The present study was aimed to investigate the mechanisms by which vitamin A plays a role in maintaining the efficiency of gastric mucosal barrier. Particularly, we measured electrical parameters and the RNA/DNA ratio of gastric mucosa isolated \textit{in vitro} from the stomach of rats in which vitamin A-deficiency was induced by means of a vitamin A-free diet and then abolished by means of a massive vitamin A supplementation. Pair-fed vitamin A-nondepleted rats and normal rats fed \textit{ad libitum} on a standard diet served as controls. Vitamin A status was assayed for each group of rats by measuring the hepatic content of vitamin A. We found that in gastric mucosa vitamin A-deficiency induced: 1) a decrease in both transmucosal potential difference and short-circuit current; 2) an increase in transmucosal electrical resistance; 3) a decrease in RNA content resulting in a decreased RNA/DNA ratio. Abolishment of vitamin A-deficiency restored both electrical parameters and RNA content of rat gastric mucosa. Our results stress the role of vitamin A in maintaining the efficiency of the gastric mucosal barrier. Vitamin A seems to act by stabilizing gastric electrical parameters and by controlling the protein synthesis/turnover in the surface gastric mucosal cells.

\textbf{Key words:} \textit{Rat, vitamin A, gastric mucosa, gastric barrier, electrical parameters, DNA, RNA.}

\section*{INTRODUCTION}

Both clinical observations on humans and experimental studies on animals suggest a protective action of vitamin A against gastric ulcer induced either by stress or by well-known gastro-offensive agents like nonsteroidal anti-inflammatory drugs (1, 2). Interestingly, it has been demonstrated (3) that, in an
animal model, high intake of selenium, beta-carotene, and vitamins A, C, and E reduces growth of *Helicobacter pylori* (*H. pylori*), a bacterium involved in the pathogenesis of a number of gastroduodenal diseases including peptic ulcer and gastric cancer (4, 5). Moreover, Annibale et al. (6) showed that *H. pylori* affects the plasma, intragastric and mucosal concentration of different antioxidant compounds such as ascorbic acid, alpha-tocopherol and beta-carotene suggesting a relationship between *H. pylori* infection and micronutrients in the multistep cascade leading to gastric carcinogenesis.

Retinoids prevent chemically induced gastric mucosal damage without inhibiting gastric acid secretion, thus exerting the so-called "nutritional gastric cytoprotection" (7). Mounting evidence suggests that, in epithelial tissues characterized by a rapid renewal like gastrointestinal tract, vitamin A affects cell surface phenomena such as cell-cell interaction, cell adhesion, and cell surface permeability mainly acting on either the synthesis of glycoproteins or the glycosylation of specific glycoproteins (reviewed in Ref. 8). However, the mechanisms by which vitamin A exerts a protective function on gastric mucosal barrier are still not completely understood.

This study sought to investigate the action of vitamin A on functions that are strictly linked with gastric mucosal barrier efficiency (9-11). We measured the bioelectric parameters (i.e., transmucosal potential difference (PD), short-circuit current (I\textsubscript{sc}), and electrical resistance (R)) as well as the RNA/DNA ratio and the protein content of gastric mucosa isolated *in vitro* from the stomach of rats in which vitamin A-deficiency was induced by means of a vitamin A-free diet and then abolished by means of a massive vitamin A supplementation.

**MATERIAL AND METHODS**

**Animals and Diets**

Vitamin A-deficiency was induced in 3 week-old Wistar strain male albino rats fed on a vitamin A-deficient diet (12) and individually housed in wire-bottom cages to prevent coprophagy, as previously described (13). The male rats were the offspring of females fed on the Randoin and Causeret's standard diet (14) which prevents large reserves of vitamin A accumulating in the liver (13). The rats fed on the vitamin A-deficient diet were divided in four groups. I group: vitamin A-deficient rats obtained by feeding on the vitamin A-deficient diet. II group: rats pair-fed (i.e., receiving the same amount of food eaten by paired rats the day before) with group I, but supplemented daily by 150 I.U. of vitamin A-palmitate *per os* to prevent the development of vitamin A deficiency. III group: rats which received, afterwards the occurring of vitamin A-deficiency (first period of treatment), a high amount of vitamin A (i.e., 1000 I.U. of vitamin A-palmitate *per os* daily) till disappearing of vitamin A deficiency (second period of treatment). IV group: rats pair-fed with group III, supplemented daily by 150 I.U. of vitamin A-palmitate *per os* during the first period to prevent the development of vitamin A deficiency. Like group III, group IV rats received 1000 I.U. of vitamin A-palmitate *per os* daily during the second period. A V group of rats was also used: 8-9 week-old normal Wistar rats fed *ad libitum* on Randoin and Causeret's standard diet (14).
Vitamin A-deficiency occurred after 5-6 weeks and disappeared in 3-4 weeks after vitamin A supplementation at high dose. The depletion criteria used to evaluate vitamin A-deficiency were those employed in the biological assay of vitamin A (13). Vitamin A-deficient rats showed 1) a lower weight gain in comparison to normal rats; 2) xeroftalmia; and 3) paralysis mainly localized to the hind legs. Vitamin A-deficient rats showed no evidence of any macroscopically visible alterations in the gastric mucosa (such as erosions or superficial injury) in comparison to the other groups of rats.

Vitamin A status of each group of rats was assayed by measuring the hepatic content of vitamin A by means of the Carr-Price reaction as previously described (13).

**Gastric mucosa sampling**

After overnight fasting, rats of all the groups were killed by decapitation, the stomach immediately exposed by laparotomy, and gastric mucosa samples were rapidly isolated as previously described (11). Briefly, mucosa (samples of 1.5 cm²) was stripped from the muscular and serosal layers of the gastric body following the technique of Forte et al. (15) as modified by Main and Pierce (16). The overall procedure took no more than 3-4 min.

**Measurement of PD, \( I_{sc} \), R of gastric mucosa in vitro**

Gastric mucosa samples were mounted in a double flux Ussing-type chamber allowing the exposition of 1.0 cm² of both the luminal and the serosal surface of the mucosa. Each surface was incubated with 20 ml of Krebs-Henseleit (K-H) solutions at 37°C. The serosal surface was bathed with K-H solution buffered at pH 7.35 and gassed with 95% O₂-5% CO₂, while the mucosal surface was bathed with K-H solution buffered at pH 3.2 and gassed with 100% O₂. At the end of a 40 min stabilization period, the electrical parameters were measured every 5 min for 20 min with conventional techniques as previously described (11). Briefly, PD was measured by means of two saline-agar bridges connected via calomel electrodes to a voltmeter (Ballantine mod. 3036; 100 mV end scale), while \( I_{sc} \) was measured by delivering current through two saline-agar bridges from a variable direct current source via carbon electrodes (Superpila) connected to an ammeter (500 µA end scale) so as to reduce PD to zero value. The current required to maintain zero potential was taken to be equivalent to the current continuously generated by the mucosa and is referred to as \( I_{sc} \), in accordance with Miller et al. (17). R was calculated according to Ohm's law (i.e., as the ratio of PD to \( I_{sc} \)). The R of the incubation fluid was subtracted from the calculated value of R.

**Measurement of DNA, RNA, and protein content in gastric mucosa samples**

Gastric mucosa samples were assayed for DNA content in accordance with Giles and Myers (18), for RNA content by the method of Fleck and Begg (19), and for protein content by the method of Lowry et al. (20).

**Statistics**

Results are expressed as means ± SEM. The statistical significance of the differences was evaluated by the Student's paired \( t \)-test or by analysis of variance (ANOVA) followed by Newman-Keuls' \( Q \)-test; significance was set at \( P<0.05 \).

**RESULTS**

We found that vitamin A-deficient rats obtained by feeding on the vitamin A-deficient diet (i.e., group I) exhibited a) a very low amount of vitamin A in the
liver (Table 1) that well fits with the biological evidences of vitamin A deficiency observed (i.e., low weight gain, xerophthalmia, and paralysis); b) a protein content of the gastric mucosa virtually identical to that of either pair-fed (i.e., group II) or normal rats (i.e., group V) (Table 2); c) a decreased RNA/DNA ratio of the gastric mucosa which is completely accounted for by a decrease in RNA mucosal content whereas the DNA content was virtually identical to that of either pair-fed or normal rats (Table 2). On the contrary, rats pair-fed with group I, but supplemented by vitamin A to prevent the development of vitamin A deficiency, (i.e., group II) showed a hepatic content of vitamin A five times higher than in normal rats (Table 1) while all other biochemical parameters were virtually identical to those of normal rats (Table 2). The high hepatic content of vitamin A of group II rats was most likely due to the fact that the dose of vitamin A administered (150 I.U./day) exceeded the normal requirements and therefore vitamin A accumulated in the liver, as previously described by Ceriani et al. (13). Similarly, rats in which vitamin A-deficiency was abolished by means of a massive (1000 I.U./day) vitamin A supplementation (i.e., group III) showed, like their pair-fed rats (i.e., group IV), a hepatic content of vitamin A higher than that of normal rats (Table 1) while all other biochemical parameters were virtually identical to those of normal rats (Table 2). In other words, the abolishment of vitamin A deficiency by vitamin A supplementation completely restored the normal pattern of the biochemical parameters tested restoring the RNA content of the gastric mucosa, and thus the RNA/DNA ratio, in comparison to vitamin A-deficient rats.

Table 1. Vitamin A content in the liver from different groups of rats

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Vitamin A (µg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, Vitamin A-deficient</td>
<td>0.67 ± 0.10 °</td>
</tr>
<tr>
<td>II, Vitamin A-nondepleted</td>
<td>150.21 ± 12.15 *</td>
</tr>
<tr>
<td>pair-fed with group I</td>
<td></td>
</tr>
<tr>
<td>III, Recovered from vitamin A-deficiency</td>
<td>174.43 ± 14.21 *</td>
</tr>
<tr>
<td>IV, Vitamin A-nondepleted</td>
<td>161.28 ± 12.84 *</td>
</tr>
<tr>
<td>pair-fed with group III</td>
<td></td>
</tr>
<tr>
<td>V, Normal</td>
<td>27.97 ± 2.18</td>
</tr>
</tbody>
</table>

Values are means ± SEM of 9-12 rats.
*, P < 0.05 versus normal rats
°, P < 0.05 versus the respective pair-fed rats
Table 2. Content of proteins, DNA, RNA and RNA/DNA ratio in the gastric mucosa from different groups of rats

<table>
<thead>
<tr>
<th>Group of rats</th>
<th>Proteins (mg/g wet tissue)</th>
<th>DNA (mg/100 mg proteins)</th>
<th>RNA (mg/100 mg proteins)</th>
<th>RNA/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, Vitamin A-deficient</td>
<td>103.65 ± 7.40</td>
<td>3.22 ± 0.18</td>
<td>6.34 ± 0.32*°</td>
<td>1.96 ± 0.09*°</td>
</tr>
<tr>
<td>II, Vitamin A-nondepleted pair-fed with group I</td>
<td>91.42 ± 6.51</td>
<td>3.52 ± 0.31</td>
<td>8.38 ± 0.52</td>
<td>2.39 ± 0.10</td>
</tr>
<tr>
<td>III, Recovered from vitamin A-deficiency</td>
<td>102.13 ± 8.21</td>
<td>3.27 ± 0.24</td>
<td>7.90 ± 0.42</td>
<td>2.44 ± 0.12</td>
</tr>
<tr>
<td>IV, Vitamin A-nondepleted pair-fed with group III</td>
<td>99.47 ± 7.25</td>
<td>3.31 ± 0.20</td>
<td>7.82 ± 0.26</td>
<td>2.38 ± 0.13</td>
</tr>
<tr>
<td>V, Normal</td>
<td>104.71 ± 8.23</td>
<td>3.13 ± 0.25</td>
<td>7.78 ± 0.41</td>
<td>2.47 ± 0.10</td>
</tr>
</tbody>
</table>

1 Calculated for each rat of each group.
Values are means ± SEM of 9-12 rats. *, P < 0.05 versus normal rats. °, P < 0.05 versus the respective pair-fed rats

As shown in Fig. 1A, vitamin A-deficient rats (i.e., group I) exhibited a statistically significant alteration of all the bioelectric parameters of the gastric mucosa tested in comparison to either pair-fed (i.e., group II) or normal (i.e., group V) rats. Particularly, in vitamin A-deficient rats PD and $I_{sc}$ decreased while $R$ increased. On the contrary, bioelectric parameters of gastric mucosa from rats pair-fed with group I, but supplemented by vitamin A to prevent the development of vitamin A deficiency, (i.e., group II) did not differ from those of normal rats. It is worth noting that the bioelectric parameters of gastric mucosa from normal rats (Fig. 1A) were in a physiological range (10, 11) thus assuring the good viability of the in vitro gastric mucosa preparations. Interestingly, rats in which vitamin A-deficiency was abolished by means of a massive vitamin A supplementation (i.e., group III) exhibited bioelectric parameters of gastric mucosa virtually identical to those of either pair-fed (i.e., group IV) (Fig. 1B) or normal rats (not shown). In other words, the abolishment of vitamin A deficiency by vitamin A supplementation completely restored the normal pattern not only of the biochemical but also of the bioelectric parameters.

DISCUSSION

Vitamin A plays a role in gastric mucosal protection (1, 2, 7). In indomethacin-treated rats, the number and severity of gastric mucosal lesions was dose-dependently decreased by the simultaneous treatment with vitamin A (2). Also
indomethacin-induced decrease in rat gastric mucosa levels of ATP, AMP and cAMP was dose-dependently prevented by vitamin A (2). The existence of gastric mucosal protection by retinoids may be observed in healthy persons (against indomethacin treatment) as well as in patients with either gastric or duodenal ulcer without any inhibition of gastric acid secretion (7). Moreover, the serum levels of vitamin A were significantly decreased in patients with chronic gastrointestinal inflammatory diseases, colorectal polyposis, and different gastrointestinal (e.g., esophageal, gastric, pancreatic, hepatic, and colorectal) carcinomas (reviewed in Ref. 7).

Fig. 1. Transmucosal potential difference (PD; mucosal side negative), short-circuit current (I_{SC}), and electrical resistance (R) of gastric mucosa isolated in vitro from the stomach of different groups of rats. A) ○, Group I: vitamin A-deficient; ●, Group II: vitamin A-nondepleted pair-fed with group I; △, Group V: normal. B) □, Group III: recovered from vitamin A deficiency; ■, Group IV: vitamin A-nondepleted pair-fed with group III. Values are means ± SEM of 15-18 rats. *, P<0.05 versus either the respective pair-fed or normal rats at the same time point.
In this study we investigated the effect of vitamin A deficiency by itself (i.e., in the absence of any concomitant pharmacological treatment with gastro-offensive agents or experimentally-induced gastric lesions) on functions that are strictly linked with gastric mucosal barrier efficiency (9-11). We found that vitamin A deficiency altered the bioelectric parameters of rat gastric mucosa isolated in vitro causing a decrease in both PD and $I_{sc}$ and an increase in transmucosal R. All these effects appeared to be strictly dependent on vitamin A deficiency since they completely disappeared when vitamin A deficiency was abolished by vitamin A supplementation. The increase in transmucosal R observed is especially noteworthy because it clearly suggest that vitamin A deficiency by itself does not cause a rupture of gastric mucosal barrier (condition in which also R decreases along with PD and $I_{sc}$) but rather only a partial impairment. It is indeed widely accepted that measurements of transmucosal R, unlike PD or $I_{sc}$, allow to distinguish between permeability and active transport alterations. A decreased R implies an increased tissue permeability, whereas an increased R indicates a diminished active transport rate (21-23). Mounting evidence suggests that low concentrations of damaging agents (such as alclofenac, aspirin, bile salts, ethanol, indomethacin) inhibit the active ion transports in gastric mucosal preparations thus causing a reduction in both PD and $F_{sc}$ and an increase in R (10, 17, 23, 24). The permeability changes (i.e., the concomitant decrease of PD, $F_{sc}$ and R) induced in gastric epithelium by the above damaging agents may actually be a late, second-step phenomenon, while their initial action is the inhibition of active ion transport (10, 17, 23, 24).

It is widely accepted that the movements of Na$^+$ and Cl$^-$ ions against their concentration gradients mainly determine the bioelectric parameters of mammalian gastric mucosa (11), which is predominantly a Cl$^-$-secreting, rather than a Na$^+$-absorbing, epithelium (25). It has also been reported that the acid-induced increase in transmucosal R is mainly accounted for by both Na$^+$/H$^+$ and Cl$^-$/$\text{HCO}_3^-$ exchange activity (25). These facts suggest that vitamin A might play a pivotal role in maintaining the efficiency of the transporters responsible for the aforementioned ionic exchanges, in particular the Cl$^-$/$\text{HCO}_3^-$ one.

Our findings thus suggest that vitamin A deficiency acts on the gastric mucosa by inhibiting ion active transport since it causes a decrease in PD and $F_{sc}$ and an increase in transmucosal R. The possibility arises that the gastric mucosal barrier weakened by vitamin A deficiency might become more prone to rupture by various injurious agents (e.g., nonsteroidal anti-inflammatory drugs and ethanol) or different injuring conditions (e.g., water restraint stress and ischemia followed by reperfusion). That vitamin A deficiency may exert a weakening action on gastric mucosal barrier is also supported by the findings of Gulich and Onoprienko (26) that vitamin A deficiency caused ultrastructural changes in plasma and intracellular membrane turnover with, for instance, impaired regeneration of membranes in the Golgi complex cisternae, secretory and
transport vesicles and apical part of the plasma membrane, thus resulting in a decreased resistivity to injury.

Modulation of active ion pumps in gastric mucosa may depend on cell cAMP and cGMP content and vitamin A was demonstrated to activate cAMP-dependent protein kinases, to produce a dose-dependent inhibition of ATP hydrolysis to ADP and to increase the transformation of ATP into cAMP (7, 8). It is also widely accepted that surface epithelial cells play a major role in the genesis of the bioelectric parameters of the gastric mucosa (11, 27). The decrease in PD and $I_{sc}$ as well as the increase in R we found might be also related to vitamin A deficiency-induced structural modifications of surface gastric mucosal cells at plasma membrane level (8, 26). Interestingly, we found that vitamin A deficiency caused a decrease in RNA content in rat gastric mucosa and, consequently, a decreased RNA/DNA ratio. Even though the total amount of proteins per wet weight of gastric mucosa appeared not to be altered by vitamin A deficiency, the decreased RNA/DNA ratio (which under physiological conditions is highly stable within a given tissue or organ) may be considered as a sensitive index of an altered protein synthesis/turnover (28). Moreover, it is well known that vitamin A deficiency decreases glycoprotein synthesis in many tissues (reviewed in Ref. 8). Glycoproteins, mainly sialic acid and fibronectin, play a role in the organization on cell surface of membrane protein channels and ion pumps (8). In this respect, we have previously demonstrated that vitamin A deficiency caused a decrease in the sialic acid content in rat gastric mucosa (29).

Taking that the generation of reactive oxygen species seems to play an important role in various models of gastric mucosal injury (30), retinoids may also exert a cytoprotective role by means of their anti-oxidizing activity as shown by Pohle et al. (31) for vitamin C in aspirin-induced gastric damage in humans.

In conclusion, the results here presented emphasize the pivotal role of vitamin A in maintaining the efficiency of the gastric mucosal barrier. Vitamin A seems to act by stabilizing the gastric electrical parameters (i.e., maintaining the efficiency of active ion transports) and by controlling the protein synthesis/turnover in surface mucosal cells. Vitamin A deficiency by itself results in an impairment of gastric mucosal barrier even though it does not directly cause its rupture.

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