

Transport abnormalities during intestinal anaphylaxis in the rat: Effect of antiallergic agents*

Mary H. Perdue, Ph.D., and D. Grant Gall, M.D. Calgary, Alberta, Canada

Our previous studies demonstrated that rats sensitized to egg albumin had reduced intestinal absorption of water and electrolytes in response to intraluminal antigen. The rapid onset of this effect and reduction in mucosal histamine and numbers of granulated mast cells in the lamina propria suggested a reaginic (IgE) mechanism involving mast cell mediators. In this study we examined the effect of antiallergic agents on the intestinal transport abnormalities in our model. Sensitized rats, 14 days after intraperitoneal injection of 10 µg of egg albumin plus alum had specific IgE serum titers $\geq 1:64$; control rats had no measurable IgE antibodies. Net fluxes of Na^+ , Cl^- , and H_2O were determined by in vivo perfusion during a 1-hour antigen-free period and then a 1-hour antigen period. Sodium cromoglycate, administered intravenously (20 mg/kg) or in the perfusate (5×10^{-4} mol/L) failed to prevent mucosal mast cell degranulation as evidenced by histamine release or the decrease in absorption of H_2O , Na^+ , and Cl^- induced by antigen exposure. In contrast, 10^{-3} mol/L of doxantrazole in the perfusate completely inhibited these changes. Histamine receptor antagonists, H_1 , diphenhydramine, or H_2 , cimetidine, in perfusates had no effect on the transport abnormalities. Our findings support a role for intestinal mucosal mast cells, but not connective tissue mast cells, in the pathogenesis of the intestinal dysfunction associated with mucosal IgE-mediated reactions to food proteins and suggest that mast cell mediators other than histamine are involved. (J ALLERGY CLIN IMMUNOL 76:498-503, 1985.)

We previously demonstrated that rats sensitized by intraperitoneal injection of EA responded with production of antibodies directed against EA.¹ These antibodies were heat labile (56° for 3 hours) and produced PCA reactions 72 hours after intradermal injection, suggesting that they were of the IgE class.² When segments of jejunum in these rats were perfused in vivo with EA-containing electrolyte solution, absorption of Na^+ , Cl^- , K^+ , and H_2O decreased dramatically. The transport abnormalities were specific for the sensitizing antigen, did not occur in nonsensitized control rats, nor did they reverse when the EA-

Abbreviations used

EA:	Egg albumin
PEG:	Polyethylene glycol
PCA:	Passive cutaneous anaphylaxis
DOX:	Doxantrazole
SCG:	Sodium cromoglycate
DPH:	Diphenhydramine
CIM:	Cimetidine

containing solution was replaced with EA-free solution. The rapid onset of these changes and their persistence after the antigen was withdrawn were suggestive of an immediate hypersensitivity reaction involving mediators released from mast cells. Indeed, after antigen perfusion, histamine in mucosal homogenates and numbers of darkly stained granulated mast cells in the lamina propria of sensitized rats were decreased. Concomitantly, histamine concentrations in collected perfusates increased. Although these findings implied that mast cells degranulated in response to the antigen, we had no direct evidence that mast cells or their mediators were involved in producing the transport abnormalities.

From the Intestinal Disease Research Unit, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada.

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Reprint requests: D. Grant Gall, M.D., Dept. of Pediatrics, University of Calgary Health Science Centre, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.

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Isolated mast cells have been used to examine the action of pharmacologic agents on antigen-induced degranulation. By use of the appearance of histamine in the suspending medium as a marker of degranulation, Pearce et al.³ found that rat mast cells isolated from different sources responded differently to antiallergic agents. For example, SCG blocked antigen-induced histamine release from peritoneal mast cells but not from intestinal mucosal mast cells. DOX, however, inhibited histamine release from both types of mast cells.

The present studies were designed to examine the effect of antiallergic compounds on the transport abnormalities that occur in sensitized rats in response to intraluminal antigen. Compounds that act directly on mast cells were used to determine whether these cells could be implicated. In addition, since histamine is the classical mediator usually associated with immediate hypersensitivity reactions, histamine receptor antagonists were used to examine the role of histamine in producing the abnormalities.

MATERIAL AND METHODS

Model

Hooded-Lister rats were obtained from an outbred colony maintained at our institution. Animals weighing 100 to 130 gm were sensitized by intraperitoneal injection of 10 μ g of chicken egg albumin (EA, grade V, Sigma Chemical Co., St. Louis, Mo.) as described¹ except aluminum hydroxide was used as adjuvant.⁴ Control rats were sham-treated litter mates. Thirteen days later, after a 6-hour fast, blood was obtained for determination of anti-EA antibody titers. Animals were fasted for an additional 18 hours and then submitted to the experimental protocol.

Experimental protocol

Fourteen days after sensitization, rats were anesthetized with an intramuscular dose of urethane. Temperature was maintained at 37° C by means of a heating pad and rectal temperature monitor. A laparotomy was performed, and a 15 cm segment of jejunum, starting 10 cm distal to the ligament of Treitz, was cannulated at both ends and perfused with electrolyte solution (140 mmol/L Na⁺, 10 mmol/L K⁺, 120 mmol/L Cl⁻, and 30 mmol/L HCO₃⁻) containing 10 μ Ci/L of [¹⁴C]PEG 4000 as a nonabsorbable marker.¹ After an initial 60-minute equilibration period, consecutive 20-minute samples were collected from the distal sampling site by gravity flow into chilled containers (4° C). Samples were stored at -20° C for later determination of Na⁺, Cl⁻, and [¹⁴C]PEG for each 20-minute period. Experimental rats were perfused initially, 0 to 60 minutes, with antigen-free electrolyte solution. Then the perfusate was changed to one that included 10 μ g/ml of EA, and collections were continued from 100 to 160 minutes. In the experiments on sensitized rats, the compound under study was present in the perfusate

during the equilibration, antigen-free, and antigen periods. Compounds studied and concentrations used in the perfusate were DOX, 10⁻⁴ mol/L and 10⁻³ mol/L, SCG, 5 $\times$ 10⁻³ mol/L, DPH, 5 $\times$ 10⁻⁴ mol/L, CIM, 10⁻⁴ mol/L. The dose of DPH was based on preliminary experiments in jejunal tissue from Hooded-Lister rats. In Ussing-type chambers, histamine, 10⁻⁴ mol/L, produced a significant ($p < 0.001$) increase in short-circuited current (79 ± 6.9 versus $47 \pm 4.8 \mu\text{A} \cdot \text{cm}^{-2}$, $\bar{X} \pm \text{SE}$) that could be prevented by the addition of 10⁻⁵ mol/L of DPH (41 ± 7.3 versus 40 ± 7.0). Additional studies examined the effect of intravenously administered SCG, 20 mg/kg, in 1.0 ml of saline 20 minutes before challenge with EA. Control rats were studied to determine if the agents themselves had any effect on ion absorption. Absorption during a 1-hour basal period when no agent had been added was compared with a 1-hour test period starting 40 minutes after addition to the perfusate of the agent at the concentrations indicated above. At the end of each experiment, the perfused segment was removed, length was measured, and the mucosa was scraped and homogenized.

Measurements

Specific anti-EA IgE titers were measured by PCA.² Positive sera were heated to 56° C for 3 hours, and the PCA determination was repeated. [¹⁴C]PEG was measured by β -scintillation spectrometry, Na⁺ by flame photometry, and Cl⁻ by chloride analyzer. Net water and ion fluxes were calculated by standard formulae.⁵ Mucosal histamine was assayed in mucosal homogenates by the method of Beaven et al.⁶ as previously described¹ and expressed per milligrams of homogenate protein. Protein was determined by the method of Lowry et al.⁷ Results were compared statistically by use of analysis of variance, analysis of covariance, Student's *t* test, or paired *t* test where it was appropriate.

SCG (Intal; Fisons Corp., Bedford, Mass.) was purchased from a local pharmacy. DPH and CIM were purchased from Sigma Chemical Co. DOX was a gift from Burroughs Wellcome Co. (Research Triangle Park, N. C.).

RESULTS

Thirteen days after sensitization, experimental rats had antibody titers against EA of $\geq 1:64$ as measured by PCA. The PCA reaction was eliminated when these sera were heated to 56° C for 3 hours. Sera from control rats had no anti-EA antibodies.

The effect of the addition of each antiallergic agent on absorption of water and electrolytes in control animals is illustrated in Fig. 1. Data are expressed as percent net absorption during the test hour when the agent was present compared with absorption during the basal hour when the perfusate contained no additions. As illustrated, none of the compounds studied had any effect on the basal H₂O and electrolyte transport. In control animals net absorption of Na⁺, Cl⁻,

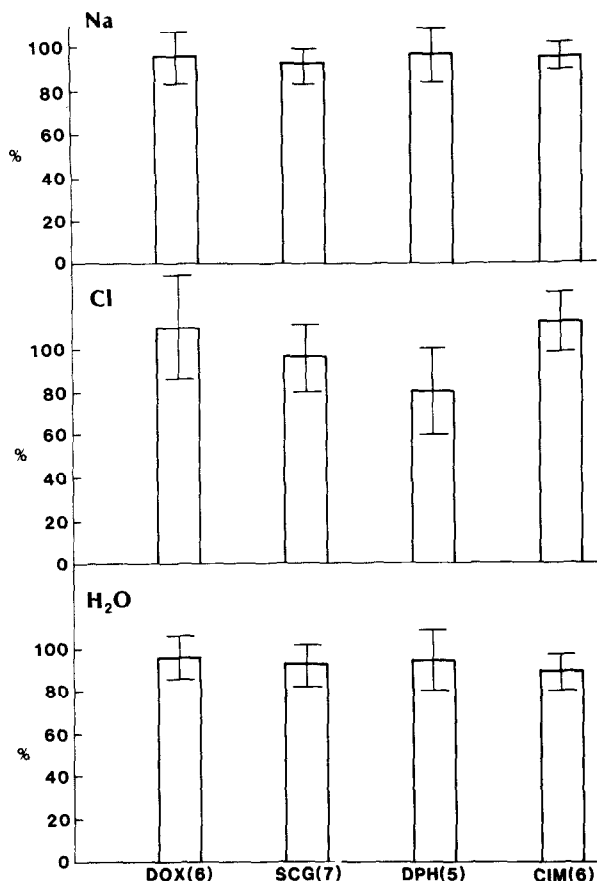


FIG. 1. Effect of antiallergic agents on absorption in control rats. Results are expressed as percent absorption during the test hour when the agent was present compared with absorption during the basal hour. Concentrations in the perfusates were DOX 10^{-3} mol/L, SCG 5×10^{-3} mol/L, DPH 5×10^{-4} mol/L, and CIM 10^{-4} mol/L. Number of animals in each group is presented in parentheses.

and H₂O was not altered by the presence of DOX, SCG, DPH, or CIM.

The effect of DOX on antigen-induced transport abnormalities is presented in Fig. 2. Water and ion fluxes were calculated for a 1-hour antigen-free period (0 to 60 minutes) and a 1-hour antigen period (100 to 160 minutes) in untreated sensitized and control rats and in sensitized animals receiving either 10^{-3} or 10^{-4} mol/L of DOX in the perfusate. For comparison, results are presented as percentage of the antigen-free period for each group. As previously demonstrated,¹ intraluminal antigen challenge in sensitized animals led to a significant ($p < 0.005$) reduction in net absorption of Na⁺, Cl⁻, and H₂O. DOX, 10^{-3} mol/L, completely abolished the transport abnormalities. Net Na⁺, Cl⁻, and H₂O fluxes in sensitized rats during antigen challenge were not significantly different from

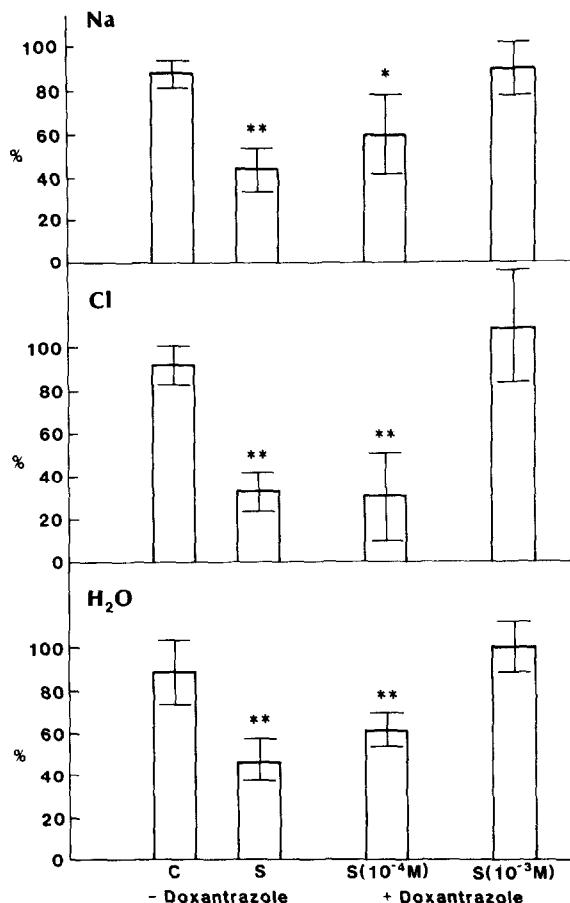


FIG. 2. Effect of DOX on absorption abnormalities in sensitized rats. Results are expressed as percent absorption during the test hour when antigen (EA, 10 μ g/ml) was present compared with absorption during the basal hour when the perfusate was antigen free. The concentration of DOX was 10^{-4} mol/L ($n = 5$) or 10^{-3} mol/L ($n = 6$) in perfusates as indicated. Values for untreated control ($n = 12$), C, and sensitized rats ($n = 15$), S, are also presented. ** $p < 0.005$ and * $p < 0.025$ compared with antigen-free hour.

values obtained during the antigen-free period or values obtained in control animals. DOX at 10^{-4} mol/L produced an intermediate response. Antigen challenge resulted in a slightly less but still significant decrease ($p < 0.05$ to $p < 0.025$) in net H₂O and ion absorption.

The effect of SCG administration during antigen perfusion in sensitized rats is presented in Fig. 3. SCG, whether in the perfusate at 5×10^{-3} mol/L or 20 mg/kg administered intravenously, did not prevent the decreases associated with antigen challenge in sensitized rats. Water and electrolyte fluxes decreased significantly ($p < 0.005$) after antigen challenge compared to the antigen-free period and were not sig-

TABLE I. Mucosal histamine after antigen challenge

Rats	Addition	n	Histamine (ng/mg)
Controls	—	6	4.16 ± 1.07
Sensitized	—	8	0.48 ± 0.13*
Sensitized	DOX	5	4.89 ± 1.34
Sensitized	SCG	6	0.83 ± 0.41*

Results are presented as mean ± SE. Rats were perfused with 10 µg/ml of EA for 60 minutes. Histamine values are expressed as nanograms per milligrams of homogenate protein. DOX was 10^{-3} mol/L in perfusates; SCG was administered intravenously 20 mg/kg 20 minutes before antigen challenge. * $p < 0.025$ when data are compared with control data.

nificantly different from those observed during antigen challenge in untreated sensitized animals.

The effect of the H_1 receptor antagonist, DPH, 5×10^{-4} mol/L, and the H_2 receptor antagonist, CIM, 10^{-4} mol/L is also presented in Fig. 3. DPH and CIM were without effect. Despite their presence, antigen challenge led to a significant reduction in H_2O and electrolyte absorption that did not differ in degree from that observed in untreated sensitized animals.

Histamine levels in mucosal homogenates prepared after antigen perfusion are listed in Table I. In the absence of any antiallergic agent, histamine levels were decreased by antigen challenge in sensitized rats compared to control rats ($p < 0.025$). The presence of 10^{-3} mol/L of DOX in the perfused solutions during antigen challenge in sensitized rats prevented histamine release. Histamine levels in mucosa in these animals were not significantly different from control values. In contrast, SCG, 20 mg/kg administered intravenously, was without effect. Antigen challenge, despite SCG, significantly reduced mucosal histamine levels in sensitized animals. These levels were not significantly different from those observed in untreated challenged experimental rats.

DISCUSSION

In this study and as previously demonstrated,¹ rats sensitized to EA consistently demonstrated abnormalities of net absorption of water and electrolytes in response to intraluminal antigen challenge. These animals had serum anti-EA antibody titers of $\geq 1:64$ as measured by PCA, and the PCA reaction was abolished by heat treatment of the sera. The PCA reaction was determined 72 hours after intradermal injection of sera. By this time any IgG antibodies would be expected to have dissociated from mast cells.⁸ These results suggest that the reaginic affect was due to heat-

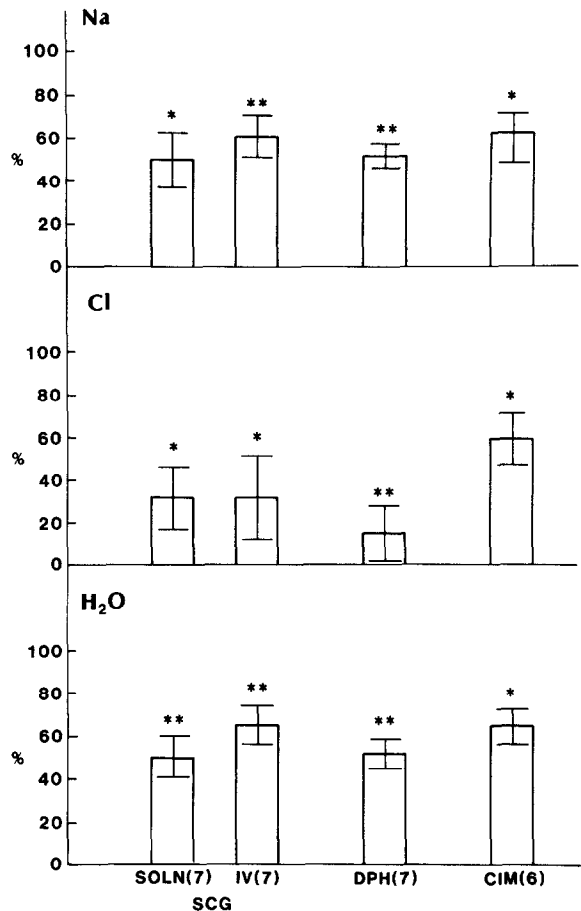


FIG. 3. Effect of antiallergic agents on absorption in sensitized rats. Results are expressed as percent absorption during the test hour when antigen (EA, 10 µg/ml) was present compared with absorption during the basal hour when the perfusate was antigen free. Concentrations in perfusates were DPH 5×10^{-4} mol/L, CIM 10^{-4} mol/L, SCG in solution (SOLN) 5×10^{-3} mol/L; SCG, intravenous 20 mg/kg. Number of animals in each group is presented in parentheses. ** $p < 0.005$ and * $p < 0.025$ compared with antigen-free hour.

labile IgE antibodies. In the previous reported study,¹ transport abnormalities were associated with reduced numbers of darkly stained granulated mast cells in the lamina propria and decreased histamine levels in mucosal homogenates of sensitized rats exposed to antigen. Results from the present study provide further evidence for a role of intestinal mast cells in the antigen-induced transport dysfunction. DOX, 10^{-3} mol/L in the perfused solutions, protected sensitized rats against the antigen-induced decrease in mucosal histamine and transport abnormalities; however, SCG, another mast cell stabilizer, was not protective. To assure that the lack of effect of SCG in the perfusate was not due to poor absorption, we also administered

the drug intravenously before antigen challenge. Intravenous administration was also without effect.

The doses of DOX and SCG used were chosen based on their effectiveness in preventing histamine release from isolated rat mast cells. DOX at 10^{-3} mol/L prevents degranulation of both peritoneal and mucosal mast cells; SCG at concentrations above 10^{-5} mol/L prevents degranulation of peritoneal mast cells but is ineffective even at high (10^{-3} mol/L) concentrations on mucosal mast cells.³ In our studies SCG was used at a concentration of 5×10^{-3} mol/L in the perfused solution to counteract poor absorption. To confirm the lack of response to SCG, we also examined the effect of systemic therapy. The intravenous dose used, 20 mg/kg, was selected to approximate a concentration of 5×10^{-4} mol/L in the extracellular fluid.

It is clear from recent reports that histamine-containing mast cells represent a heterogeneous population. Mucosal mast cells differ from connective tissue mast cells by their morphologic and staining characteristics and by their response to secretagogues and antiallergic agents. Mucosal mast cells are smaller and have fewer granules that contain less histamine and serotonin and no heparin.⁹ Polymyxin and 48/80, which induce degranulation of connective tissue mast cells, are without effect on mucosal mast cells.^{9, 10} DOX but not SCG prevents antigen-induced histamine release from gut-associated mucosal mast cells, whereas both DOX and SCG are protective in studies on connective tissue peritoneal mast cells. If mast cells in situ respond in a similar manner, our results suggest that the transport abnormalities in sensitized rats are due to release of one or more mediators from mucosal mast cells in the intestine.

We also evaluated the effect of antihistamines. Histamine has been demonstrated to produce net fluid secretion in the intestine. In *in vitro* rabbit ileum 10^{-4} mol/L histamine produces an increase in short-circuited current and secretion of Cl^- ions. This effect is prevented by 10^{-4} mol/L of DPH but not 10^{-4} mol/L of CIM.¹¹ Similarly, in guinea pig ileum increases in short-circuited current produced by 10^{-4} mol/L of histamine are abolished by 10^{-5} mol/L of pyrilamine, another H_1 receptor antagonist.¹² We confirmed similar findings in *in vitro* rat jejunum (methods). The increase in short-circuited current produced by 10^{-4} mol/L of histamine was blocked by 10^{-5} mol/L of DPH but not 10^{-5} mol/L of CIM. In our *in vivo* perfusion studies the antigen-induced transport abnormalities in sensitized rats were not prevented by an even higher concentration of DPH, 5×10^{-4} mol/L, or CIM, 10^{-4} mol/L. Although we did demonstrate

depletion of mucosal histamine and therefore presumably mast cell release, the antihistamine results suggest that histamine plays a minor, if it plays any role, in the transport abnormalities induced by intestinal anaphylaxis. It is possible that local concentrations of histamine released from mast cells may have been insufficient to produce an H_1 antagonist inhibitable effect. Antihistamines are effective only against the action of histamine and not against the actions of other mast cell autacoids. At least 20 chemical mediators of immediate hypersensitivity have been defined,¹³ many of which have potent effects on the intestinal epithelium. Besides histamine, serotonin, vasoactive intestinal peptide, arachadonic acid metabolites, and acetylcholine are released when mast cells degranulate and produce changes in intestinal ion transport.¹⁴⁻¹⁶ Our findings indicate that one or more of these mediators are probably more important than histamine in producing the intestinal transport pathophysiology that occurs in intestinal anaphylaxis.

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Where does cat allergen 1 come from?

K. Bartholomé, M.D.,* W. Kissler, M.D.,** H. Baer, Ph.D.,***

E. Kopietz-Schulte, B.Sc.,* and U. Wahn, M.D.*

Bochum, West Germany, and Bethesda, Md.

Cat allergen 1, the major allergen from cats, has been demonstrated to be present in surface washing and in saliva. With the use of an immunohistologic technique with monospecific anti-CA 1, we demonstrated two different sources of the protein, mucous salivary glands and hair roots, where it originates from sebaceous glands. (J ALLERGY CLIN IMMUNOL 76:503-6, 1985.)

Aqueous extracts prepared from cat hair and dander have been demonstrated by crossed-immunoelectrophoresis to contain at least 18 different proteins, 12 of them binding to IgE antibodies of cat-sensitive individuals.¹ CA 1, an antigen with a molecular weight of 32,000 daltons, with an isoelectric point of its major component of 3.8 has been demonstrated to be the major allergen from cats.²⁻⁷ It has been isolated and purified.^{8,9} CA 1 is present in epidermal scrapings, in surface washes,¹⁰ and in cat saliva.¹¹ It is not found in urine obtained by bladder puncture¹² but is frequently found in voided urine¹¹⁻¹³; it has not been found in serum.^{11,12}

It has been hypothesized that CA 1 is mainly produced in the saliva and transferred to the pelt by grooming or licking¹⁴; however, Brown et al.¹¹ re-

Abbreviation used

CA 1: Cat allergen 1

cently found its concentration in relation to cat albumin to be higher in cat pelt than in saliva. The authors could not rule out a possible extra salivary source of CA 1 in the pelt.

Our study was performed in order to investigate the presence of CA 1 in salivary glands and skin sections. With the use of an immunohistologic technique, the presence of CA 1 in tissues can be demonstrated. Evidence is presented that sources of CA 1 are both the hair roots and mucous salivary glands.

MATERIAL AND METHODS

Animals

Specimens of skin and salivary glands were obtained from adult domestic cats after the animals had been sacrificed at the end of experiments not related to this study.

Immunohistology

The tissue, deep frozen in liquid nitrogen, was cut into slices (7 µm) without supplementary agents. Slices were put on a microscopic slide and incubated with a monospecific antiserum to CA 1 for 1 hour at 37° C at various concentrations. Monospecific antiserum to CA 1 was pre-

From the *Universitätskinderklinik, Bochum, West Germany.

Pathological Institute, Ruhr University, Bochum, West Germany, and *Office of Biologics, Food and Drug Administration, Bethesda, Md.

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Reprint requests: Priv. Doz. K. Bartholomé, M.D., University Children's Hospital, Alexandrinenstrasse 5, D-4630 Bochum, West Germany.