ENTODERMAL ANTIGEN IN THE HUMAN ORAL MUCOSA, ORGANS, AND SECRETIONS

V. N. Isaev, Z. Kh. Mamilyaeva, and T. P. Séédre

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In dogs with spontaneous stomatitis an additional component appears in ectodermal derivatives (the mucous membrane of the cheek, tongue, and hard palate), which is not found in the homonymous regions of the normal oral mucosa [5].

The object of this investigation was to study the antigenic spectrum of the human oral mucosa and to compare the additional antigen in man and animals (dogs).

EXPERIMENTAL METHOD

As immunizing material a saline extract of the mucous membrane of the human soft palate was used; as experimental investigations have shown, this extract contains the maximal amount of the antigen to be studied.

Saline extracts of the mucosa from different parts of the cheek, of the soft palate, the hard palate, the lateral surface and dorsum of the tongue, and the gums, and also extracts from various organs, all of human origin — esophagus, stomach, small and large intestine, kidney, liver, spleen, lung, skin, and heart — were used as test material. Blood serum and various secretions, bile, mucus from different parts of the intestine, milk, and saliva from the parotid, submandibular, and sublingual human salivary glands, also were investigated.

Saline extracts of human organs and tissues were prepared by the usual method with slight modifications [1]. Total protein in the extracts was determined by the biuret method; its content was 7-10 mg/ml [7].

Hyperimmune antisera were obtained by immunization of rabbits directly into the lymph nodes [2] with Freund's complete adjuvant (from Difco, USA). Rabbit antisera were absorbed by two methods: by addition of lyophilized saline extracts of various organs and tissues, and also by means of sorbents prepared by the method of Avrameas and Ternynck [9]. Altogether 200 saline extracts of various organs and tissues and 60 rabbit antisera against the mucosa of the human soft palate were investigated.

EXPERIMENTAL RESULTS

Antisera obtained against the mucosa of the human soft palate reacted with the formation of several precipitation bands, both with other regions of the oral mucosa and with human blood serum and also with antigens from other organs: antigens of the liver, kidneys, lungs, heart, and spleen (Fig. 1). A step-by-step investigation of the antiserum yielded a monospecific antiserum against the mucosa of the human soft palate (Fig. 2). This monospecific antiserum reacted with a saline extract of the mucosa of the posterior regions of the cheek, but did not react with saline extracts of the mucosa of the anterior regions of the cheek, the, tip, dorsum, and lateral surface of the tongue, or mucosa of the hard palate and gum, all of human origin. After detection of the additional entodermal antigen in the antigenic spectrum of the human oral mucosa, a comparative study was made of the distribution of this antigen in the healthy man and dog. The results are given in Table 1. They show that antigen of the mucosa of the soft palate (described as entodermal under these experimental conditions) is present in both dog and man in homonymous regions of the oral mucosa.

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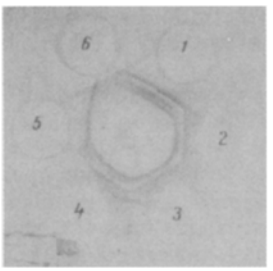

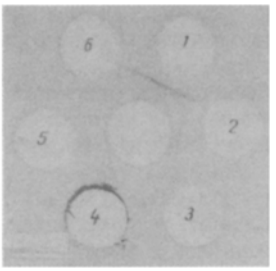

Fig. 1 Fig. 2

Fig. 1. Antiserum against mucosa of human soft palate (before absorption). Central well contains antiserum against mucosa of human soft palate. Peripheral wells contain: 1, 2, 3, 4, 5) antigens of human soft palate, kidney, liver, spleen, and lung, respectively; 6) human blood serum.

Fig. 2. Monospecific antiserum against mucosa of human soft palate. Central well contains antiserum against mucosa of soft palate after absorption. Peripheral wells contain: 1, 2, 3, 4, 5) antigens of human soft palate, kidney, liver, spleen, and lung, respectively; 6) human blood serum.

TABLE 1. Comparative Distribution of Entodermal Antigen in Healthy Man and Dog

Test material	Man	Dog
Mucosa:		
of soft palate	+	+
hard	−	−
Cheek, anterior region	−	−
Tongue:		
tip	−	−
root	+	+
dorsum	+	+
lateral surface	−	−
Gum	−	−
Esophagus	+	+
Stomach	+	+
Small intestine	+	+
Large intestine	+	+
Liver	−	−
Spleen	−	−
Kidney	−	−
Skin	−	−

To determine the content of this entodermal antigen in the test extracts, semiquantitative analysis was carried out by Zil'ber's method [4] with maximal dilution of the test system.

These investigations showed that in dog tissues entodermal antigen was present in titers of 1:8 to 1:256; in the direction from the mouth toward the distal parts of the gastrointestinal tract its content gradually fell to reach a minimum in the gastric mucosa, after which it increased again toward the distal end of the intestine. In the corresponding human tissues the content of entodermal antigen was somewhat less than in dogs. For instance, in the mucosa of the human large intestine this antigen could be detected only by means of immunoautoradiography.

The study of entodermal antigen in human secretions revealed an important feature: Just as in animals it was absent from saliva in the efferent duct of the parotid salivary gland but was constantly present in saliva from the submandibular and sublingual salivary glands.

TABLE 2. Comparative Physicochemical Properties of Entoderman Antigen from Man and Animals

Procedure, property	Entodermal antigen	
	human	dog
Procedure:		
boiling for 10 min	Inactivates	Inactivates
trypsin	No effect	Partly inactivates
pronase	Inactivates	Inactivates
amido black 10B	Stains	Stains
azocarmine	"	"
Electrophoresis — movement; immunoelectrophoresis — relative mobility	Toward anode 0.3	Toward cathode 0.5
Preparative electrophoresis — location	Zone of γ-globulins	Zone of α_2-macroglobulins
Property of accumulating in mucosa of entodermal origin in chronic inflammatory conditions	Present	Present

The antigen was not detected in milk.

The content of entodermal antigen in mucus from the gastrointestinal tract varied in different parts: The maximal content of antigen was found in the large intestine, minimal in the small intestine. The entodermal antigens detected in different parts and secretions of the intestine were immunologically completely identical.

In view of data on the distribution of lysozyme in the mucosa of the gastrointestinal tract [8] it was decided to study whether the entodermal antigen contained in these tissues is in fact lysozyme. The results showed that the two are not identical. Entodermal antigen likewise is not identical with antigen of human mucus-forming cells which itself is identical with carcinoembryonic antigen [6].

A study of the physicochemical properties of human entodermal antigen showed (Table 2) that in nature it is a protein, for it does not withstand heating to 75°C for 5 min, it is inactivated by pronase, and it stains with amido black 10B and azocarmine. To isolate the entodermal antigen, the original material consisted of saline extracts, secretions, and mucus containing different quantities of this antigen. On fractionation of saline extracts, secretions of glands, and mucus from different parts of the gastrointestinal tract in polyacrylamide gel they separated into 8-13 visible components. They were fewest in number when saliva from the submandibular salivary gland was used, most numerous in the case of mucus from the intestine. Analysis of the results of electrophoresis showed that all the original materials are suitable for isolation of entodermal antigen, but that its content is highest in mucus from different parts of the gastrointestinal tract. Because of the large quantity of impurities, saline extracts of tissues are least suitable for the isolation of entodermal antigen.

The study of the immunoelectrophoretic mobility of the isolated antigen showed that it is localized to the fraction with relative mobility of 0.3-0.4. Preparative and analytical electrophoresis in polyacrylamide gel showed that human entodermal antigen is localized in the γ-globulin zone, but canine entodermal antigen lies closer to the α_2-macroglobulin zone.

Experiments on isolation and purification of the entodermal antigen consisted of two stages: preparation of partially purified samples of entodermal antigen and their subsequent purification. After each stage of purification the fractions obtained in the preparative apparatus were tested by the agar diffusion test for the presence of entodermal antigen, then dialyzed against distilled water, and freeze-dried. The antigen thus obtained was kept at 2-8°C and, when necessary, used in the diffusion test and for electrophoresis. It preserved its properties for a long time. Proteins were fractionated by disc electrophoresis in polyacrylamide gel [3].

Clinical and experimental studies of entodermal antigen thus showed that it is present in the human oral mucosa in the same regions as those where it was first found in animals (dogs); the physicochemical properties of human and canine entodermal antigen also are similar in many respects. This suggests a possible general biological rule for its distribution in at least two representatives of mammals.

These investigations also showed that in man, entodermal antigen accumulates in saliva of the submandibulal and sublingual salivary glands during chronic recurrent diseases and remains in it in considerable quantities at all stages of development of the disease.

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EFFECT OF SOME NEW ANTIHISTAMINE DRUGS ON IMMUNOLOGIC REACTIVITY

M. D. Mashkovskii, I. G. Veksler, M. É. Kaminka, and V. A. Yakimenko

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Many observations have shown [1, 2, 4, 5] that determination of the number of antibody-forming cells (AFC) and rosette-forming cells (RFC) in the blood and immunocompetent organs and detection of allergic reactions of delayed-type hypersensitivity (HDT) are objective tests with which to study immunologic reactivity and its changes under the influence of pharmacologic intervention.

The object of this investigation was an experimental study of the effect of new antihistamine drugs — phencarol (quinuclidyl-3-diphenylcarbinol) and bicarphen [quinuclidyl-3-di(ortho-tolyl)carbinol hydrochloride] — by comparison with the action of dimedrol (diphenhydramine hydrochloride) on immunologic reactivity *in vivo*. According to the results of pharmacological studies, phencarol has higher antihistamine activity than dimedrol, whereas bicarphen differs from phencarol in its longer antihistamine action and its high antiserotonin activity [3].

EXPERIMENTAL METHOD

Male BALB/c mice (135 animals) weighing 18-20 g and light colored guinea pigs (40 animals) weighing 350-400 g were used. The drugs were injected into the stomach in a volume of 0.2 ml of 1% starch solution (bicarphen) or distilled water (phencarol and dimedrol).

Immunologic reactivity was determined as the number of AFC in the mouse spleen, revealed by the method of local hemolysis in gel [7] in response to intraperitoneal injection of the test antigen ($5 \cdot 10^8$ sheep's red blood cells), and the number of immune RFC in the spleen,

Laboratory of Pharmacology, S. Ordzhonikidze All-Union Pharmaceutical Chemistry Research Institute, Moscow. Department of Protective and Regulatory Mechanisms in Carcinogenesis, R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev.

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