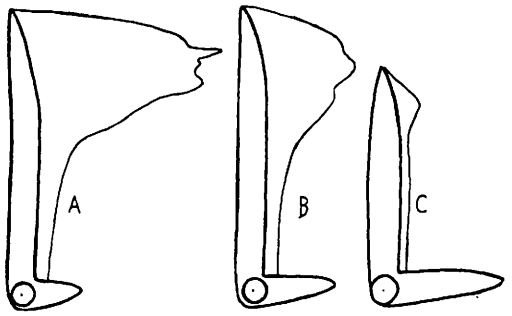

FIG. 2. Tracings of 2-dimensional filter paper chromatograms. A. = albumins, 100 + γ-globulins, 20; B. = albumins, 60 + γ-globulins, 60; C. = albumins, 20 + γ-globulins, 100. Quantities are in μg.

resulting pattern when 120 μg of Cohn's "albumins" of method 10(5), called "Fraction V," was applied to the paper. In "B" 120 μg of Cohn's "γ-globulins" of method 10(5), called Fraction II, was applied. In "A," the upper flag-like portion in the first dimension of the pattern is characteristic, under the conditions of the experiment, for the albumins fraction. In "B" the lower streak, in the second dimension, is typical for the γ-globulins fraction. In both instances there is movement in both dimensions.

These patterns are in harmony with the 3 patterns shown in Fig. 2. "A" in Fig. 2 represents a mixture of 100 μg of albumins and 20 μg of γ-globulins. "B" in Fig. 2 shows a mixture of 60 μg of albumins and 60 μg of γ-globulins. "C" represents 20 μg of albumins and 100 μg of γ-globulins. It may be seen that 20 μg quantities of either albumins or γ-globulins may readily be detected in the presence of 100 μg of the albumin fraction.

Summary. When albumins (Fraction V) were mixed with the γ-globulins (Fraction II) there occurred a typical separation of a portion of each plasma fraction. Our investigations indicate that 2-dimensional filter paper chromatography may have a definite value in protein chemistry, and in clinical research, even if complete separation of proteins is not possible at present.

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Gastric Ascorbic Acid in the Gastritic Rat.* (19550)

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Ascorbic acid, instrumental in the formation and maintenance of intercellular substance, may also play a role in gastrointestinal homeostasis. It has been demonstrated that ascorbic acid deficiency in guinea pigs results in a high percentage of gastric ulcers(1). Gastric cancer patients utilize more ascorbic acid than patients of other malignant or non-malignant diseases(2).

In the rat, in which abundant biosynthetic ascorbic acid is available, spontaneous and induced gastric disorder is rare(3). A eugenol-induced gastritis(4) is perhaps the only readily reproducible example of a gastric, glandular dyscrasia.

The purpose of the present investigation was to study the ascorbic acid in the tissue of the fore- and glandular stomachs of eugenol-treated, gastritic rats and comparable con-

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TABLE I. Effect of Eugenol Administration on Gastric and Adrenal Ascorbic Acid.

Group	No. of rats	Treatment	Chem. form.	Mean ascorbic acid — Stomach — Glandular mg %	Glandular µg	Forestomach mg %	Forestomach µg	Adrenals, mg %
I	10	Water st*	ox	2.2	24	3.3	11	15.8
			red	22.2	244	11.6	38	364.8
			tot	24.4§	268	14.9§	49	380.6§
II	7	Eugenol st	ox	2.9	32	2.7	9	16.6
			red	16.6	182	16.3	53	261.9
			tot	19.5	214	19	62	278.5
III	5	" di†	tot	24.8§	281	15.1§	50	266.6
IV	5	" gi‡	tot	19.4	213	18.8	62	290.4

* By stomach tube. † By duodenal inj. ‡ By gastric inj.
§ Comparison of corresponding totals from groups I and II and comparison of totals of groups III and IV are statistically significant. $P<.01$.

trols. The adrenals were also studied as a reference organ.

Methods. Male Sprague-Dawley-Holtzman rats, weighing 200-250 g received Purina Laboratory Chow and tap water *ad libitum.* Eugenol (4-allyl-2-methoxyphenol) as an aqueous 1.0% emulsion, or distilled water was administered daily in 3 ml doses for 7 days. The rats were divided into 4 groups according to the manner in which the eugenol emulsion or distilled water was introduced into the gastrointestinal tract. Group I: Distilled water was administered by stomach tube. Group II: Eugenol emulsion was administered by stomach tube. Group III: The rats were subjected to a) light ether anesthesia, b) a 2-3 cm abdominal incision, and c) injection of eugenol emulsion into the upper duodenal lumen. The musculature and skin were sutured with silk thread. This operative procedure was repeated daily for 7 days. Group IV: The rats were subjected to the same daily operative procedure as those of group III, but eugenol emulsion was injected into the gastric lumen. On the eighth day the animals were sacrificed by cervical dislocation. The forestomach, glandular stomach and adrenals were observed grossly, excised, weighed, and subjected to analysis for total and oxidized ascorbic acid(5). The value for reduced ascorbic acid was taken as the difference between the total and oxidized values.

Results and discussion. Weight loss was not observed in any animal. The results of the ascorbic acid determinations appear in Table I. The ascorbic acid in the stomach and adrenals was found predominantly in the reduced form. In the water-fed controls (I), the reduced ascorbic acid accounted for 91% of the total ascorbic acid found in the glandular stomach, 78% of that found in the forestomach, and 96% of that found in the adrenals. The concentration of total ascorbic acid was found to be significantly higher in the glandular stomach than in the forestomach. The glandular stomach of the rat is relatively less susceptible to spontaneous and induced dyscrasia than the forestomach(3). The possibility of a relationship between the pathological susceptibility of gastric tissue and the amount of reduced ascorbic acid present in the tissue must be considered.

Introduction of eugenol emulsion to the gastric lumen by stomach tube (II) resulted in a grossly recognizable gastritis which was distinctly absent in the water-fed controls. The total ascorbic acid concentration in the glandular stomach was significantly decreased in the gastritic rats (II), while in the forestomach the concentration was significantly increased. The total amount of ascorbic acid in the complete stomach of the gastritic group was 13% less than the total amount found in the water-fed controls. The percent of the total amount of ascorbic acid, found in the reduced form in the forestomach, glandular stomach, and adrenals of the gastritic group (II) was not significantly different from the percentage found in the water-fed controls.

Administration of eugenol emulsion by

stomach tube (II) resulted in a significant decrease in adrenal ascorbic acid. This reduction is suggestive of a stress phenomenon occurring after intestinal absorption. The possibility that the decrease of ascorbic acid in the stomach could also have been a systemic effect led us to place the eugenol directly in the duodenum. If the gastric effect observed in group II was systemic in nature, the ascorbic acid in the stomach should be decreased. Injection of eugenol emulsion into the duodenal lumen (III), did not evoke a grossly recognizable gastritis and the concentrations of ascorbic acid found in the fore- and glandular stomachs were almost identical to those of the water-fed controls. Injection of eugenol into the duodenum avoided direct gastric mucosal contact with the eugenol, but allowed intestinal absorption, as evidenced by the decrease in the adrenals. Since the adrenal decrease in ascorbic acid occurred in the absence of anesthesia and operative procedure, the reduction is probably related to the systemic presence of the eugenol.

The injection of eugenol emulsion into the gastric lumen (IV) resulted in a grossly recognizable gastritis and changes in gastric ascorbic acid concentration practically identical to those observed in the eugenol-fed, gastritic group, but significantly different from those in the duodenum-injected group. It may be concluded that the changes in the gastric ascorbic acid during a eugenol-induced gastritis, as well as the gastritis itself, are associated with the direct contact of the eugenol with the gastric mucosa, and not a systemic effect after gastrointestinal absorption.

Summary. Concentrations of reduced, oxidized, and total ascorbic acid in the forestomach, glandular stomach, and adrenals of the rat have been reported. Direct contact of a eugenol emulsion with the gastric mucosa resulted in a gastritis and a 13% decrease in the total gastric ascorbic acid. Systemic absorption of the eugenol, avoiding direct gastric contact, did not induce these changes. A decrease in adrenal ascorbic acid after eugenol administration suggests a systemic stress effect also associated with the presence of the eugenol, but distinct from the direct gastric effect.

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The Action of Threonine in Inducing an Amino Acid Imbalance.* (19551)

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Of several amino acids reported to produce growth inhibition when fed in excess with a 9% casein ration, threonine appears to be one of the most effective in this respect(1). Since the addition of either niacin or tryptophan to this ration overcomes the inhibition produced by threonine, it appeared that the effect of the threonine might be due to an inhibition of the synthesis of tissue pyridine nucleotides from the already limiting amounts of tryptophan in the 9% casein ration. The

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