It is noteworthy that this group of labelled neurons appeared when HRP was injected near the sphenopalatine ganglion, although in 1 case a neuron was observed at the same position after severance of fibres of the vidian nerve. The morphology of these neurons in the Golgi material was very similar to that revealed by the HRP group. Dendrites of these neurons were in relation to the cells of the subnucleus oralis and nucleus principalis; their axons went out with the facial root. No axons were observed entering the subnucleus oralis or nucleus principalis.

Discussion. The existence of a certain group of cells situated in the ventrolateral part of the reticular formation has been previously demonstrated[3-5]. This 'ventrolateral group' was considered to be part of the superior salivary nucleus[10], but in fact both salivary nuclei are located in the brainstem in a more dorsomedial position[2,3]. However, the precise location of the neurons innervating the oral and nasal mucosae had not yet been established. Our findings suggest that this ventrolateral group has this function. The presence of labelled neurons after HRP injection in the vicinity of the sphenopalatine ganglion strongly favours the hypothesis that the HRP was transported by the vidian, great petrosal and intermediate nerves as far as the brainstem. Moreover, the findings in the 1st group suggest that at least some of the fibres may run with the trigeminal nerve. Indirect Wallerian degeneration of vidian and intermediate nerve fibres would stop at the sphenopalatine ganglion, but that of trigeminal fibres would not. These degenerated fibres were present in the ventrolateral reticular formation. The close interrelationships between the ventrolateral group and the trigeminal nuclei were demonstrated by the Golgi material. This might explain the vasomotor phenomena which sometimes occur in trigeminal neuralgia. Moreover, physiological findings[11] suggest that this zone is involved in general autonomic efferent reactions. Histochemical studies show this zone to be extremely coincident with Dalström and Fuxe's A5[12,13], containing catecholamines and acetylcholine.

Taken together, these facts indicate that this ventrolateral group is an important individual parasympathetic center in the brainstem, functioning as an area of somatic and autonomic integration.

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The role of vitamin E in preventing the hemolysis of kid and chick erythrocytes with Tween 20

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Summary. Erythrocytes of vitamin E-deficient kids and chicks were hemolyzed at concentrations of Tween 20 above 1%. In vivo or in vitro uptake of vitamin E by the erythrocytes, or the addition of dithiothreitol or 2-mercaptoethanol, prevented the hemolysis with Tween 20.

Several hemolytic procedures[1-3] have been used in vitro to estimate the vitamin E status of rats. However, no such procedures have been reported for ruminants. In our preliminary work we tried to find a specific hemolytic agent for the erythrocytes of vitamin E-deficient kids, and among several detergents tested, Tween 20 seemed to be a promising one. In this paper we examined the hemolytic procedure with Tween 20 and confirmed its usefulness as a tool for detecting vitamin E deficiency in kids and chicks.

Materials and methods. 3-day-old Japanese meat-type kids were bottle-fed either a vitamin E-deficient or a vitamin E-supplemented diet reconstituted with warm water in the ratio of 1:5. 60 g of diet were given daily until the animals were 2 weeks old, then 75 g were given per day. The basic composition of these diets was: 87.2% dried skim milk, 10.0% lard, 2.0% lecithin, and 0.8% vitamin-mineral supplement[4] devoid of vitamin E. Only the vitamin E-supplemented diet contained 100 mg dl-α-tocopherol/kg. Rats and chicks were fed the same diets with the addition of 5% cellulose.

The standard hemolytic procedure used for the kids was as follows: 25 volumes of saline-phosphate buffer (pH 7.4)[2] containing 0.5 mM Na EDTA was added to 1 volume of heparinized blood and the mixture was centrifuged at 1000 × g for 10 min. The cell pellet was resuspended with the same volume of the above buffer and stored at 4 °C. A 0.5 ml aliquot of the cell suspension was mixed with 0.5 ml of 5% (v/v) Tween 20 in 0.9% saline and incubated at 37 °C for 15 min. Immediately after incubation, 2.5 ml of ice-cold saline-phosphate buffer was added to the incubation mixture and centrifuged. The optical density of the supernatant was measured at 540 nm. Percentage hemolysis was calculated by dividing the above optical density by the optical density of the completely hemolyzed sample and multiplying by 100. For the hemolytic tests of rat and chick

Means and standard deviations of percentage hemolysis with Tween 20 of erythrocytes of kids fed vitamin E-deficient or vitamin E-supplemented diets

Period of diet	Percentage hemolysis	
	Vitamin E-deficient diet	Vitamin E-supplemented diet
2 weeks	35±11 (4)	3±2 (3)
3 weeks	69± 7 (4)	7±5 (3)
4 weeks	79± 2 (4)	7±1 (3)
9 weeks	90± 4 (4)	5±3 (3)
14 weeks	89± 1 (3)	7±1 (2)

Figures in parentheses are number of animals used.

erythrocytes the final concentration of Tween 20 was 1% instead of 2.5%. Plasma vitamin E contents were measured by the fluorometric method[5]. 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 m units/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-dificient 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.[6] previously reported that dog erythrocytes were hemolyzed at concentrations of Tween 20 above 0.1%. Although the addition of 40 µg of dl-α-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[1]

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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[2-7] 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[8,9].

Although a number of our observations[10-13] 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[14,15] 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[16]. 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: $p < 0.001$, $t = 8.17$.