began soon after the addition of Cu. The initial release rate of Cd depended on the Cu concentration, as shown in the figure, c. The release rate of Cd was so rapid at 0.49 µg/ml of Cu concentration that 63% of the Cd taken up by the cells was released in 2 h. Cd was not released from the cells into the fresh medium without the addition of Cu, and the release of Cd was very slow even in the presence of EDTA[14]. After the uptake of Cd by the cells, the culture medium was replaced with fresh medium not containing Cd. The release of Cd was again observed on the addition of Cu, as shown in the figure, d. From the comparison between figures c and d it seemed likely that the rate of Cd release did not depend on the concentration of Cd outside the cells. The inhibition of Cd uptake by Cu may be explained by the relationship between the rate of the uptake of Cd and that of the release of Cd in the presence of Cu. The organ distribution of Cd and Cu may be explained by this kind of interaction of Cd and Cu[16-18].

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The effect of urethane on histamine-induced contraction of guinea-pig tracheal smooth muscle

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Summary. Urethane possesses a direct depressant action on histamine-induced contractions of guinea-pig tracheal smooth muscle both in vivo and in vitro.

Urethane anesthesia appears to potentiate indirectly histamine-induced broncho-constriction through a reduction of sympathetic bronchodilator tone[1]. Since urethane depresses contraction of vascular smooth muscle induced by vasoactive agents[2-6] it seemed worthwhile to determine whether or not it also has a direct inhibitory effect on histamine-induced contractions of guinea-pig tracheal smooth muscle as compared to a competitive (receptor) antagonist (i.e. chlorpheniramine)[7] of histamine in this preparation.

Materials and methods. In vitro experiments: Male albino guinea-pigs weighing 300-400 g were stunned and bled, and the whole trachea rapidly removed and placed in oxygenated (96% O_2+4% CO_2) Krebs solution of the following composition in mM: NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.5, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11. The trachea was carefully cleaned of adhering connective tissue. Five rings of tracheal tissue were cut and arranged to form a chain as described by Castillo and De Beer[8]. This chain was mounted in a 5-ml organ bath (heated at 37 °C by means of a Julabo Paratherm III water bath) under a constant load of 1 g and attached to an isometric force transducer (MARB 79 Tl). Contractile tone and its variations were delivered to a MARB 776 DC preamplifier and recorded on a Hewlett Packard 7402 A polygraph. After a stabilization period of 1 h concentration response curves (CRC) to histamine were constructed according to Van Rossum[7] at 15-min intervals, until 2 or more reproducible curves were obtained. The effect of urethane and chlorpheniramine were evaluated after a 15-min incubation period.

In additional experiments, after a 1-h stabilization period, tracheal chains were exposed to a high K^+ Ca^{++} free depolarizing solution (mM composition; NaCl 69, $NaHCO_3$ 25, KCl 54,7 $MgSO_4$ 1.5, KH_2PO_4 1.2, glucose 11, EDTA 0.77) for 1 h with successive washings with this solution every 15 min.

A cumulative CRC to $CaCl_2$ was then obtained at the end of the which $CaCl_2$ was washed out with normal Krebs solution, reincubated for 60 min and the high K^+ Ca^{++} free depolarizing procedure repeated again as described above.

A 2nd cumulative CRC for $CaCl_2$ was then obtained in the presence of urethane or chlorpheniramine added to the

Table 1. Effect of urethane aerosol on histamine-induced bronchospasm in conscious guinea-pigs

Treatment	No. of animals	Total dose nebulized (mg/kg)	Time of appearance for histamine-induced bronchospasm (sec, mean ± SE)
Controls	10	– –	62.7 ± 3.5
Urethane	8	10	83.5 ± 3.8[a]
Urethane	8	50	109.2 ± 6.7[b]
Urethane	8	100	139.0 ± 12.3[b]
Chlorpheniramine	8	0.35	504.8 ± 34.1[b]

[a] Significantly different from controls $p < 0.02$; [b] significantly different from controls $p < 0.01$.

muscle bath 15 min before the 1st dose of $CaCl_2$. The effects of urethane and chlorpheniramine on the CRCs to histamine and $CaCl_2$ are expressed as mean ±SE and statistical analysis was performed by means of Student's t-test for paired data. pA_2 and pD'_2 values for competitive and non competitive antagonism were calculated as appropriate, according to Van Rossum[7].

In other experiments the effects of urethane were evaluated against the contraction produced by exchanging the Krebs solution for a high K^+ solution of the following composition (mM): NaCl 69, $NaHCO_3$ 25, KCl 54.7, $MgSO_4$ 1.5, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11. The resulting contracture was allowed to reach a steady state and challenged with cumulative concentrations of urethane and chlorpheniramine. The potential antagonism of these latter was expressed as percent inhibition of the initial tone.

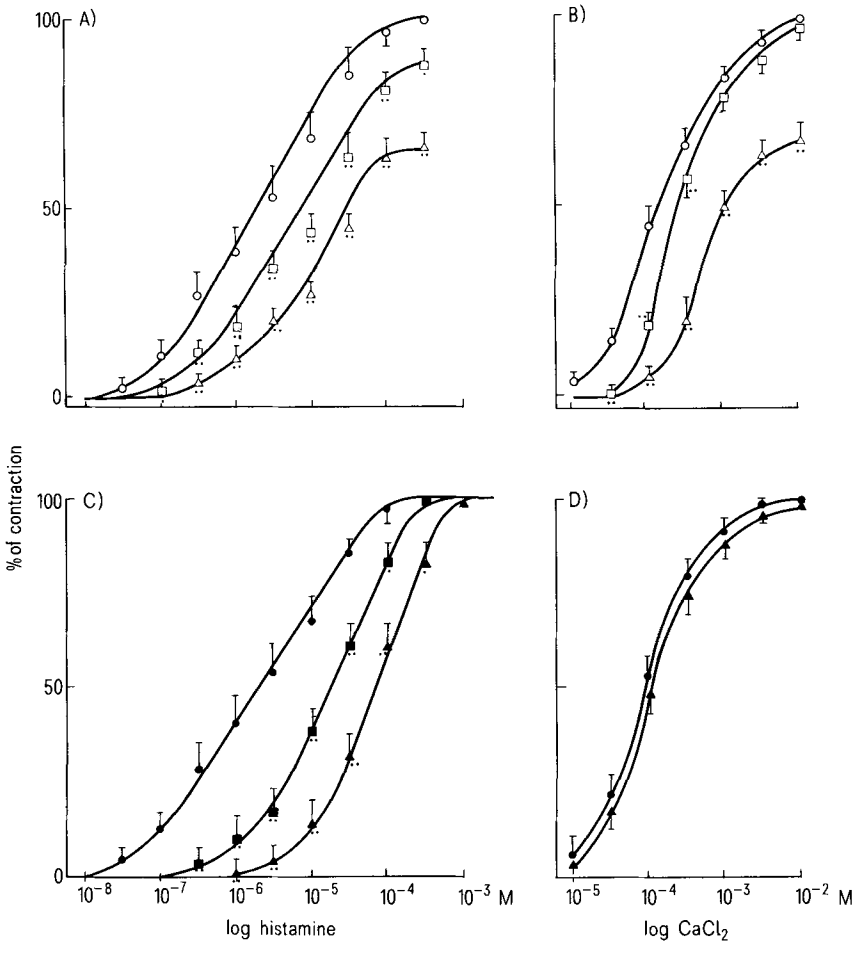
Linear regression analysis was calculated according to the method of minimum squares. ED_{50} and its 95% confidence limits was calculated according to Litchfield and Wilcoxon[9].

In vivo experiments: Male albino guinea-pigs weighing 250–300 g were confined to a glass cylinder (25×8×8 cm) and exposed for 5 min to an aerosol (0.15 ml/min at 0.2 kg/cm^2) of either saline controls or concentrations of urethane which corresponded roughly to the subanesthetic doses of 10,50 and 100 mg/kg or concentration of chlorpheniramine which corresponded roughly to a dose of 0.35 mg/kg. Shortly after, both controls and urethane-treated animals were exposed to a 0.2% histamine HCl aerosol which produced a respiratory distress characterized by an increased rate and depth of respiration culminating in a typical convulsive cough whose appearance was chosen as the end-point of this test. Statistical analysis of the data was performed by means of Student's t-test for unpaired data.

Results. Urethane ($5 \cdot 10^{-2} - 1 \cdot 10^{-1}$ M, n=6) produced a prompt and marked decrease in resting tone of tracheal smooth muscle in vitro, a significant shift to the right of histamine CRC and a reduction in the maximal tension attainable (fig., A). Urethane ($5 \cdot 10^{-2}$ M) significantly antagonized $CaCl_2$-induced contraction at the lower and intermediate doses of the CRC, without a reduction of the maximal tension attainable, which was significantly decreased at $1 \cdot 10^{-1}$ M (n=6) (fig., B). The evaluation of urethane effect on histamine and $CaCl_2$ CRC in terms of non competitive antagonism[7], yielded pD'_2 values of 0.62±0.08 (mean ±SE) and 0.75±0.1 respectively, which did not differ significantly from each other. Chlorpheniramine ($1 \cdot 10^{-7} - 3 \cdot 10^{-7}$ M, n=4) (fig., C) produced a significant parallel shift to the right of histamine CRC, but maximal contractile tension was still attainable by increasing histamine concentration; the evaluation of this effect in terms of competitive antagonism[8] yielded a pA_2 value of 7.91±0.09 (mean ±SE). On the other hand chlorpheniramine, ($3 \cdot 10^{-7}$ M, n=4), had no significant effect on $CaCl_2$-induced contraction of tracheal smooth muscle (fig., D).

Urethane ($1 \cdot 10^{-2} - 1 \cdot 10^{-1}$ M, n=4) antagonized high K^+-induced contractions in a concentration-dependent manner; its relative ED_{50} and 95% confidence limits were $3.32\ (1.59-6.95) \cdot 10^{-2}$ M, while chlorpheniramine ($3 \cdot 10^{-7}$ M, n=3) had no inhibitory effect.

Subanesthetic doses of urethane produced a slight but still significant and dose-related delay in the appearance of histamine-induced bronchospasm, while chlorpheniramine



Effect of urethane on histamine (A) and $CaCl_2$ (B) CRC in guinea-pig tracheal smooth muscle. ○, Control; □, urethane $5 \cdot 10^{-2}$ M; △, urethane $1 \cdot 10^{-1}$ M. Effect of chlorpheniramine on histamine (C) and $CaCl_2$ (D) CRC in guinea-pig tracheal smooth muscle. ●, Control; ■, chlorpheniramine $1 \cdot 10^{-7}$ M; ▲, chlorpheniramine $3 \cdot 10^{-7}$ M. · $p < 0.05$; · · $p < 0.01$.

(0.35 mg/kg) had a similar but much more marked effect (table).

Discussion. Douglas et al.[10] observed a reduced effect of i.v. and aerosolized histamine on the dynamic compliance of urethane-anesthetized as compared to conscious guinea-pigs. Our results agree well with these findings, and demonstrate that urethane has a direct depressive effect on histamine-induced contractions of guinea-pig smooth muscle at some step(s) beyond H_1 receptor activation.

The observation that, even when administered in subanesthetic doses, aerosolized urethane produces a delay in histamine-induced bronchospasm, indicates that, under appropriate experimental conditions, the direct effect of urethane on histamine-induced contractions of bronchial smooth muscle is a depressive one. In fact the 'topical' application of aerosolized urethane rules out the intervention of a centrally-mediated[11] reduction of sympathetic bronchodilator tone[1].

Urethane-induced depression of tracheal smooth muscle resting tone in vitro could likewise indicate that the increase in pulmonary airway resistance in urethane anesthetized as compared to conscious guinea-pigs depends upon an indirect effect of this anesthetic[1].

Urethane antagonism toward cardiovascular responsiveness to noradrenaline[3-6] and chronotropic response to isoprenaline[12] could be explained by an inhibition of agonist induced transmembrane Ca^{++} influx[13]. This mechanism could likewise explain our observation concerning the depressive effect of urethane on histamine-induced contraction of guinea-pig tracheal smooth muscle. In fact: a) unlike chlorpheniramine, a competitive H_1 receptor antagonist[8], urethane proved to be a non competitive antagonist, b) unlike chlorpheniramine, urethane antagonized $CaCl_2$-induced contractions in a high K^+ Ca^{++} free medium (a similar effect has been observed in rat aorta and portal vein[13]); c) urethane but not chlorpheniramine antagonized high K^+-induced contractions, which appear to be produced by a voltage-dependent activation of Ca^{++} channels in the cell membrane[14] leading to an inward movement of Ca^{++} responsible for smooth muscle contraction[15].

In addition, histamine-induced contractions of guinea-pig tracheal smooth muscle are known to be closely dependent upon the influx of Ca^{++} from the extracellular space, since they are markedly depressed in Ca^{++} free medium[16].

In conclusion, the direct effect of urethane on histamine-induced contractions of guinea-pig tracheal smooth muscle both in vitro and in vivo is of a depressive type, and it could be tentatively attributed to an interference with some basic cellular mechanism of Ca^{++} transmembrane movement.

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Is vitellogenin a cuticular component of the female locust?

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Summary. One major antigen, present in female cuticle, female blood and eggs, is revealed by the antiserum against soluble cuticular proteins of adults locusts, and by all the antisera against vitellin or vitellogenin. It is not revealed by the specific antiserum against diglyceride-binding lipoprotein. The presence of this major antigen in the cuticle depends on the presence of vitellogenin in the blood.

In insects, proteins are important cuticular components[1,2]. While the question of their site of synthesis remains unanswered, there is some evidence that a relationship exists between the hemolymph and cuticle proteins. For instance, in *Locusta migratoria*[3], *Periplaneta americana*[4], *Manduca sexta*[5], and *Calliphora vicina*[6], radiotracer studies have shown that some blood proteins can be transported into the cuticle; moreover, it has been demonstrated by immunodiffusion techniques that some cuticular proteins are immunologically similar to blood proteins[5,7,8]. The existence of sex-linked proteins in the cuticle of adults was reported for the first time in *Locusta migratoria*[9] by means of electrophoretic and immunodiffusion analysis.

The aim of our study was to elucidate the origin of one major female specific cuticular protein.

Material and methods. Rabbit antisera against soluble cuticular proteins of adult male and female locusts (AC), blood of adult females (AH) and eggs (AOe) were prepared according to the immunization schedule of Harboe and Ingel[10]. The supernatant (AOe*) of the AOe serum precipitated with male blood was equivalent to antivitellin (fig. 1). Specific antivitellin (AV) and specific anti-diglyceride-binding lipoprotein (A-LPI) sera were kindly given to us by Proff. Appelbaum and Emmerich, respectively.

Abdominal cuticles were carefully cleaned from adhering tissues, blood and cells, with small pieces of cotton mois-