pH 6.5. Sections were postfixed in buffered 1% osmium tetroxide for 2 h at 4 °C, dehydrated in a graded series of alcohols and embedded in epon. These sections were cut with glass knives on a LKB ultratome, stained with uranyl acetate and lead citrate and examined in a HU-12 electron microscope. Some of the sections cut at 30 µm were also incubated in the same medium but at pH 5.5 or in Allen's incubation medium[2] (pH 5.5). The sections were washed in distilled water, immersed in dilute ammonium sulfide and mounted in glycerine jelly for light microscope observations. The reaction product was present in the endoplasmic reticulum and nuclear envelope in all cell types of the epididymis, i.e. the principal cell, halo cell, basal cell, clear cell, smooth muscle cell and fibroblast (figures 2–4). The amount of the reaction product was scanty and was similar in all cell types in the initial segment and part I (figure 2), and apparently more abundant in the principal cell than in other cell types in parts II, III and IV (figures 3 and 4). The basal cell, halo cell, clear cell, smooth muscle cell and fibroblast showed similar amounts of deposits in all parts of the epididymis. The reaction product was also observed on the stereocilia of the principal cell and sometimes in the lysosomes in the principal and basal cells. The product on the stereocilia was abundant in the initial segment, in small quantities in part I and was not seen in the other parts.

In order to ascertain whether the reaction product is due to G6Pase activity, control experiments were carried out as described previously[9,10] at light and electron microscopic levels. Immersion of the glutaraldehyde-fixed sections in 0.1 M acetate buffer (pH 5.0) at 37 °C for 15 min before incubation in the reaction medium and the use of β-glycerophosphate in place of glucose 6-phosphate in the reaction medium, resulted in complete absence of the reaction product except on the stereocilia of the principal cell and in some lysosomes in the principal and basal cells. Pre-incubation of the sections in 0.25 M sucrose containing 1 mM Cu^{2+}, 10 mM Zn^{2+}, 10 mM F^- or 10 mM CN^-, and then incubation in the reaction medium containing equal moles of these ions, caused a loss of the histochemical staining except on the stereocilia, although no staining was seen on the stereocilia with CN^-. These results indicate that the reaction product in the endoplasmic reticulum and nuclear envelope is due to G6Pase activity, but the deposition of final product on the stereocilia of the principal cell and in the lysosomes in the principal and basal cells is probably related to non-specific phosphatase or acid phosphatase activity.

There were no deposits in the Golgi complex, mitochondria, plasma membrane and other organelles in these cells, including the supranuclear vacuoles of the principal cell. Under the light microscope the staining for the activity appeared more intense in sections incubated in the regular medium (pH 6.5) than in those incubated in the medium at pH 5.5 or Allen's medium (pH 5.5).

G6Pase is known to be present in almost all cell types in various organs[4,8,10–17]. The functional role of this enzyme in the liver and kidney is to release glucose into the blood by hydrolyzing glucose 6-phosphate produced via gluconeogenesis and glycogenolysis[3]. However, the role of the enzyme in other cell types is unknown. In the terminal segment and caudal half of the middle segment of epididymis, the activity of G6Pase is apparently higher in the principal cell than in other cell types. The epididymis is the site of maturation (initial and middle segments) and storage (terminal segment) of the spermatozoa[7]. The principal cell might, therefore, release glucose for use by the spermatozoa. On the other hand, the role of the enzyme in the basal cell, halo cell, clear cell, smooth muscle cell and fibroblast might be simply to regulate the concentration of glucose 6-phosphate in these cells, hydrolyzing any excess, as postulated for the enzyme in the tracheal epithelial cell[15].

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Cimetidine prevents the induction of unresponsiveness by feeding T-dependent antigens[1]

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Summary. Bovine γ globulin (BGG) or ovalbumin (OVA) given to mice orally induced virtually complete unresponsiveness to later parenteral immunization. When these antigens were given to mice orally, following cimetidine treatment, and the animals were parenterally immunized later, enhanced response to the challenge antigen occurred.

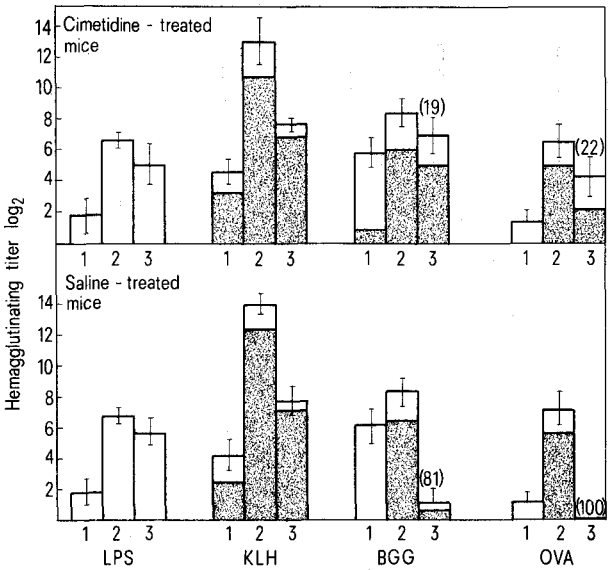
The receptor for histamine, designated H2, can be blocked with the drug cimetidine, which causes marked inhibition of gastric acid secretion[2–4]. Suppressor T cells (T_S) also bear this receptor and there is evidence that cimetidine may modify these cells and under some conditions produce an immunostimulatory effect [5,6]. Suppressor T cells have been shown to be involved in the specific unresponsiveness which follows the oral administration of certain antigens[7,8].

The present study was undertaken in order to investigate the influence of cimetidine on the humoral immune response to T-dependent and T-independent antigens given orally in mice.

Materials and methods. Randomly bred Swiss mice weighing 18–20 g were used. The antigens, *E. coli* lipopolysaccharide (LPS) - Difco Laboratories; Keyhole limpet hemocyanine (KLH) - A grade, (Calbiochem, San Diego, Calif.);

bovine-γ-globulin (BGG) - Cohn fraction II (Sigma, St. Louis, Mo.); ovalbumin (OVA) - grade V (Sigma), were administered by intragastric intubation under light anaesthesia with a syringe to which a blunt metallic cannula was attached. Saline suspension of cimetidine (ground film-coated tablets) in 0.1 ml (100 mg/kg) was injected by intragastric intubation 2 h prior to the antigen injection. The passive hemagglutination titer of sera from individual mice was determined. KLH, BGG and OVA were coated on to sheep red blood cells by the method of Daniel and Stavitski[9]. LPS was coated as previously described for brucella lipopolysaccharide[10]. At the same time, passive hemagglutination was performed in a diluent containing 0.05 M mercaptoethanol.

Results and discussion. There was no difference in the hemagglutinating antibody response between the cimetidine- and saline-treated mice when LPS or KLH were given p.o. on day 0, and the mice subsequently immunized i.p. on day 7 (figure). The animals showed an enhanced response to the subsequent parenteral immunization. In contrast, 17 out of 21 mice (81%) fed BGG and 12 out of 12 mice (100%) fed OVA were totally unresponsive, as indicated by hemagglutinating titers 14 days post oral feeding and 7 days post parenteral immunization. Cimetidine given p.o. prior to the intragastric feeding prevented the establishment of specific unresponsiveness. Only 19% of BGG-treated mice and 22% of OVA-treated mice showed an unresponsive state following i.p. immunization. No difference was found between the cimetidine- or saline-treated groups when these antigens were given i.p. on day 0. The hemagglutinating antibodies of LPS-treated mice were mercaptoethanol-sensitive, whereas the KLH antibodies were mostly mercaptoethanol-resistant. After oral feeding of BGG and OVA to cimetidine-treated animals and after subsequent parenteral immunization mercaptoethanol-resistant antibodies appeared (figure). These antibodies are therefore of the IgG type and are indicative of a secondary immune response. The action of cimetidine is not due to its inhibition of production of gastric acid, since feeding antigen in sodium bicarbonate produced the same state of unresponsiveness, as found in the control animals (results not shown in this paper). A number of studies have shown that exposure to protein or particulate T-cell-dependent antigens by the digestive route may result in diminished responsiveness to later immunogenic encounters with specific antigen[11,12]. However, animals immunized orally with bacterial antigens showed an enhanced response to subsequent parenteral immunization[13]. The failure of the bacterial antigens to induce hyporesponsiveness is probably a reflection of their T-cell-independent nature. In our study also, LPS and KLH, both T-independent antigens[14,15] elicited an elevated immune response. On the other hand, OVA or BGG (T-dependent antigens) produced a state of unresponsiveness. Cimetidine given p.o. prevented the induction of the unresponsive state. The mechanism/s underlying this inhibition is/are unknown. Antigen-specific T_S cells occur in the spleen and lymph nodes of OVA-fed mice. These T_S inhibit the induction phase of sensitization[16]. As T-suppressor cells bear H2 receptors, their inhibition by cimetidine might result in an enhancement of a delayed type hypersensitivity reaction and antibody production against T-dependent antigen[4,5,17]. The findings of Ngan and Kind[7] and Mattingly and Waksman[18] that Peyer's patches from antigen-fed animals contained transferable T_S, which were first detected in Peyer's patches and mesenteric lymph nodes, and then migrate to the spleen and peripheral lymph nodes, allow the interpretation that inhibition of T_S is probably the cause of the immunostimulating effect of cimetidine given orally. Whatever the exact mechanism/s for the inhibition of orally-induced unresponsiveness by cimetidine may be, it is clear from the present data, that humoral immune response to T-dependent antigens can be induced p.o. The current wide use of cimetidine in the human population should prompt further study of its effect on reaction to food antigens.



Effect of intragastric pre-treatment with cimetidine on subsequent humoral antibody response by feeding T-dependent or T-independent antigens. Groups of 10-20 mice were treated as follows: *1* given saline p.o. on day 0 (0.1 ml); *2* given antigen i.p. on day 0 (LPS, 50 µg; KLH, 100 µg; BGG and OVA, 200 µg); *3* given antigen p.o. on day 0 (200 µg in 0.1 ml). All the animals received i.p. injection of antigen on day 7 (LPS, 50 µg; KLH, 100 µg; BGG and OVA, 200 µg) and were bled on day 14. Cimetidine or saline were given per os (100 mg/kg in 0.1 ml) on day 0, 2 h prior to the treatment with antigen or saline. □, Mercaptoethanol-sensitive hemagglutinating antibodies; ▨, mercaptoethanol-resistant hemagglutinating antibodies. Numbers in parentheses indicate the percentage of unresponsive mice. Data are mean ± S.E.

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