eggs derived from makisterone A treated females and controls. At the given temperature (26.5 °C), makisterone A accelerated embryonic development for 3.1±0.4 h compared with controls. (Number of tests: n=6; total of tests eggs: t_t=514; total of control eggs: t_c=420.) Test eggs also hatched earlier than control eggs, but the difference was not as pronounced as at evagination, namely 1.7±0.4 h. This means that eggs with a high content of exogenous makisterone A (respectively RIA active metabolites) develop faster during early embryogenesis but more slowly during late embryogenesis as compared with nontreated eggs.

These observations were confirmed when the eggs were incubated at room temperature (fig. b), and at low (17°C) temperature (fig. c). The experiment in figure b was repeated twice; t_t=172; t_c=200. The experiment in figure c was also repeated twice; t_t=216; t_c=342. In all repeats, there was no great variation from figures b and c.

Maternal treatment with exogenous 20-hydroxyecdysone had no influence on the timing of early embryonic development (fig. d), although the content of RIA active ecdysteroids was 2.5 times higher than after makisterone A treatment. Late embryonic development, however, was prolonged for about 2 h compared with controls. (The experiment of figure d was repeated twice with identical results; t_t=190 and t_c=300.)

It is shown (fig. a) that maternal makisterone A treatment accelerates the early development during 3.5 h but that the embryo hatches only 1.3 h earlier than controls. Hence, late embryonic development must have been slowed down in comparison to controls. In this respect - prolongation of late embryonic development - makisterone A and 20-hydroxyecdysone had a similar effect, but only makisterone A accelerated early embryonic development. The slowdown of late embryonic development is not yet understood. Kaplanis et al.[5] suggested that makisterone A might be the biologically active ecdysteroid in the embryo of *Oncopeltus fasciatus*. Evidence that makisterone A might also be the active ecdysteroid in reproducing females was found previously[9,11]. It is shown here that only exogenous makisterone A applied to the mother can stimulate early development. This effect may indicate a role of maternal ecdysteroids which are regularly found in normal eggs (see Hoffmann et al.[12] for review). There have been only a few indications for such a function of ecdysteroids[13]. Future studies will have to reveal whether makisterone A stimulates specific processes of the early differentiation, or whether growth and/or differentiation in general are influenced.

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Stimulation of H^+ ion secretion from the isolated mouse stomach by sodium fluoride

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Summary. The effect of sodium fluoride on H^+ ion secretion was investigated in the isolated distended mouse stomach. It was found that sodium fluoride on its own caused dose-related stimulation of H^+ ion secretion. Sodium fluoride did not inhibit H^+ ion secretion induced by histamine. The possible mechanisms involved are discussed. It is considered that sodium fluoride might stimulate H^+ ion secretion by causing histamine release and by increasing cyclic AMP formation in the intact gastric mucosa.

It has been shown that instillation of sodium fluoride (NaF) into the cat gastric lumen produced marked reduction in the output of H^+ ions secreted in response to histamine[2] and to gastrin[3]. Most recently, Reed and Smy[4] studied the effects of NaF on gastric acid and electrolyte output in the anaesthetized cat. They found that in the histamine-stimulated stomachs, the NaF-induced inhibition of H^+ ion secretion was accompanied by reduction of blood flow. To eliminate possible complicating factors involved in in vivo experiments, the isolated distended mouse stomach known to be a useful preparation for quantitative studies[5] was used in the present work to investigate the mode of action of NaF on in vitro gastric acid secretion.

Materials and methods. Gastric acid secretion was studied according to the method described previously[5]. Fed mice (Charles River) of either sex, about 20 g b.wt, were used. While the animal was anaesthetized with ether, the stomach was exposed, polythene cannulae were tied into the cardiac and the pyloric region and the oesophagus was ligated. The stomach was then isolated and placed immediately in an organ bath containing 30 ml of serosal solution maintained at 37 °C and gassed vigorously with 95% O_2+5% CO_2. The pyloric cannula was connected to a perfusion pump and the cardiac cannula was connected to a pH electrode unit adjusted to raise the intragastric pressure to 18 cm H_2O to achieve the distension effect. The stomach lumen was continuously perfused with warm oxygenated mucosal solution at 1 ml/min and the perfusate was passed over the pH electrode (a micro, dual electrode) and then collected into a vessel at 15 min intervals and titrated to pH 7.0 with 10^{-2} M NaOH. The pH readings were continuously noted as a function of time with a pen recorder and the volumes of titrant used were recorded on a titrigraph every 15 min.

The buffered serosal solution contained in mM/l: NaCl, 118, KCl, 4.8, KH_2PO_4, 1.2, $MgSO_4$, 1.2, $CaCl_2$, 1.3 and Na_2HPO_4, 16. The unbuffered mucosal solution had the same composition as the serosal solution except that KH_2PO_4 and Na_2HPO_4 were omitted and the concentration of NaCl was 135 mM/l. Both solutions contained 15 mM/l of glucose.

After setting up the preparation, the initial rate of H^+ ion secretion was allowed to stabilize for 40 min before the effect of drugs was studied. NaF or histamine acid phosphate (dose expressed as base) was dissolved in warm saline and added in a volume of 0.3 ml to the serosal solution.

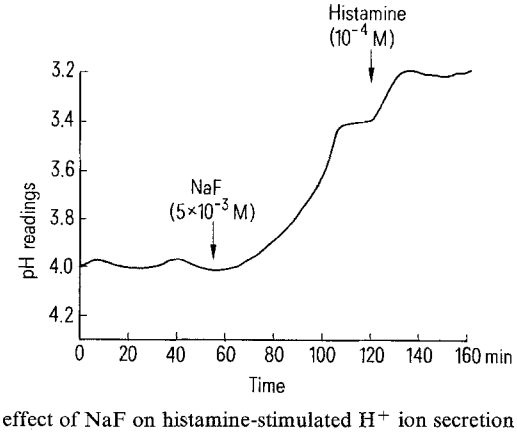
Since the initial rate of acid secretion was usually stable with small variations between different preparations, the responses to drugs are simply expressed as pH values or as acid output µmole/15 min (total acid output during the 15 min test period). Student's t-test was used to evaluate the results and a p-value of less than 0.05 was considered significant.

Results and discussion. The effect of histamine on H^+ ion secretion was studied in 12 stomachs in the absence and presence of NaF. It was found that NaF did not inhibit the stimulatory effect of histamine on H^+ ion secretion. One of the typical experiments is illustrated in the figure. It is seen that in the stomach treated with NaF (5×10^{-3} M), H^+ ion secretion rose slowly from the initial value of pH 3.96 to a stable peak response of pH 3.4 about 50 min after NaF administration. A further increase in H^+ ion secretion was obtained upon the addition of histamine (10^{-4} M). Peak acid output (as µmole/15 min) in the 6 stomachs in response to histamine (10^{-4} M) alone was 6.7 ± 0.3, as compared to that of 11 ± 0.5 in the other 6 stomachs treated with NaF (5×10^{-3} M) plus histamine (10^{-4} M). In another series of experiments, 30 stomachs were used to investigate the effect of NaF at various concentrations (7.5×10^{-4} to 5×10^{-2} M) on H^+ ion secretion, with another 6 saline-treated stomachs serving as the controls. The results presented in the table show that NaF at 10^{-3} to 10^{-2} produced dose-dependent increase in H^+ ion secretion. However, the stimulatory effect of NaF decreased as the concentration of NaF exceeded 10^{-2} M.

Effect of NaF on H^+ ion secretion in the isolated distended mouse stomach

Conditions		Acid output (µmole/15 min)	
Saline control	(6)	1.5 ± 0.1	
NaF 7.5×10^{-4} M	(6)	1.6 ± 0.1	NS
NaF 10^{-3} M	(6)	4.4 ± 0.4	*
NaF 5×10^{-3} M	(6)	5.3 ± 0.3	*
NaF 10^{-2} M	(6)	6.8 ± 0.5	*
NaF 5×10^{-2} M	(6)	3.8 ± 0.5	*

Values are mean ± SE. Number of experiments are indicated in parenthesis. * $p < 0.001$ vs control; NS = not significant.



The effect of NaF on histamine-stimulated H^+ ion secretion in the isolated distended mouse stomach.

The present finding that NaF did not inhibit the stimulatory effect of histamine on H^+ secretion is not in agreement with published data that instillation of NaF into the cat gastric lumen decreased histamine-induced H^+ ion output[2-4]. The differences in the experimental conditions employed by different workers (such as the differences between in vivo and in vitro preparations, site of NaF action and species used) might have caused the discrepancy. There has been very little, if any, published work on the effects of NaF on in vitro mammalian gastric acid secretion and it is thus interesting to see that NaF on its own produced stimulation of H^+ ion secretion in the isolated distended mouse stomach. Since it is known that NaF causes histamine release from the isolated rat mast cells[6], NaF might have caused histamine release from the mouse stomach mast cells, which could in turn stimulate H^+ ion secretion. It has been shown recently that NaF increased cyclic AMP levels in the intact cells[7,8]. It is thus possible that NaF stimulated H^+ ion secretion by elevating cyclic AMP levels in the intact mouse gastric mucosa. The effects of NaF on the metabolic processes in the gastric mucosa should also be considered as it is well-known that NaF affects the activity of various enzymes[9]. For instance, NaF has been found to be a specific inhibitor of glycolysis in the rat adipose tissue[10]. There is also evidence that NaF stimulated glucose oxidation and phospholipid turnover[8]. Since the secretion of HCl by the gastric mucosa is obligatorily dependent on oxidative metabolism[11], it would be of interest to investigate the effect of NaF on substrate oxidation in the gastric mucosa. It has been shown that NaF is a reactivator of phosphorylated acetylcholinesterase as well as a neuromyal facilitator[12,13]. There is also evidence that NaF antagonized acetylcholine desensitization of amphibian neuromyal junction[14]. In view of the fact that acetylcholine is a potent stimulant of gastric acid secretion in various species, it would be pertinent to study the action of NaF as related to the stimulatory effect of acetylcholine on gastric acid secretion.

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