Stomach Ulcers in Vitamin A-Deficient Syrian Golden Hamsters

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Abstract. Stomach ulcers of various stages occurred in seven adult male Syrian golden hamsters fed on a vitamin A-free diet for seven months. The ulcers were located in the glandular portion close to the forestomach. These hamsters showed food retention containing various amounts of hair in the stomach. Histopathologically, the early ulcerogenic change was focal necrosis of the glandular epithelium, which resulted in erosion and ulcer, with leukocytic infiltration and edema of the surrounding tissue. The most advanced ulcers exhibited perforation of the gastric wall resulting in adhesive peritonitis. In an additional experiment in that hamsters were fed on a diet mixed with their own hair, no gastric food retention nor morphological alterations of the glandular stomach were observed. It was suggested that the induction of stomach ulcers might be a synergistic effect of the necrotic changes in the glandular epithelium and the food retention caused by vitamin A-deficiency state.

It is well known that vitamin A deficiency produces various lesions showing growth retardation, nyctalopia, keratomalacia, xerophthalmia, and keratinizing squamous metaplasia of mucous membranes in mammals [15]. The effects of vitamin A deficiency have been also examined on various organs of hamsters by many workers [1, 6, 8, 19]. However, there has been no report on stomach ulceration in hamsters, although several investigators have reported the ulcerogenic effect of vitamin A deficiency on rats [1, 14] and chicks [4, 12].

We encountered stomach ulcers of hamsters fed on a vitamin A-free diet in the experiment carried out as a part of the continuing project concerning the effect of

cigarette smoke inhalation on the respiratory tract. It was primarily considered that the occurrence of the stomach ulcer might be attributed to vitamin A deficiency. These hamsters, however, also had various amounts of hair in the gastric contents, forming large boluses in a few animals. Since we could not ignore the role of hair as a mechanical irritant, a further experiment subjecting hamsters to a diet mixed with hair was performed to clarify the pathogenesis of the stomach ulcer.

Materials and Methods

Experiment I

Fourteen adult male Syrian golden hamsters (*Mesocricetus auratus*) purchased from Engles La-

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Table 1. Composition of the diets (g/100 g)

Component	Standard laboratory chow		Vitamin A-free diet
	Purina	Oriental	
Protein	23.4	24.1	24.0 (vitamin-free casein)
Fat	4.5	4.6	10.0 (vegetable oil)
Fiber	5.2	4.2	8.0 (alphacel)
Ash	7.3	7.1	4.0 (salt mixture U.S.P.XIV)
Nitrogen-free extract (NFE)	50.8	53.0	53.0 (sucrose)
Moisture	8.8	7.0	—
Vitamin A	1,500 IU	1,000 IU	1.0 (vitamin diet fortification mixture except vitamin A)

boratory Animals (Farmersburg, Indiana, USA) were used.

The hamsters weighing from 100 to 110 g were divided into two groups of equal numbers. One group was fed on a vitamin A-free powdered diet (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio, USA) for seven months, whereas the other group (control) was maintained on a standard Purina laboratory pelleted chow (Ralston Purina Company, St. Louis, Missouri, USA) for the duration of the experiment. The composition of the diets is shown in Table 1. Both groups were supplied with water ad libitum. The hamsters were individually housed in suspended cages with wire mesh bottoms.

The hamsters were anesthetized with sodium pentobarbital injected intraperitoneally and blood samples were collected from all animals by cardiac puncture with heparinized syringes for vitamin A analysis. Vitamin A analysis was carried out according to the method of Neeld and Pearson (1963) with tribluoroacetic acid as the reagent. After gross examination, the stomach was fixed in a 10% neutral-buffered formalin solution along with other organs. Several pieces of the stomach were embedded in paraffin and the sections were stained with hematoxylin-eosin (H-E) and periodic acid-Schiff (PAS) stains.

Experiment II

Fourteen male Syrian golden hamsters obtained from Nippon Institute for Biological Science (Ohme, Tokyo) were used at 7 weeks old. The hamsters were divided into two groups of 7 animals each. One group (control) was fed on a standard Oriental powdered chow (Oriental Yeast Co., Ltd., Itabashi, Tokyo) and the other group maintained on the standard diet containing 1% (weight/weight) hair of hamsters for the same duration (7 months) as in experiment I. The composition of the diet is also shown in Table 1. Food and water were provided

ad libitum and the hamsters were individually housed in an animal room maintained at a temperature of $24 \pm 1^\circ\text{C}$ and a relative humidity of $55 \pm 5\%$. Body weight and food and water consumption were measured weekly during the experimental period.

The hamsters of both groups were killed at the termination of the experiment. After gross examination, half of the stomach specimen was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for scanning electron microscopic observation. The specimen was examined and photographed with a Hitachi S-430 scanning electron microscope. The remaining half side of the stomach was fixed in a 10% formalin solution with other organs and tissues and placed on a routine process for light microscopy. The paraffin sections were stained with H-E and PAS.

Results

Experiment I

The control group of hamsters displayed no remarkable change in external features as well as in the gross and histological observations of the stomach. All the hamsters on vitamin A-free diet showed several clinical, gross and histopathological changes.

Clinically, the hamsters showed loss of hair and/or coarsened, matted coat. Some animals also showed mild xerophthalmia.

Macroscopically, stomach ulcers varying in number, degree and size (2–3 mm in diameter) were found in all hamsters as shown in Table 2. The lesions were located in the glandular stomach mostly close to the junction of the forestomach (Fig. 1), and

Table 2. Gross and histological findings in the stomachs of hamsters fed on vitamin A-free diet

Animal No.	Food retention	Number of ulcers	Extent of ulcer
1	+	2	Early and moderate
2	++	1	Moderate
3	+++ (bolus)	1	Advanced
4	+	2	Early
5	++	2	Moderate
6	+++ (bolus)	1	Perforated
7	+++ (bolus)	1	Perforated

+ ; Slight, ++ ; Moderate, +++ ; Severe.

were gray in color, occasionally with hemorrhages. Two hamsters showed perforated ulcers accompanied with adhesive peritonitis. There was retention of food containing various amounts of hair in the stomach (Table 2). Three cases each had a large bolus in the retained food. All the animals showed also splenomegaly, discoloration of the kidney and atrophic testis.

Microscopically, the lesions in the glandular stomach ranged from focal necrosis of the epithelium to perforated ulcer. The former was accompanied by leukocyte infiltration and edema of the surrounding tissue (Figs. 2 and 3). The necrotic masses frequently showed detachment from the mucosa resulting in erosion and ulcer (Fig. 4). Epithelial regeneration was also observed, but not prominent. Solitary necrosis and multinucleation of the parietal cells were scattered in the relatively normal mucosa (Figs. 5 and 6). In one case, calcification was observed in the necrotic area of the ulcer bed (Fig. 7). In advanced stomach ulcers, including perforated ones, the surrounding tissue or the base was thickened, associated with granulation tissue. The inflammatory reaction extended to the omentum, spleen, liver capsule and pancreatic interstitium. The mucosa surrounding the ulcer frequently showed intestinal metaplasia (Fig. 8). An increase in mucus-

secreting cells was demonstrated in PAS-stained sections of the metaplastic regions.

The forestomachs (esophageal diverticula) adjacent to the ulcer showed hyperkeratosis, sometimes accompanied by epithelial desquamation, leukocyte infiltration and submucosal edema (Fig. 7).

The histological findings in other organs and tissues including squamous metaplasia of the respiratory tract will be reported elsewhere.

The hamsters fed on vitamin A-free diet showed a significant ($p < 0.05$) decrease in the plasma levels of vitamin A compared with those of the controls. The mean values ($\mu\text{g}/100\text{ ml}$) in the former hamsters and the controls were 16.41 and 27.78, respectively.

Experiment II

Group mean body weight and food and water consumption of the hamsters fed on the diet mixed with hair were slightly lower (by about 5–10%) than those of the controls during the experimental period. Mean daily hair intake of the group was 65 mg per hamster.

Neither clinical changes nor gross abnormalities of the stomachs were observed in both groups except that considerable amounts of hair were noted in the gastric contents and feces of the hamsters fed on the diet containing hair.

Small erosive lesions were found at light and scanning electron microscopic levels in two of the hamsters fed on the diet mixed with hair. These lesions were accompanied by leukocytic infiltration and located in the forestomach, but not in the glandular stomach. Occasionally, hair shaft sticking on the forestomach mucosa was also found by scanning electron microscopy. In the control group, one hamster also showed minute erosion in the forestomach. No morphological alterations were noted in the

glandular stomach of either group.

Discussion

Recently, protective effects of vitamin A on the gastric mucosa from experimental stress ulceration have been shown by several investigators [9, 13, 16]. Chernov et al. [3] reported that there was a dramatic reduction of serum vitamin A levels in 29 of 35 patients who had been hospitalized with severe burns or other major injuries and that such patients often developed serious gastric ulcers. As a result of sudden depletion of serum vitamin A level, the authors believed that the mucous cells were adversely affected, underwent autolysis and failed to replicate normally. This results in development of mucosal erosions followed by ulceration and hemorrhage. Inthorn et al. [10] also have observed a significant reduction of serum vitamin A levels in rats having undergone hypoxic stress and suggested that gastric ulceration secondary to the stress could be reduced by parenteral application of vitamin A prior to or with the onset of the stress. Thus, vitamin A deficiency may play an important role in development of gastric ulcers.

The present study revealed serial ulcerogenic changes of the gastric mucosa in the vitamin A-deficient hamsters. It was considered that mucosal necrosis resulting in erosion and ulcer might principally be due to functional disorder of the epithelial cells caused by vitamin A deficiency. Hayes [7] reported a shift in the type of glycoprotein synthesis in the intestinal mucosa associated with loss of mucous cells in vitamin A-deficient rats and postulated that vitamin A deficiency caused interruption of differentiation of the post mitotic cells and favored squamous metaplasia or loss of mucous production. In PAS-stained sections of the stomach of the present cases, no remarkable

difference was observed between vitamin A-deficient hamsters and the controls, although the mucosa exhibiting intestinal metaplasia showed increased mucus-secreting cells (PAS positive). On the other hand, multinucleation and necrosis of the parietal cells were also observed in the vitamin A-deficient hamsters. Vitamin A deficiency may disturb the function and differentiation of the parietal cells. However, it remains obscure whether these parietal cell alterations led the mucosa to necrosis or not. Further ultrastructural investigations on the gastric epithelial cells are required to understand the histological evidence of necrotic processes leading to erosion and ulcer.

All the vitamin A-deficient hamsters showed also gastric food retention with hair mixture. The food retention might be due to vitamin A deficiency, since the hamsters ingesting hair in our second experiment showed no gastric food retention. Vitamin A deficiency may be responsible for physiological or functional disturbance of the stomach. Johnson [11] found high incidence of gastric retention (64%) in his patients with gastric ulcers and suggested that gastric retention was an etiologic factor for development of gastric ulcers.

The various amounts of hair observed in the gastric contents of the vitamin A-deficient hamsters may be the result of epilation and licking. It is possible that these hamsters licked their own coat which may have become loose as a result of vitamin A deficiency. Pappenheimer and Larimore [18] noticed ulcerative lesions in the fore-stomachs of the rats fed on diets deficient in various elements of nutrition and the stomachs frequently contained hair in considerable amounts. Furthermore, they demonstrated experimentally that the gastric lesions were produced by adding chopped hair to the food even in the control rats

fed on a complete diet. From such facts, it was concluded that rats fed on improper diets tended to eat hair, and the ingested hair was an important factor in causation of the lesions. The authors stated also that it was probable that the deficient diets in some way intensified the injury caused by hair. However, the location of stomach ulcers observed in the present experiment was different from that seen in rats [18]. In addition, no morphological alterations were noted in the glandular stomachs of the hamsters ingesting hair in our second experiment. Therefore, the presence of hair in the stomach might not have been an important factor for development of glandular stomach ulcers in the vitamin A-deficient hamsters. However, it is still required to clarify the role of hair for development of gastric ulcers, since Nakamura and Ishitani [17] suggested that the existence of hair in the stomachs of so-called "white veal" calves might be an important factor for gastric (abomasal) ulceration.

In the first experiment of the present investigation, it was not known whether the physical difference between vitamin A-free (powder) and control (pellet) diets influenced induction of the gastric ulcers. Flatlandsmo and Slagsvold [5] have studied the effect of physical structure of the diet on development of gastric ulcers in swine and stated that the pelleted diet appeared to be the most consistent factor related to the lesions. Since the control hamsters fed on the standard diet (powdered) in our second experiment were not affected with stomach ulcer, the physical form (powder) of the vitamin A-free diet may not have been a contributory factor for development of ulcers.

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要 約

ハムスターにおけるビタミンA欠乏性胃潰瘍の発生について：原田孝則・山城茂人・P.D. MEADE・P.K. BASRUR（ゲルフ大学獣医学部），真板敬三・白須泰彦（残留農薬研究所）——ビタミンA欠乏飼料で7カ月間飼育したハムスター全例（7例）に胃潰瘍の発生を認めたので，その発生機序について病理学的に検討した．これらのハムスターの胃は，食物停滞のため拡張し，内容物中に被毛が種々の程度に混在していた．潰瘍性病変は，全て腺胃部に局限し，特に前腺胃移行部に好発した．組織学的には，粘膜の局限性壊死・びらんにとどまるものから大型の穿孔性潰瘍に至るものまで種々の過程の潰瘍性病変が認められた．これらの潰瘍は，ビタミンA欠乏による胃の機能障害に起因する食物停滞および粘膜上皮の壊死性変化の共力作用の結果生じたものと推察した．なお，胃内容物中の毛の存在も潰瘍発生における物理的要因として無視できなかったため，ハムスターに1%の割合で被毛を混餌投与する追加実験を行ったが，被毛摂取による胃の食物停滞および腺胃部粘膜の組織学的変化は観察されなかった．

Explanation of Figures

- Fig. 1. Ulcer (arrow) in the glandular stomach of a vitamin A-deficient hamster (No. 2). Note thickening of the gastric mucosa surrounding the ulcer.
- Fig. 2. Focal necrosis with leukocytic infiltration in the glandular stomach epithelium of a vitamin A-deficient hamster (No. 4). H-E. $\times 120$.
- Fig. 3. Submucosal edema in the glandular stomach of a vitamin A-deficient hamster (No. 1). H-E. $\times 120$.
- Fig. 4. Desquamative necrotic mass in the glandular mucosa of a vitamin A-deficient hamster (No. 1). H-E. $\times 270$.
- Fig. 5. Solitary necrosis of parietal cells in the glandular stomach of a vitamin A-deficient hamster (No. 4). H-E. $\times 480$.
- Fig. 6. Multinucleated parietal cells in the glandular stomach of a vitamin A-deficient hamster (No. 4). H-E. $\times 480$.
- Fig. 7. Stomach ulcer in the glandular region adjacent to the forestomach of a vitamin A-deficient hamster (No. 5). It is associated with calcification in the necrotic area. H-E. $\times 120$.
- Fig. 8. Intestinal metaplasia of the glandular mucosa seen in the vicinity of ulcer in a vitamin A-deficient hamster (No. 3). H-E. $\times 48$.