

erythrocytes the final concentration of Tween 20 was 1% instead of 2.5%. Plasma vitamin E contents were measured by the fluorometric method<sup>5</sup>. Plasma creatine kinase activities were measured by using the Boehringer assay kit at 37 °C.

**Results and discussion.** Plasma vitamin E contents for the kids fed a vitamin E-deficient or a vitamin E-supplemented diet were 3–5 and 10–20 µg/ml, respectively, and plasma creatine kinase levels increased from 100 to 1200 munits/ml during the experimental period only in the vitamin E-deficient kids. As shown in the table, percentage hemolysis increased only in samples from the vitamin E-deficient kids and reached a maximum value of about 90%, whereas in samples from the control kids percentage hemolysis was less than 10% throughout the experimental period.

Under the experimental conditions of the standard hemolysis, hemolysis of vitamin E-deficient kid erythrocytes began at 1% Tween 20 and was completed at 2.5% Tween 20. Under the same conditions Tween 40, 60 and 80 did not induce hemolysis. Rat erythrocytes were hemolyzed easily irrespective of their vitamin E status, but chick erythrocytes responded in the same way as kid erythrocytes. Krantz et al.<sup>6</sup> previously reported that dog erythrocytes were hemolyzed at concentrations of Tween 20 above 0.1%. Although the addition of 40 µg of dl- $\alpha$ -tocopherol to the incubation

mixture could not prevent the hemolysis, overnight incubation of vitamin E-deficient blood with 250 µg of tocopherol/ml at 37 °C or intravenous injection at 2–3 h before blood sampling of 30 mg tocopherol/kg b.wt prevented the hemolysis completely. The addition of dithiothreitol or 2-mercaptoethanol to the incubation mixture containing 2.5% Tween 20 prevented the hemolysis of vitamin E-deficient kid erythrocytes completely at 0.25 and 0.8 mM, respectively. The effect of dithiothreitol was nullified if N-ethylmaleimide was present at twice the concentration of dithiothreitol.

Vitamin E may play a structural role in protecting membrane lipoproteins against solubilizing and oxidative damage in the presence of Tween 20.

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## Effect of daily parenteral injection of betamethasone on histamine concentration of gastric tissue in albino rats<sup>1</sup>

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**Summary.** Gastric tissue histamine concentration was determined in albino rats following daily parenteral injection of betamethasone over a period of 12 days. The result shows a highly significant fall in gastric tissue histamine concentration in comparison with that in saline-treated albino rats over a similar period.

A number of observations<sup>2–7</sup> indicate that the hypothalamus is involved in stress and that it acts on gastric glands via vagal and adrenal pathways. That both these pathways are important in influencing gastric ulcer production in situations of stress is evident from our observations that subdiaphragmatic vagotomy or bilateral adrenalectomy could reduce the gastric ulcer index to a very low level<sup>8,9</sup>. Although a number of our observations<sup>10–13</sup> strongly indicate that vagal pathways influence gastric secretion by controlling the release of histamine from the stomach wall, our knowledge about the mechanism of adrenal action in influencing gastric secretion is not very clear. We suggested earlier<sup>14,15</sup> that adrenal action on gastric glands is also probably mediated by the release of histamine from gastric mucosal mast cells.

With a view to understanding the mechanism of action of the adrenals on gastric glands, the present experiment was planned to study the effect of betamethasone injection on gastric tissue histamine concentration in albino rats.

**Materials and methods.** 19 colony-bred albino rats of both sexes, weighing 110–160 g, housed in separate cages, were divided into 2 groups. The 1st group of 10 rats served as a control; they were injected parenterally daily for 12 days with 0.5 ml saline, while in the 2nd group of animals, containing 9 rats, 0.4 mg of betamethasone (Betacortril, Pfizer) in 0.5 ml saline was injected parenterally daily for 12 days. Following these injection schedules, the animals in both groups were fasted for 24 h; they were allowed only water. After this period the animals were sacrificed by a

blow on the head and sectioning of their carotid arteries, following which their stomachs were removed, cut along the greater curvature and cleaned. Each stomach was weighed dry, cut in to fine pieces in 1 N hydrochloric acid (2 ml per g of tissue) and ground up, with a little previously cleaned and dried sand, in a mortar. 10 ml of distilled water per g of tissue was added during grinding. The extract was put in a conical flask and boiled for 1 min. Before assaying, it was filtered, neutralized and made up to a given volume<sup>16</sup>. Histamine concentration was estimated by the 3-point biological assay method using the terminal portion of ileum of a 24-h-fasted medium sized guinea-pig in a thermostatic organ bath at 37 °C. Atropinized Tyrode solu-

Effect of betamethasone injection on gastric tissue histamine concentration in albino rats

Serial No.	Experimental situation	No. of animals	Mean gastric tissue histamine concentration (µg/g) ± SD
1	Control animals with daily saline injection for 12 days	10	12.92 ± 2.52
2	Animals with daily betamethasone injection for 12 days	9	5.03 ± 1.52

Student's t-test between experiments 1 and 2:  $p < 0.001$ ,  $t = 8.17$ .

tion was used as bath fluid and the presence of histamine was confirmed by mepyramine maleate, 0.2 ml,  $2.5 \times 10^{-6}$  M. Histamine concentration was calculated and expressed in  $\mu\text{g}$  per g of tissue.

**Results.** The result of the experiment is summarized in the table. It can be seen that daily intramuscular administration of 0.4 mg betamethasone over a period of 12 days leads to a highly significant reduction in gastric tissue histamine concentration in albino rats.

**Discussion.** It has been reported that about half of the whole-body histamine formation takes place in the stomach<sup>17</sup>. A strong positive correlation between mast cell

population and tissue histamine concentration has been shown to exist<sup>18</sup>. We have reported earlier that following betamethasone injection the gastric mucosal mast cell population is greatly reduced, whereas bilateral adrenalectomy causes an increase in their number<sup>14</sup>. An increase in gastric tissue histamine concentration following bilateral adrenalectomy has also been observed in albino rats<sup>19</sup>. Therefore, in view of the above observations and in the light of the present findings, it appears that betamethasone injection leads to the liberation of histamine from the gastric mast cells and thereby reduces the gastric tissue histamine concentration.

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## Taurine concentrations in the aqueous humor and plasma of anesthetized rabbits<sup>1,2</sup>

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**Summary.** Aqueous humor taurine concentrations were found to be significantly higher ( $p < 0.01$ ) than that of the plasma in anesthetized rabbits. Topical application of 2 mg terbutaline lowered intraocular pressure ( $p < 0.001$ ), but did not alter aqueous taurine content.

Taurine has become the subject of increasing interest in the visual sciences since its recognition as one of the amino acids classified as putative inhibitory neurotransmitters<sup>3-5</sup>, and has been found in high concentrations in the lens and retina<sup>3,4,6</sup>. In rabbits the concentration of most amino acids is higher in the aqueous humor than in the plasma<sup>7</sup> however, the respective concentrations of taurine apparently have never been reported. Moreover, it has been reported that catecholamines can limit production and release of certain amino acids from striated muscle<sup>8</sup>, apparently owing to stimulation of  $\beta$ -adrenergic receptors and adenylyl cyclase. It has further been shown that  $\beta_2$  agonists are potent ocular hypotensive agents<sup>9</sup> and can increase cAMP content of aqueous humor<sup>10</sup>. Thus, it appears reasonable to determine whether a correlation exists between a  $\beta_2$ -mediated decrease in intraocular pressure (IOP) and changes in aqueous taurine levels. The purposes of this study were to determine the relative concentrations of taurine in the plasma and aqueous humor of anesthetized rabbits, and to describe the concomitant effects of  $\beta_2$ -selective terbutaline on IOP and aqueous taurine concentrations.

**Materials and methods.** IOP was measured in mm Hg with an Alcon applanation pneumatonograph and taurine was measured in nM/ml with a Beckman amino acid analyzer in 3-4 kg male albino New Zealand rabbits anesthetized

with pentobarbital. About 300  $\mu\text{l}$  aqueous humor was aspirated with a 25 gauge needle inserted through the cornea on a plane parallel with that of the iris. 3 ml blood obtained by cardiac puncture was immediately mixed with 300  $\mu\text{l}$  EDTA solution (12 mg/ml), then centrifuged for 10 min at  $1000 \times g$  to obtain plasma. Plasma and aqueous

Table 1. Taurine levels (nM/ml) in plasma and aqueous humor of 8 anesthetized rabbits

Plasma	57.7 $\pm$ 5.2 (8)
Aqueous	75.2 $\pm$ 3.3* (16)

Mean  $\pm$  SE (n), \*  $p < 0.01$ .

Table 2. Aqueous taurine levels (nM/ml) and IOP (mm Hg) 1 h after administration of 2 mg topical terbutaline or vehicle

	Taurine	IOP
Vehicle (bilateral)	93.1 $\pm$ 3.5 (4)	21.4 $\pm$ 0.7 (10)
Terbutaline treated eye	86.2 $\pm$ 8.9 (4)	16.6 $\pm$ 0.8* (7)
Terbutaline fellow eye	91.6 $\pm$ 8.5 (4)	23.0 $\pm$ 0.8 (7)

\*  $p < 0.001$ .