control and experimental samples were compared by Student's t-test.

Results. The erythropoietic response of rodents to hypoxia is well documented[16–18]: Hypoxia stimulates the production of erythropoietin[19], which in turn stimulates maturation of bone marrow[20] and hastens the release of reticulocytes into the peripheral blood[21]. This process is followed in the table, as hematocrit versus days of hypoxic exposure. The hematocrit gradually rises throughout the period of exposure, significantly so ($p < 0.05$) by day 1. Concurrently, a sharp rise in methemoglobin, significant ($p < 0.05$) by 12 h, peaks at 24 h ($p < 0.001$) and returns to control level by day 6.

Discussion. Normal methemoglobin levels are maintained by a balanced cycle of oxidation and reduction of hemoglobin and methemoglobin, respectively[22]. Hypoxic stress causes methemoglobin levels to elevate rapidly, significantly so ($p < 0.05$) by 12 h, far faster than the corresponding erythropoietic response (table)[18]. This drastic rise could be explained by the fact that deoxygenated hemoglobin, prevalent under hypoxia, oxidizes more readily to methemoglobin than does oxygenated hemoglobin[23]. Alternatively, a decrease in NADH levels or in NADH methemoglobin reductase activity could account for a diminished reduction of methemoglobin to hemoglobin[22]. The return of normal methemoglobin levels by the 6th day of hypoxia could occur by the reversal of these processes after the hematocrit has stabilized at a new high (table). This relationship corroborates the observation of Gourdin et al.[13] mentioned earlier.

Although observed in high-altitude residents[13], methemoglobin has not been examined for acclimative significance. Combined with what is now known about this process, our data permit the following description: In lowland natives, hypoxia evokes a rapid rise in methemoglobin (table), which increases the strength of the affinity between hemoglobin and oxygen[12], which in turn saturates the blood with oxygen for delivery to the tissues, which then facilitates survival[10] until gradual erythropoiesis can increase the blood's oxygen-carrying capacity by enlarging the erythron (table)[18]. Methemoglobin levels can only then return to normal as 2,3-DPG levels rise[4]. The process eventually leads to the high hematocrit and low hemoglobin-oxygen affinity characteristic of sojourners in high altitude.

Research is needed to determine whether a corresponding shift in the hemoglobin-oxygen dissociation curve does occur and whether elevated methemoglobin does affect survival. Meanwhile, we conclude that the dramatic though transient rise in methemoglobin revealed in our study is an effective short-term physiologic adjustment of a lowland native to an oxygen-deficient environment.

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Oxatomide, a new orally active drug which inhibits both the release and the effects of allergic mediators

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Summary. Oxatomide is a new potent inhibitor of anaphylactic and allergic reactions. After oral administration, the compound both inhibits the release of endogenous histamine and prevents the effects of exogenous histamine, at comparable doses. The combination of these effects appears to be the basis of the effectiveness of oxatomide in allergic reactions and may lead to clinical applications different from classical antihistaminics and from cromoglycate.

Allergic reactions centre on mast cells. It has long been recognized that the potent spasmogenic agent, histamine, is stored in this type of cell[1]. Antibodies of a special class, identified as IgE or reaginic antibodies, bind slowly but tightly to its surface[2] and subsequent contact of the allergen with the sensitized mast cell is a powerful, specific trigger for the release of intracellular histamine[3]. The final physiological responses depend on the topographical relation between the discharging mast cells and the mediator-sensitive smooth muscle. An unexpected feature of this relation in space is the recent finding that histamine-containing cells are found within the lumen of human bronchi[4].

The rational treatment of allergic patients started with the introduction of antihistaminic drugs in 1942[5]. After 30 years of use, however, it is widely accepted that the therapeutic effectiveness of the classical H_1-antagonists in allergic conditions has been disappointing in many respects[6] and of no or little interest in a major allergic condition, that of asthma[7]. The relative failure of these compounds in preventing human allergic bronchocon-

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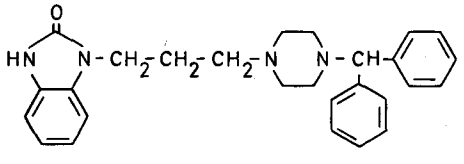
striction has focused attention on the release of mediators. Cromolyn sodium has been developed on the basis of its activity on this earlier step of the reaction sequence which follows allergen exposure[8] and many new compounds that inhibit histamine release from mast cells but do not antagonize histamine, have been announced recently.

Oxatomide, the new compound we present here, is different from both types of well-known drugs. Its effective anti-allergic activity after oral administration appears to result from simultaneously inhibiting mast cell discharge and antagonizing whatever histamine is set free. Chemically, oxatomide or R 35 443 is an original compound, corresponding to 1-{3-[4-(diphenylmethyl)-1-piperazinyl] propyl}-1,3-dihydro-2H-benzimidazol-2-one (figure). The compound is currently prepared by alkylating 1,3-dihydro-2H-benzimidazol-2-one with 1-bromo-3-chloropropane, whereafter the chloropropylderivative is coupled with 1-(α, α-diphenylmethyl)-piperazine. Both the alkylating and the coupling step occur under 1-methylethenyl-protection of the 2nd benzimidazolinone nitrogen and the protecting group is removed by acid hydrolysis. Chemically pure oxatomide is a white to slightly beige powder which is practically insoluble in water.

Oxatomide was very effective in protecting guinea-pigs from anaphylactic shock, according to the procedure previously used to study cinnarizine and flunarizine[9]. Oxatomide prevented lethal anaphylaxis at least as effectively as the simultaneously induced histamine paw oedema. Animals prepared for active anaphylaxis were protected not only from acute respiratory failure but, at slightly higher doses, also from the later, slowly developing, 'protracted' shock. In all these experiments, the anaphylacric reaction of guinea-pigs is mediated by IgG_1-antibodies. In humans IgG-dependant hypersensitivity is clinically important[10], but the major mast cell fixing antibody is of the IgE-type. Oxatomide was then studied in a widely used model of sensitization with IgE antibodies, passive cutaneous anaphylaxis (PCA) in the rat. In our procedure, reactions to histamine were induced in the skin of the animals at the same time as PCA reactions. The effect of orally administered oxatomide was dose-dependent and parallel for both reaction types in the range of 1.25 to 40 mg/kg; this latter dose virtually totally prevented dye accumulation in the reactive skin zones. Cromolyn sodium at the dose of 40 mg/kg, injected i.v. just before the challenge with histamine and with antigen, produced a significant reduction of the PCA reaction intensity, with no effect on the histamine reactions. Orally administered mepyramine, diphenhydramine and doxanthrazole were inactive at the dose of 40 mg/kg, against either reaction type.

The activity of oxatomide was further explored in dogs. Although anaphylaxis was first described in dogs[11], animals of this species have rarely been used to study the activity of new compounds against hypersensitivity reactions. It is, however, in this species that the study of oxatomide clearly revealed its unusual combination of activities.



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Chemical structure of oxatomide.

Many dogs acquire hypersensitivity to worm allergens[12], and beagles responding with the rapid formation of a large bleb upon intradermal injection of diluted *Ascaris suum* coeloma fluid, were selected for the oxatomide study. The oral dose of 10 mg/kg, administered 4 h before the challenge, virtually totally suppressed the oedema of this allergic reaction, and 2.4 mg/kg was the calculated ED_{50}. Simultaneous evaluation of the response to histamine indicated that a dose of 3.6 mg/kg is required to reduce the oedema by half. From the quantitative comparison it again appeared unlikely that the pronounced anti-allergic activity of oxatomide could result from histamine antagonism alone. Experiments were then set up to study the effect of oxatomide on histamine release. It is known that the surfactant Cremophor EL^R is a potent histamine releaser in dogs[13] and histamine plasma levels were measured[14] before, and at various times after, the i.v. injection of diluted Cremophor in dogs. 5 min after the injection, histamine reached about 40 times the circulating baseline level and oral administration of oxatomide 4 h before the challenge strongly limited the response. Reduction to half the control value required a dose of 2.75 mg/kg. In a separate experiment, it was verified that oxatomide does not change the clearance of i.v. injected histamine; and it is therefore concluded that the lower histamine levels after oxatomide administration in the Cremophor study reflect protection of tissue histamine stores. This protection was observed at the ultrastructural level in the peribronchiolar mast cells of guinea-pigs subjected to systemic anaphylaxis. After oxatomide treatment, these cells remain largely unchanged, whereas in the corresponding controls degranulated mast cells were recognized by the appearance of nuclear microtubules and swollen mitochondria[15].

Studies on the guinea-pig ileum revealed oxatomide to be a potent antagonist of histamine with a dual type of action, competitive at low doses and noncompetitive at higher doses. This mixed type of antagonism is qualitatively unlike mepyramine. Also the anti-serotonin activity of oxatomide on isolated arteries was pronounced; in anaesthetized guinea-pigs serotonin-induced bronchoconstriction was antagonized as effectively as histamine-induced bronchoconstriction. These effects were slow in onset but long-lasting, which suggests tight binding of oxatomide to pulmonary and other tissue. Oxatomide did not exhibit H_2-antagonism on the guinea-pig atrium.

Oxatomide produced maximal suppression of immediate hypersensitivity reactions at doses which did not induce any behavioural change in the same species. Absence of CNS effects was confirmed in specific tests for neuroleptic, analgesic, anticonvulsant and antidepressant activity. In the cardiovascular evaluation, the only effect occurring after high i.v. or oral doses was a slight and transient decrease in blood pressure. In accordance with experiments on isolated tissues and on haemostasis, this effect appears to be due to peripheral vasodilation. Oxatomide had no

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effect on platelet function, plasma coagulation and fibrinolysis, and did not affect gastrointestinal function or chronic arthritis in rats. High doses produced a progressively more pronounced depressant behavioural effect, but acute toxicity was low. The large safety margin of oxatomide was confirmed in the elaborate chronic toxicity studies in rats and dogs.

The pharmacological profile of oxatomide clearly warranted studies in man. It soon appeared that, upon oral administration, the new compound was very effective in reducing wheal and flare responses to allergens in atopic patients. Allergic rhinitis and conjunctivitis disappeared within 3 days in 60% of the large group of patients who were taking 20 mg of oxatomide 3 times a day in a double-blind study[16]. In asthmatic children, oxatomide and cromolyn sodium proved to be effective inhibitors of exercise-induced asthma. Oxatomide was superior to cromolyn sodium, as the airway obstruction was significantly inhibited at all post-exercise time intervals, and as the peak decrease of the 1st sec forced expiratory volume was significantly smaller[17].

As is expected from a potent H_1-antagonist, oxatomide prevents the effects of histamine on the isolated guinea-pig ileum, in pulmonary tissue and in the skin of various species. At the same time, oxatomide differs from classical antihistamine agents in several respects. In vitro the new compound exhibits a slowly developing but sustained activity which is partly non competitive. Oxatomide's high activity in rats, guinea-pigs and dogs is obtained after oral administration and suppression of anaphylactic or allergic reactions in the 3 species is pronounced after a dosage at least as low as that required for the suppression of histamine-induced reactions. This pronounced anti-allergic activity appears to be linked to the inhibition of histamine release. However, by contrast to cromolyn sodium and to other similarly acting compounds, oxatomide is highly active for a long time after oral administration. Furthermore the new compound is active against immediate hypersensitivity reactions, irrespective of sensitization by IgG or IgE homocytotropic antibodies. In conclusion, 2 important mechanisms for the inhibition of hypersensitivity reactions appear combined at effective doses of oxatomide, inhibition of mediator release, as well as potent antagonism of their spasmogenic activity. In animals, virtually total abolition of anaphylactic and allergic reactions is thus obtained at doses free of behavioural effects and far below toxic levels. The 1st clinical observations in man indicate that this pharmacological profile may result in a novel type of effective antiasthmatic drug.

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Radioimmunoassay of some hormones simultaneously measured in serum and breast cyst fluid

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Summary. Blood and breast cyst fluid were drawn simultaneously for hormonal determination. There was no difference between serum and cyst fluid values of PRL and TSH. A significant difference was noted for LH ($p < 0.01$) and FSH ($p < 0.05$), serum concentrations being higher than cyst fluid concentrations.

There is a high incidence of breast cyst, but little has been published regarding the hormonal composition of this easily obtainable cyst fluid[3]. Benign cystic disease of the human breast is not generally considered in itself to be a precancerous lesion, but earlier studies have suggested that women who develop benign breast disease are at increased risk of later acquiring breast cancer[4–7]. The frequency of benign disease in breast tissue removed at mastectomy is reported to be 39%, and mammary carcinoma developed in the affected cases 1.73 times as often as it did in the general population[8]. Women with cystic disease have about 4 times the breast cancer rate of comparable women without cystic disease[9]. Another study reported also shows the prevalence of malignant transformation in the total series of 876 cases to be 3.1%[10]. The presence of intracystic carcinoma is very rare[11–13].

These observations have led to 2 hypotheses on the mechanism of possible association between cystic mastitis and cancer[14]. According to the first, cystic disease could be a premalignant condition that either predisposes to neoplatic change or is an early manifestation of malignant change. According to the second, benign and malignant breast diseases could have factors in common, such as hormonal pattern. In line with the latter hypothesis, we

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2 The authors are indebted to Miss J. Meister, E. Dullaert and J. Beck for their skillful technical help. We are also indebted to the National Institutes of Health, NIAMDD and the World Health Organization, International Laboratory for Biological Standards.

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