indomethacin on the gastric mucosal damage (group A vs. F); b.) the possible role of gastric hypersecretion on the development of gastric mucosal damage (without application of any drug) (group A vs. B); c.) the increase of gastric volume (without any increase of gastric H^+ secretion) (group B vs. C); d.) the actions of sodium salicylate on the gastric secretory volume, acid output and mucosal damage (group C vs. D); f.) the effect of indomethacin on the gastric secretory responses (volume, acid output) and on gastric mucosal damage in 4 hr pylorus-ligated rats (group C vs. E); g.) the comparison of the damaging effect of indomethacin without (group F) and with (group E) gastric hypersecretion; h.) the biochemical correlation (as members of membrane-bound ATP-dependent energy systems) in gastric mucosa or gastric physiological (pathological) events (group A vs. F).

The gastric hypersecretion (group C) alone can not produce gastric mucosal lesion, which is associated with an extremely increased ATP-ADP transformation (and with the decrease of ATP-cAMP transformation) (114), meanwhile it is inhibited by surgical vagotomy (117). If the animals are treated with ig. saline solution (2 ml) (group B) thereafter neither the gastric secretory volume nor the acid output will not change in comparison with only pylorus-ligated (group C) rats, furthermore the changes in ATP-dependent energy systems are the same. If animals received 200 mg/kg sodium salicylate ig. dissolved in 2 ml 150 mmol/l HCl, then the gastric acidity (output) decreased significantly ($p<0.001$), while the

volume of gastric secretion increased ($p<0.001$) and gastric mucosal lesions appeared (group C vs. D). The tissue level of ATP was higher in sodium salicylate treated rats than in pylorus-ligated+ig. saline solution treated animals. Indomethacin (without any surgical intervention) produced gastric mucosal damage in association with similar changes in the ATP-dependent energy systems as those were in pylorus-ligated (group B) and pylorus-ligated+ig. with saline solution treated animals. It means that indomethacin increases the ATP-ADP transformation (in association to a decreased ATP-cAMP transformation). The indomethacin treatment (group E) did not modify the gastric secretory responses (volume and acid output) in pylorus-ligated animals in comparison those with only pylorus-ligated rats.

These results demonstrate that neither gastric secretory responses nor changes in the gastric mucosal ATP-dependent energy systems did not cause separatedly gastric mucosal injury. The significant part of gastric H^+ diffused back, meanwhile the volume of gastric secretion (dominantly water) increased. Salicylic acid is a weak acid, and it's dissociation is inhibited in the presence of HCl. The undissociated salicylic acid is able to absorb from the stomach and the gastric mucosa is precipitated in this way.

The mitochondrial ATP is a common substrate for membrane ATP-ase and for adenylate cyclase in presence of Mg^{++} (82,85,86,87) and furthermore, these enzymes can be stimulated or inhibited by various hormones, mediators and drugs (82,85,86,87).

The actual levels of membrane bound ATP dependent energy systems are as equilibrium their extens of ATP breakdown and of resynthesis. The tissue level of ATP resynthesis is impaired under the circumstances of tissue hypoxia which can be detected biochemically by the together presence of a: a significant increase in tissue level of lactate, and b: failure of ATP resynthesis due to impaired oxidative phosphorilation by tissue hypoxia. The

significant decrease of tissue ATP level was observed alone in many experimental models, but without any increase in the tissue level of lactate (see details in references 82,85,87).

The measured ATP, ADP and cAMP (in case of normal oxidative phosphorilation) indicate the equilibrium between extents of ATP-ADP and ATP-cAMP transformations. These responses show that a feedback mechanism exists between the two membrane-bound ATP-dependent energy systems. The system is characterized mainly by the following: a: ATP is a common substrate for both the membrane (Na^+-K^+ dependent ATPase and adenylate cyclase); b: drugs which stimulate membrane ATP-ase activity inhibit the transformation of ATP into cAMP by adenylate cyclase; c: drugs, mediators and hormones inhibiting membrane ATP-ase activity stimulate adenylate cyclase activity and vica versa; d: the increased transformation of ATP into ADP indirectly inhibits the transformation of ATP to cAMP; e: the decrease of transformation of ATP into ADP by various agents leads to indirect stimulation of the transformation of ATP to cAMP; f: the increase in ATP transformation to cAMP leads to an indirect inhibition of ATP into ADP (82), g: cAMP and AMP inhibit directly the transformation of ATP to ADP by inhibition of membrane ATPase (85); h: the inhibition of the transformation of ADP into cAMP is the result of the indirect stimulation of the transformation of ATP to ADP, i: the extent of cAMP transformation to cAMP through regulation of phosphodiasterase by drugs may regulate both ATP-ADP and ATP-cAMP regulation; j: the molar dose of drugs regulate the membrane ATPase are smaller molar concentrations then those effecting or modifying the adenylate cyclase activity (82,85,86,87).

The analysis of membrane-bound ATP-dependent energy systems in animals treated with sodium salicylate (group D) and indomethacin (group E) in pylorus-ligated rats indicate an increased extent of ATP-ADP transformation after indomethacin treatment in comparison with that of sodium salicylate treated animals. There is a limited (or decreased)

metabolic adaptation in the ATP energy system after sodium salicylate treatment (79). The prostaglandin levels decreased after indomethacin treatment, which is associated with a significant decrease of cAMP in the gastric mucosa. In the sodium salicylate treated animals decreased the metabolic adaptation significantly, meanwhile the ATP-ADP transformation increased in indomethacin treated animals, however PGs. decreased

The results of membrane-bound ATP dependent energy systems were expressed as means (100 %) +/- SEM (%) in the control animals (group A). The changes of membrane bound ATP dependent energy systems were expressed in percent values (means+/- SEM) of control animals in other groups, and the p values were calculated between the controls (A) vs. others (B,C,D). this analysis of the results offered an excellent possibility to approach the final results produced by different extracellular and intracellular regulatory feedback mechanisms in the gastric mucosa. The careful analysis of the biochemical observations indicate that the first lines of ATP breakdown are in the levels of ATP-ADP and ATP-cAMP (because tissue level of lactate was unchanged in the gastric mucosa under these experimental circumstances). The extents of ATP-ADP transformation increased in pylorus ligated and alone IND-treated animals (B and D), while the extent of ATP-cAMP transformation was decreased in this group (in comparison of their extents in the control group). According to cellular biologists, the 60-75% of total cellular energy is used for the intact function of sodium and potassium pump system in the cells.

The noxious agents (Na-salicylate and IND) can modify the membrane bound ATP-dependent energy systems in different direct and indirect pathways. Both energy liberation pathways, namely ATP-ADP and ATP-cAMP, are significantly inhibited by sodium salicylate. These effects can be understood by a physiochemical property of the sodium salicylate because the extent of dissociation of a week acid (e.g. salicylic acid) is inhibited in presence of strong acid (HCl in pylorus ligated rats) (20,21). This undissociated week

acid absorbs from the stomach, producing a direct damage of cell plasma membrane (including membrane ATPase and as well as adenylate cyclase). The actual metabolic state of gastric mucosa in rats treated with sodium salicylate or IND differ significantly from each other.

Vitamin A and β-carotene prevented dose-dependently the IND-induced gastric mucosal damage in animals. Our results indicated that these compounds produce dose dependent changes of membrane-bound ATP- dependent energy systems.

After IND treatment the extent of ATP-ADP transformation increased significantly, while the ATP-cAMP transformation and the cAMP-AMP tarnsformation decreased significantly. When the animals were treated with vitamin A or beta-carotene then the extent of ATP-cAMP increased significantly, however the cAMP-AMP transformation increased.

These results prove that the mucosal protective effects of vitamin A and beta-carotene is a cAMP-dependent mechanism. On the other hand, these scavenger materials are kept a regulatory mechanism between the membrane-bound ATP-dependent adenosine compounds by the way of extra and intracellular pathways.

Previous studies havr proved that carotenoids have no inhibitory effects on gastric acid secretion neither in animals (42) nor in humans (48), however β-carotene was able to prevent the gastric mucosal damage in different experimental models such as ethanol-, hydrochloric acid- or indomethacin-induced mucosal damage. The ulcer healing effect of vitamin A was proven in randomized, multicenter clinical studies (97). The gastroprotection induced by carotenoids does not depend on the presence of vitamin A activity, β-ionone ring, number of unsaturated links or chemical structure of terminal part of molecules (48,74). Moreover, the analysis of gastric mucosal antioxidant mechanisms and free radical generation during β-carotene-induced gastric mucosal protection

suggested that the scavenger character of β-carotene might partially explain its protective effect (129).

Our investigations have shown the deficiency of certain serum carotenoids in different GI diseases. Five of the measured seven compounds were depleted in CD patients, while the serum level of vitamin A and β-carotene decreased significantly in patients with UC, furthermore vitamin A deficiency has been proved in patients with HCV hepatitis and alcoholic cirrhosis. Vitamin A and zeaxanthin were depleted first of all in the serum of patients with colorectal polyp and different GI malignant diseases.

Plasma retinol levels fall only in case of exhausted liver reserves (vitamin A is stored almost entirely in liver), therefore the screening of serum levels is not the most sensitive marker of vitamin stores but it is a simple and informative method to check vitamin A deficiency (61).

Hypovitaminosis in CD patients may be induced by several mechanisms: poor intake, malabsorption, bacterial overgrowth of the small intestine and increased requirement of the inflammatory process (45,64,106). Also disturbed zinc metabolism might contribute to the pathogenesis of vitamin A deficiency because of the depression of protein synthesis and the depression of particular impaired synthesis of praealbumin and retinol binding protein (112). Former studies (45,64,102,112) and the other checked serum parameters, furthermore the correlation between serum vitamin A level and CDAI indicate that our finding is unambigously a consequence of CD.

However, because many factors are related to the serum concentrations of vitamins, we are cautious in drawing any conclusions. We did not evaluate the vitamin intake in this study, therefore it is rather difficult to determine causal relationships - we could not

conclude which of the above mentioned factors was more important in decreasing of carotenoid levels.

Further studies, including the composition of vitamins in the diet and investigation of vitamin absorption are necessary to clarify the vitamin status in CD. These approaches seem to reveal more information to the necessity of intensive nutritional care concerning vitamins and, if necessary, as to the dose of replacement from the clinical point of view.

Although the laboratory parameters of UC patients did not indicate malnutrition, the decrease of carotenoids in the serum of patients is highly likely the consequence of the inflammatory process or poor intake.

The decrease of vitamin A level in patients with HCV hepatitis and liver cirrhosis may be the consequence of impaired storage capacity of the liver.

Significant decrease of vitamin A and zeaxantin was found dominantly in the serum of patients with different malignant gastrointestinal diseases (except of zeaxanthin in patients with pancreas adenocarcinoma). Similar results were found in cases of colorectal polyps with different histological stages. No significant changes were observed in the serum level of the other carotenoids.

It is rather difficult to determine causal relationships in the present study the based on these results but the decrease in serum level of vitamin A and zeaxanthin may offer relation of cause and effect between their serum levels and development of colorectal polyps and GI cancers in patients. Earlier the decrease of serum level of vitamin A has been emphasised in development of different malignancies (included GI malignancies) in Basle study and other prospective studies (110,118). Our study indicated the existence of the same negative correlation between the decrease of vitamin A and zeaxanthin in the patients' sera vs. colorectal polyps and different GI carcinomas.

These observations indicate:

1. The serum level of vitamin A decreases in patients with different histological stages of colorectal polyps, proceeding the progression of colorectal cancers;

2. It was surprising that the serum levels of vitamin A precursors (α- and β-carotene) were normal in the patients with GI cancer and colorectal polyps, when the serum level of vitamin A decreased significantly. At this moment we have no explanation why these provitamins are not able to transform into vitamin A (disorders of enzymatic transformation or decrease of vitamin A binding capacity, etc.). Probably these events are in the background why vitamin A decreased in the sera of patients with adenoma and GI cancer.

3. Accepting the fact that colorectal polyps represent precancerous state for colon tumor and the same clinical correlation exists between carotenoids vs. colorectal diseases (polyps and cancers), furthermore between carotenoids vs. the other malignant GI diseases, the decrease of vitamin A and zeaxanthin may be one of the nutritional promoting compounds in the development of these diseases;

4. Zeaxanthin does not have vitamin A activity, so surprisingly the supposed preventive effect is not only vitamin A activitie's consequence.

The effect of carotenoid supplementation on precancerous lesions and cancer incidence has been investigated in numerous clinical trials in the last two decades. I would like to cite several trials related to this topic, some of them support our thesis, the other part seems to be contradictious at first.

Four small trials in human suggest that antioxidants can reduce colorectal epitethelial cell proliferation. In a small randomized, placebo-controlled clinical trial in colorectal adenoma patients (n=41), those given 30 000 IU vitamin A, 70 mg α-tocopherol plus 1000 mg

ascorbic acid had a 45% greater reduction than those given placebo in the labeling index (LI) of the upper 40% of the colonic crypts (95). In an uncontrolled trial (n=10), the labeling index dropped 33% in sporadic adenoma patients given 200 μg of selenium for 1 month (16). In a one month randomized placebo-controlled four-armed parallel group trial in sporadic colorectal adenoma patients (n=48), those given 750 mg vitamin C had a 54% drop in LI, those given 9 mg β-carotene had a 41% drop, and those given 160 mg α-tocopherol or placebo had no change (15). Finally, in a trial in familial polyposis patients (n=17), those given 3.0 g ascorbic acid daily had a 19% greater reduction in the labeling index than those on placebo (14).

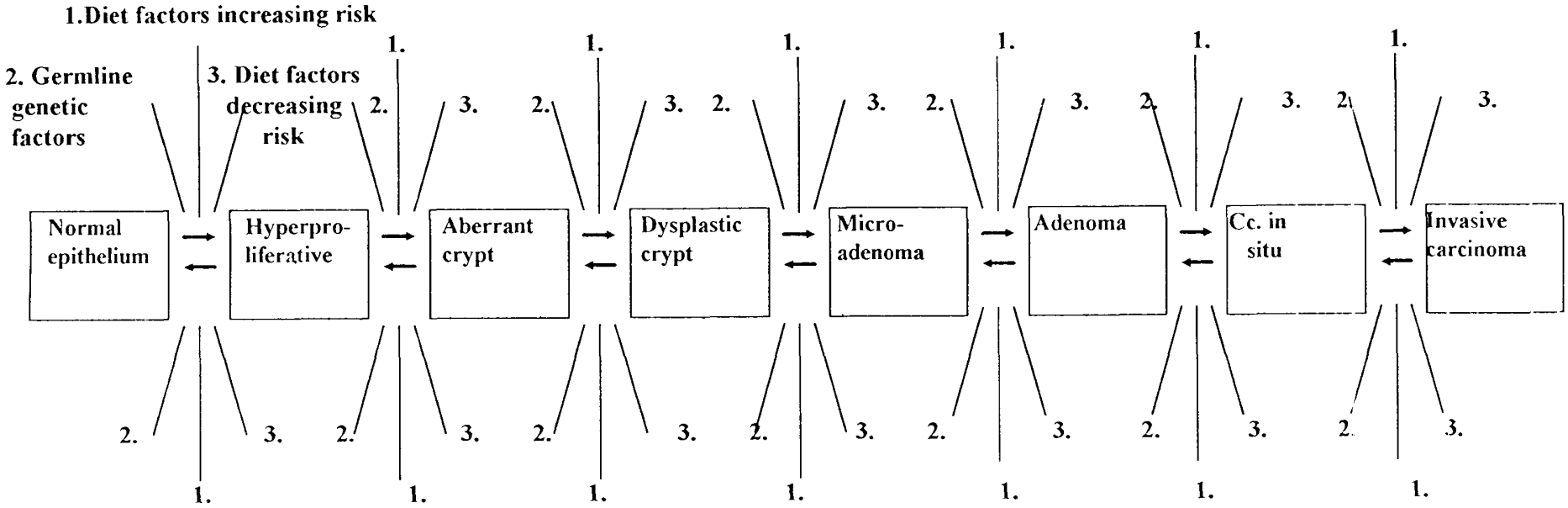
Antioxidant micronutrients have reduced polyp formation in small trials in humans, but not in all larger full-scale trials. In the most striking randomized, placebo-controlled clinical trial of polyp recurrence, sporadic colorectal adenoma patients (n=209) treated with 30,000 IU vitamin A, 70 mg vitamin E plus 1.0 g vitamin C daily over 18 months had a polyp recurrence rate of 5.7% vs 35.9% for those on placebo ($p<0.001$) (107). In a pilot randomized, placebo-controlled trial, sporadic colorectal adenoma patients (n=129) treated with 400 mg vitamin E plus 400 mg ascorbic acid daily over 2 years had a polyp recurrence rate of 41.4% vs 50.7% for those on placebo (not statistically significant) (65). In three small trials of polyp recurrence in familiar polyposis patients, two testing 3.0 g ascorbic acid daily (19,95) and the other testing 4.0 g ascorbic acid plus 400 mg α-tocopherol daily (24) small reductions in polyp formations were suggested. However more recently the lack of efficacy of administering 400 mg vitamin E , 25 mg β-carotene, and 1000 mg vitamin C have been reported in reducing adenoma recurrence over a 4-yr period (34). This was an excellent study (n=751) that did exactly what it was designed to do: determine the efficacy of the above treatment regimen on colorectal polyp recurrence in estabilished polyp formers over a relatively brief period of time. Based on what was known at the time the study was

originally conceived, these results would have led to the conclusion that antioxidants would be ineffective in colon cancer prevention. We know now that this is not the case, and that the question is still rather wide open. One reason that this study (34) may not have yielded hypothesized results is that when originally designed it did not take into account the findings (131) that multiple slowly accumulated genetic alterations are usually required to produce a colonic neoplasm in the non-FAP (Familial Adenomatous Polyposis) patient. It would be unrealistic to think that every potential protective factor can protect against cancer by inhibiting every step in this pathway and antioxidants are no exception. If a patient already has several cell lines with the requisite accumulated genetic changes to commit the cell lines to adenoma formation, there may be no mechanism whereby antioxidants could block the growth of the commited lines. We know that patients with incident adenoma are likely to form recurrent adenoma: thus, it is likely that by the time a patient has an incident adenoma, other cell lines are genetically altered to form adenomas also. We also know that the natural history of adenoma growth is rather slow. This all means , if a protective agent exerts it's action prior to full genetic commitment of a cell to adenoma formation, a reduction in polyp recurrence may not be seen until a substantial proportion of the already commited cell lines have „played out", and it may take longer than the 4 yr follow up of the above mentioned study. So antioxidants may not be excluded as protective agents against colon cancer based on similar theoretical grounds.

Colon carcinogenesis can be thought of as a long term, multistep process, in which multiple somatic genetic defects are accumulated. The possibility exist that various factors can can influence the occurence or even the reversal of each of the steps (Fig. 36.). By inference, the posibility exists that, even after several steps have occured the entire process can be reversed. The likelihood of reversing the entire process is greater when few rather than many steps have occured. A given environmental (dietary) factor (e.g. vitamin A) may

play a role at one or more than one steps. Regarding to the above cited studies and and our result carotenoids and other antioxidants may inhibit the first 3-4 steps. Furthermore a magic bullet approach to prevention is inadequate in this matter (10.).

Fig. 36. A model of diet and nutrition in the etiology and prevention of colorectal cancer



The outcome of some prospective studies (including the particularly conclusive Basie study) support the potential chemopreventive role of carotenoids. It has been demonstated that lower levels of retinol and carotene et entry were associated with a significantly increased relative risk for bronchus cancer, colorectal cancer and all other cancers. At base-line of the study, all principal antioxidant micronutritients of 2974 clinically healthy males were assayed prior to measurable antioxidant decay in frozen plasma (118,119). The 12-year follow-up study detected that the mortality from all cancers increased overmultiplicatively at simultaneous occurence of low levels of retinol and carotene. This was reconfirmed by the 17-year evaluation with regard to overall cancer mortality and further specified for an overmultiplicative increase of mortality from bronchus cancer at low lewels of the checked compounds.

Few studies indicated a positive correlation between carotene intake and carcinogenesis. The ß-Carotene and Retinol Efficacy Trial (CARET) (38,94) randomized 18 314 men and women at high risk for lung cancer because of cigarette smoking history or occupational asbestos exposure to daily treatment with a combined supplement containing ß-carotene (30 mg) and vitamin A (25 000 IU) or placebo. The trial was terminated early, after approximately 4 year of treatment based primarily on interim data analyses suggesting it was unlikely any material benefits would emerge with continuation to its scheduled termitation, but also besause of emerging data compatible with another findings (The Alpha-Tocopherol, ß-Carotene Cancer Prevention Study) (39,40). Specifically, there was a nonsignificant 26% increase in cardiovascular disease mortality among those assigned the supplementation combination. There was a statistically significant 28% increased risk of lung cancer in the treated group and a significant 17% increase in the total mortality (38,94). Independent effects of the study interventions could not be established because the study agent were given combined.

The Physicians Health Study (PHS) (115) randomized 22 071 US male physicians to alternate daily 50 mg ß-carotene and 325 mg Aspirin, both active treatment, or both placebo. There was no significant effect of ß-carotene on total cancer incidence.

Several problems have been pointed out with these three studies. If the effect of ß-carotene (or other antioxidants) is to protect against the initiation of carcinogenesis, all three trials were much too short in duration. However ß-carotene is only one among the hundreds of identified carotenoids, only ß-carotene and vitamin A were tested in these studies. It is likely that a balanced mixture of carotenoids and other vitamins (as found in vegetables and fruits) is able to prevent carcinogenesis. Moreover, there is a possibility that a large intake of ß-carotene might inhibit the absorption of other protective substances. The evidence on this is still conflicting. Two studies have shown that supplementary ß-carotene was associated with a decrease in serum lutein and zeaxanthin (41).

Missing correlations between retinol intake and cancer risk may not be generalized (136,140). First, the benefits of an increased vitamin A intake may only be expected if a prevalent suboptimal supply is corrected, as actually true for any essential nutrient (41). Second, cancer risk is unlikely to be reduced by any single "magic bullet" nutrient (10).

Despite a large number of studies demonstrating protection by carotenoids, the exact mechanisms through which these compounds influence carcinogenesis have not yet been proved, but numerous mechanisms of action have been hypothesised. Their beneficial effects originate partially from the vitamin A activity, on the other hand they are connected with the structure of carotenoids. Retinol dependent pathways: Retinoids exert most of their effects by modulating gene expression (35). These effects depend on retinoid acid and retinoid X receptors, which act as transcription factors (62). Retinoids regulate the growth, morphogenesis and differentiation of cells. They have a variety of

effects on the cell membrane (128) and they can influence different enzymes (27,126). These compounds exert also immunomodulatory effects (62).

Depends on the stucture: Carotenoids are non-enzymatic antioxidants (36), therefore they are able to prevent genetic changes by preventing DNA damage caused by free radicals (122). They modulate membrane functions and stabilise initiated cells in promotional phase of carcinogenesis.

Our study provides new data supporting the importance of nutrients in the polyp cancer sequence in colorectal cancer. These results indicate that the deficiency of carotenoids may be one of the factors in the development of colorectal cancer arising from adenomas and other GI cancers, furthermore not only vitamin A activity is responsible for this supposed preventive effect in carcinogenesis.

6. NEW RESULTS

1. Vitamin A and β-carotene decreased dose-sependently the number and severity of gastric mucosal lesions.
2. The gastric cytoprotective effect of retinoids produces a dose dependent inhibition on the extent of ATP transformation to ADP, meanwhile it produces an increase in the transformation of ATP into cAMP, indicating their intracellular metabolic effects.
3. The significant decrease of five serum carotenoids have been proved in CD patients (vitamin A, zeaxanthin, lutein, α-, β-carotene), furthermore a strong correlation between vitamin A level and CDAI.
4. Our investigations have shown the deficiency of certain serum carotenoids in other inflammatory (ulcerative colitis - vitamin A and β-carotene) and premalignant GI diseases (colorectal polyps-vitamin A and zeaxanthin).
5. Vitamin A and zeaxanthin were depleted in the serum of patients with different GI malignant diseases (esophagus, gastric, pancreas, hepatocellular, colorectal carcinoma) except of zeaxanthin in patients with pancreas adenocarcinoma.
6. The serum levels of vitamin A precursors decreased only in patients with IBD-s significantly.
7. Furthermore vitamin A deficiency has been proved in patients with HCV hepatitis (the similar result of patients with alcoholic cirrhosis is well known as a consequence of exhausted liver reserves).

Further studies, including the composition of vitamins in the diet and investigation of vitamin absorption are necessary to clarify the vitamin status in IBDs. These approaches

seem to reveal more information to the necessity of intensive nutritional care concerning vitamins and, if necessary, as to the dose of replacement from the clinical point of view

Our data support the importance of micronutrients in colorectal polyp-cancer sequence. We plan to measure the tissue levels of carotenoids in premalignant and malignant GI diseases.

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