The Effect of pH on Mast Cell Damage by Antihistamines

MOTA and DIAS DA SILVA[1] have studied the effect of pH upon histamine release from guinea-pig lung by antihistamines. They have found that histamine release by antihistamines is dependent upon pH, being greater at pH 8.5 and negligible at 6.5. Since antihistamines are rather strong organic bases, this increase in histamine release at a higher pH could have been due to an increase in the concentration of the non-ionized base. In the present paper the possible relationship between the degree of ionization of antihistamines and their action on mast cells was studied.

Material and methods. The experimental procedure used was similar to that previously described[2]. Pieces of rat and guinea-pig mesentery were incubated at different pH in tyrode containing antihistamines, for 30 min at 37 °C. The bicarbonate-phosphate buffer of the tyrode was omitted and *Tris* HCl (trihydroxymethylaminomethane) 0.01 *M* used instead. The pH of the tyrode was adjusted potentiometrically and measured again at the end of each experiment. Fixation and staining of the pieces of mesentery, and assessment of mast cell damage were performed as described by MOTA and DIAS DA SILVA[1]. Results are given as percentages of mast cells presenting granule extrusion (rat), and as mean content of mast cells (guinea-pig). In the experiments in which the inhibitory action of histamine was studied, pieces of mesentery were pre-incubated for 20 min in tyrode containing histamine-diphosphate previously alkalinized with NaOH 0.1 *N*, and incubated for 30 min more after addition of the antihistamine. The following drugs were used: promethazine hydrochloride (Phenergan, Rhodia), chlorpromazine hydrochloride (Amplictil, Rhodia), diphenhydramine hydrochloride (Benadryl, Parke-Davis), chlorcyclizine hydrochloride (Perazil, Burroughs Wellcome), antazoline (Antistine, CIBA) and histamine-diphosphate (Sigma).

Results and discussion. The widespread rat and guinea-pig mast cell damage induced by antihistamines described previously was confirmed[1,2]. However, the present experiments show that this damaging action is highly dependent upon pH. In concentrations which caused maximal damage to mast cells at pH 8.4, antihistamines were ineffective when the pH of the experimental fluid was lowered. Although lowering the pH totally inhibited the action of all antihistamines tested, the pH at which this occurred varied with the different antihistamines used. In the rat, the action of antazoline (0.5 m*M*) was inhibited at pH 8.3–8.2, of chlorcyclizine (0.1 m*M*) at 8.0–7.9 and of diphenhydramine (1.0 m*M*) at 7.9–7.8 (Figure 1). In the guinea-pig, the action of diphenhydramine (0.66 m*M*) and of promethazine (0.1 m*M*) was inhibited at pH 8.1–8.0, of antazoline (0.8 m*M*) at 8.0–7.9, of chlorcyclizine (0.1 m*M*) at 7.7–7.6, and of chlorpromazine (0.1 m*M*) at 7.4–7.3 (Figure 2).

The above results showing that the action of antihistamines is inhibited by a rather slight change in pH suggest that these drugs should be mainly in a non-ionized form to damage mast cells. That mast cell damage by antihistamines might be induced by the undissociated molecule mainly is further supported by the fact that by raising the concentration of chlorcyclizine (in the rat and guinea-pig) and of diphenhydramine (in the guinea-pig), it was possible to overcome totally the inhibition caused by pH 7.0 (unpublished results). Probably this non-ionized lipoid-soluble form is important for the penetration of antihistamines in mast cells. However, once the base has penetrated the cell, the damaging action of antihistamines on mast cells would be induced by the ionized molecule, assuming that the pH inside the cell is 7.0. Results similar to ours in which histamine release by diamines[3] and by ammonia[4] was sharply increased by an increase in pH have been described.

Because it was found that the action of antihistamines on mast cells can be inhibited by rather slight changes in pH, the inhibitory effect of histamine on mast cell damage induced by antihistamines[2] was re-examined. This effect could have been due to a lowering of the pH by the strongly

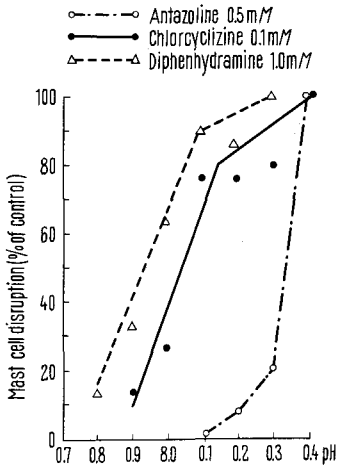

Fig. 1. Effect of pH on rat mast cell damage by antihistamines. Controls were the percentages of mast cells disrupted by antihistamines at pH 8.4.

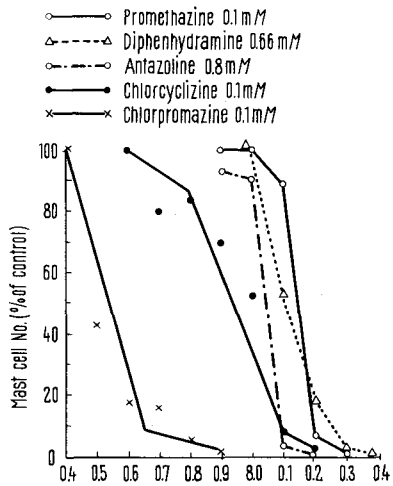

Fig. 2. Effect of pH on guinea-pig mast cell damage by antihistamines. Controls were the numbers of mast cells counted in pieces of mesentery incubated in tyrode alone.

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acidic histamine-diphosphate used in those experiments. This was found to be the case, since in experiments in which the pH was maintained at 8.4, histamine did not inhibit either the action of diphenhydramine on rat, or of diphenhydramine and of promethazine on guinea-pig mast cells. These results show that the inhibitory effect of histamine on mast cell damage by antihistamines was probably due to a lowering of the pH of the experimental fluid. Histamine by itself does not seem to inhibit mast cell damage induced by antihistamines[5].

Resumen. Se demuestra que la acción de antihistamínicos sobre los mastocitos de la rata y del cobayo depende del pH. Los antihistamínicos estudiados fueron activos en pH 8.4, pero fueron totalmente inhibidos cuando se disminuyó el pH. También se verificó que la histamina no antagoniza la acción de los antihistamínicos sobre los mastocitos cuando el pH fue mantenido en 8.4.

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The Synteratogenic Effect of Lead and Cadmium

The increasing importance of environmental pollution with heavy metals such as lead[1] and cadmium[2] should alert us to the possible effects of these metals on mammalian reproduction. Previous experimental data relating to the effect of heavy metals upon embryonic development in the pregnant hamster have revealed a striking site-specific teratogenic effect for both lead[3] and cadmium[4]. The i.v. injection of cadmium sulfate causes a high incidence of facial abnormalities and a few other malformations including exencephaly and anophthalmia. Under identical experimental conditions the teratogenic effect of various lead salts has been mainly confined to the developing tail bud and associated caudal vertebrae.

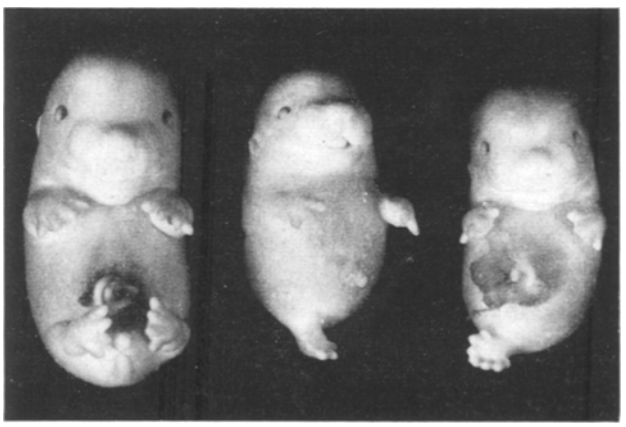
In the present experiments an attempt has been made to combine the teratogenic stimuli of cadmium and lead in order to produce a combination of congenital defects which would reflect the separate teratogenic actions of these agents on diverse parts of the developing embryo. The results were quite different than expected and represent an extremely interesting example of the complex interaction of teratogenic agents.

Female hamsters were bred under direct observation during the early evening hours. The day following the evening of breeding was designated as the first day (day 1) of gestation. On the morning of the eighth day of gestation the animals were anesthetized with pentobarbital and injected i.v. with distilled water (controls), cadmium sulfate, lead acetate or combinations of cadmium sulfate and lead acetate in the amounts and combinations shown in Table I. 4 or 5 days later, on the twelfth or thirteenth day of pregnancy, the maternal animals were killed and the embryos recovered. These embryos were examined carefully for gross external malformations. The number of resorption sites were counted and recorded.

The teratogenic effects of both cadmium and lead when injected separately into pregnant hamsters corresponded well with previous data in that cadmium caused anterior malformations[4] (exencephaly, cleft lip/palate, microphthalmia) only, while lead caused tail malformations only[3]. The combination of these agents, however, revealed that the frequency and severity of the clefts in the lip and palate caused by cadmium are reduced in the presence of lead, while the posterior tail malformations caused by lead appear to be potentiated in the presence of cadmium. Sympodia, a severe caudal malformation of the lower extremities (Figure) was never seen in the animals treated with lead only but did appear with a relatively high frequency when cadmium was added to the lead (Table II).

The teratogenic interaction of other agents has been demonstrated for insulin and 2-deoxy-D-glucose[5] but not for any heavy metals. One can speculate that the teratogenic effect of either of these metals may be due to a direct effect on embryonic tissues, a block in placental transfer of some essential metabolite, or an induced defect in maternal metabolism which secondarily affects the differentiating embryonic tissue.

One obvious possibility which bears further investigation is the well-known importance of heavy metals in the function of several metallo-enzymes which may have



13-day-old fetal hamsters. Animal at left is from a control animal and is normal. The other 2 are littermates from a mother treated with cadmium sulfate (2 mg/kg) and lead acetate (50 mg/kg) on the eighth day of gestation. Both show the same degree of sympodia. 4 other littermates had the same defect. × 3.

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