

predominant component. The mouse serum interferons appeared less heat stable than chick interferon and component A more sensitive to high pH. The characteristics of these interferons were compared with the properties of the spectrum of interferons produced in various animal species by various microbial agents.

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Histamine Liberation by Gastric Mucosa of Pylorus-Ligated Rats Damaged by Acetic or Salicylic Acids.* (30784)

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When the oxyntic gland area of the gastric mucosa is damaged by irrigation with acetic or salicylic acids, there is an increased output of water, plasma proteins, glucose, sodium and potassium from the mucosa(1,2,3). Stimulation of acid secretion and hyperemia may also occur. Many of the effects of damage could be the result of intramucosal liberation of histamine.

The gastric mucosa contains a large amount of histamine, some in the region of the oxyntic cells and some in the mast cells(4), and it has frequently been suggested (*e.g.*, 5,6) that histamine is liberated within the mucosa during stressful or damaging situations. If histamine liberation occurs during damage, the total histamine content of the mucosa should fall, and histamine should be present in the interstitial fluid. Since other constituents of interstitial fluid appear in gastric contents after damage, one would expect that if

histamine is also dissolved in the interstitial fluid, it too would be detectable in gastric contents. In the experiments reported here we show that when the stomach of the pylorus-ligated rat is damaged with acetic or salicylic acids, the histamine content of the mucosa decreases and that histamine appears in the gastric contents.

Materials and methods. Male and female rats weighing between 150 and 260 g were fasted overnight without restraint. A rat was anesthetized with sodium pentobarbital, and anesthesia was maintained for the duration of the experiment. The stomach was exposed by midline incision and gently ligated at pyloric and gastroesophageal junctions. The stomach was rinsed twice with 154 mM NaCl injected and removed through the fore-stomach. Then one of four solutions was injected at volume equal to 15% of body weight. The abdomen was closed by clips, and the fluid was left in the stomach 90 min after which the animal was decapitated and the stomach immediately removed.

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TABLE I. Histamine Found in Oxyntic Gland Area of Glandular Stomach and in Fluid Contents of Stomachs of Pylorus-Ligated Rats; Stomach Filled with Test Solution for 90 min. Means and standard deviations.

Test solution	Tissue histamine		Fluid histamine		
	n	$\mu\text{g/g wet wt}$	n	$\mu\text{g total}$	n_o^*
154 mM NaCl	12	33.2 ± 9.3	11	$.12 \pm .18$	5
100 mM HCl	11	35.3 ± 11.1	11	$.08 \pm .12$	5
100 mM acetic acid	11	$20.4 \pm 5.8^\dagger$	11	$.30 \pm .19^\dagger$	0
20 mM salicylic acid in 100 mM HCl	10	$23.1 \pm 9.4^\ddagger$	13	$.20 \pm .11^\S$	2

* Number of instances in which fluid contained no detectable histamine.

† Different from corresponding NaCl- and HCl-treated rats at $p < 0.01$.

‡ Different from corresponding NaCl-treated rats at $p < 0.05$.

§ Different from corresponding HCl-treated rats at $p < 0.02$.

The stomach content was measured to the nearest 0.1 ml and at once combined with 5 ml concentrated HCl to begin the histamine extraction procedure described by Code and McIntire(7). The stomach tissue was trimmed in order to retain only the oxyntic gland area. The tissue sample was quickly weighed and immediately immersed in 10% trichloroacetic acid, ground with powdered glass in a mortar and extracted as directed by Code and McIntire(7). Histamine content of the final extracts of both fluid and tissue was determined by assay using an isolated terminal segment of guinea pig ileum suspended in atropinized Tyrode's solution (8). Potassium concentrations of final extracts were measured by internal standard flame photometry and found to be well below the level which would affect the isolated ileum(9). All results are expressed as micrograms of histamine base. The smallest amount of histamine detectable in any sample was 0.020-0.025 μg , and quantities below this amount are recorded as zero.

The four fluids used were (1) 154 mM NaCl, (2) 100 mM HCl, 15 mM NaCl and 78 mM mannitol, (3) 100 mM acetic acid, 15 mM NaCl and 78 mM mannitol, and (4) the second solution in which was dissolved 20 mM salicylic acid.

Results. Results are given in Table I. Irrigation with 100 mM HCl had no effect on tissue or fluid histamine content as compared with irrigation with 154 mM NaCl. (The high mean and standard deviation given for the fluid histamine in the 154 mM NaCl experiments were caused by a single high value.) This is consistent with the fact that

100 mM HCl in itself does not damage the gastric mucosa. Acetic acid, which diffuses rapidly into the mucosa and increases its permeability to HCl and constituents of interstitial fluid(1), caused a fall in tissue histamine and the unequivocal appearance of histamine in gastric contents. Salicylic acid, which also diffuses through the mucosa and increases its permeability(3), likewise decreased tissue histamine content. Histamine was more frequently present in the salicylic acid solution than it was in 154 mM NaCl or 100 mM HCl, and its mean concentration was significantly higher than in the HCl solution.

The amount of histamine appearing in the fluid was far less than the total histamine disappearing; presumably the rest was carried away by the blood.

Discussion. These experiments show that when the oxyntic gland area of the rat gastric mucosa is damaged by acetic or salicylic acid its histamine concentration falls and that the histamine content of the gastric lumen rises. We infer from the latter fact that some of the histamine liberated from the mucosa is likewise present in the interstitial fluid of the mucosa. Since histamine causes both vasodilatation and increased capillary permeability, its presence in interstitial fluid would probably result in the flow of a large volume of plasma-like fluid from mucosal capillaries into the interstitial spaces and through the mucosa rendered more permeable by damage into the gastric lumen. This accounts for the inflammation, edema and increased volume output frequently observed when the mucosa is damaged. Increased interstitial concentra-

tion of histamine should also stimulate secretion by the oxyntic cells. However, in the presence of damage acid secretion is difficult to detect, for the plasma-like fluid buffers and dilutes hydrogen ions, and increased mucosal permeability allows hydrogen ions to disappear by diffusing back into it(10).

Summary. The stomachs of anesthetized, pylorus-ligated rats were filled with 154 mM NaCl, 100 mM HCl, 100 mM acetic acid or 20 mM salicylic acid in 100 mM HCl. After 90 min the fluids were removed, and they as well as the oxyntic gland area of the stomachs were analyzed for histamine. There was a rise in the frequency with which histamine could be detected in the acetic and salicylic acid solutions as compared with NaCl or HCl, and its mean concentration also increased. Gastric tissue exposed to acetic acid or salicylic acid contained about two-thirds as much histamine as did tissue exposed to NaCl or HCl. We infer that during damage histamine is present in the mu-

cosal interstitial fluid where it can exert its characteristic physiological effects.

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A Liver Glycogen Assay of Anti-Inflammatory Steroids on Chicks and Embryos. (30785)

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There is a continuous interest in increasing sensitivity and accuracy of hormonal bioassays. Detection of trace amounts of hormones utilizing biological tests may offer advantages over certain chemical determinations, which are many times non-specific, and may yield misleading results by measuring chemically similar but biologically different metabolic products. Therefore, a sensitive assay for corticosteroids and synthetic anti-inflammatory steroids was sought. The present study is based on earlier reports on the high sensitivity of chicken embryos to natural corticosteroids, as represented by suppression of growth(1,2), and on a more recent study of Guzman-Garcia *et al*(3) including observations on water, electrolyte, and carbohy-

drate metabolism of the developing chick embryo as influenced by synthetic corticosteroids. The latter publication indicated that some of the fluorinated anti-inflammatory steroids are extremely potent in influencing growth as well as liver glycogen content, and suggested that by further analysis of the liver glycogen, selecting optimal dose and time intervals, a sensitive bio-assay procedure can be developed.

Methods. (a) Fertilized white Leghorn eggs were obtained at 9 to 10 days of incubation (Kimber Farms, Inc., Fremont, Calif.) and were inoculated at the 9th, 12th, 13th, or 16th day of incubation into the yolk sac. Steroids were dissolved in sterile corn oil and were injected in a volume of 0.05 ml. The embryos were sacrificed 1-7 days after the injections. Livers were dissected free, and

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