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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structural similarities between threonine and 3-hydroxyanthranilic acid could conceivably account for this inhibition in that threonine might act as an inhibitor of the enzyme system which converts 3-hydroxyanthranilic acid to the niacin structure.

Employing the technics worked out in this laboratory(2-7) for studying the relationship between dietary tryptophan and niacin and liver pyridine nucleotides (PN), the authors have investigated the effect of excess dietary threonine on these conversions. If threonine produces an imbalance by inhibiting the conversion of 3-hydroxyanthranilic acid to PN, the synthesis of PN from dietary tryptophan and 3-hydroxyanthranilic acid should be inhibited by threonine while that from niacin should not.

Experimental and results. In these studies 2 types of experiments were carried out. In one group of experiments, rats were first depleted of liver PN by feeding a non-protein ration. This ration was then supplemented with niacin, tryptophan, or 3-hydroxyanthranilic acid, with and without excess threonine in each case. After the period of supplementation liver PN concentrations were determined. In these experiments the rations were fed *ad libitum*. In the second group of experiments rats, depleted of liver PN, were fed a 9% casein ration with and without excess threonine to study the effects of threonine on liver PN concentrations using rations ordinarily employed in amino acid imbalance studies. In these experiments a force-feeding technic was employed to keep food consumption the same in all groups.

Ad libitum experiments. Male, weanling Sprague-Dawley rats were fed a non-protein ration for 14-21 days to deplete them of liver PN(3). The ration consisted of sucrose 87.5%, corn oil 5%, Salts IV(8) 4%, complete vitamin mix excluding niacin(9) 2%, and sulfasuxidine 1.5%. The sulfasuxidine was included to depress intestinal synthesis of niacin. After the depletion period, the rats were separated at random into 8 groups and fed the non-protein ration supplemented for the respective groups as follows: Group I, no supplement; Group II, 0.163% niacin; Group III, 0.270% DL-tryptophan; Group IV, 0.202% 3-hydroxyanthranilic acid; Group V, 1.76% DL-threonine; Group VI, 1.76% DL-threonine + 0.163% niacin; Group VII, 1.76% DL-threonine + 0.270% DL-tryptophan; and Group VIII, 1.76% DL-threonine + 0.202% 3-hydroxyanthranilic acid. The niacin, tryptophan, and 3-hydroxyanthranilic acid were fed at equimolar levels, and DL-threonine was included at 11 times the molar level of the other supplements to insure an excess of the inhibitor.

After the various groups were fed their respective supplemented rations for 4-7 days, the animals were sacrificed and liver PN determined by the method of Feigelson, Williams and Elvehjem(7). These experiments were repeated in their entirety. In the first series of experiments, in which a 21-day depletion period was employed, many of the animals died during the subsequent supplementation period. Therefore, in the second experiments, a 14-day depletion period was used in order to decrease the mortality during the supplementation period. The results of both studies were averaged together and are reported in Table I. The figures in the last column were calculated by subtracting the endogenous PN values, *i.e.*, the negative controls, from the total value of each of the other groups. Therefore, the actual PN contribution of each supplement in the presence and absence of threonine can be compared. From the results in the table it appears that in the absence of threonine liver PN is readily synthesized from niacin and tryptophan and to a smaller but significant extent from 3-hydroxyanthranilic acid. The poor contribution of the 3-hydroxyanthranilic acid may be due to its instability in the ration. When threonine was included with these supplements, liver PN was synthesized just as well from all the supplements as when threonine was omitted. Therefore, the hypothesis that threonine induces growth inhibition by interfering with the enzymatic synthesis of PN from tryptophan via 3-hydroxyanthranilic acid is not supported by these experiments.

Force-feeding experiments. In producing growth inhibition in typical imbalance experiments, excess threonine is usually fed with a 9% casein-sucrose ration + 0.2% cystine.

TABLE I. Effect of Threonine on Conversion of Niacin, Tryptophan, and 3-Hydroxyanthranilic Acid to Liver Pyridine Nucleotides (*Ad libitum* Experiments).

Group	No. of animals	Supplement, %	Pyridine nucleotides (γ/g liver)	Difference in supplemented groups and the negative control (γ/g liver)
I	17	0	625	
II	14	.163 niacin*	1165	540
III	14	.270 tryptophan*	960	335
IV	14	.202 3-hydroxyanthranilic acid*	725	100
V	17	1.76 DL-threonine	600	
VI	16	1.76 " + .163 niacin*	1250	650
VII	18	1.76 " + .270 DL-tryptophan*	920	320
VIII	14	1.76 " + .202 3-hydroxyanthranilic acid*	775	175

* These supplements were added at equimolar levels.

TABLE II. Effect of Threonine on Formation of Liver Pyridine Nucleotides from Tryptophan in a 9% Casein Ration (Force-Feeding Experiments).

Group	No. of rats	Ration	γ PN/g liver Exp. 1	γ PN/g liver Exp. 2	Avg of Exp. 1 and 2
I	16	9% casein basal	553	535	544 ± 23*
II	16	" " " + .180% DL-threonine	457	428	442 ± 25*

* Standard errors of the mean.

Therefore, it is possible that in the preceding experiments the levels of tryptophan and 3-hydroxyanthranilic acid were too high in comparison with the level of threonine and that the effects of the threonine were reversed by the supplements. In the following experiments a ration known to produce an amino acid imbalance with excess threonine was employed(1). In this ration the only source of tryptophan was that in the 9% casein, and no niacin was included. In order to overcome the effects of the low food intake on this ration when threonine is included, a force-feeding procedure was undertaken to insure comparable food intake in all groups.

Male, weanling Sprague-Dawley rats were depleted of liver PN by feeding a non-protein ration for 14 days. The animals were then divided into 2 groups and force-fed a 9% casein-sucrose ration + 0.2% cystine(1) with and without 0.180% DL-threonine, respectively, for 10 days. Since threonine is known to produce an imbalance when included in this ration, the effect of threonine on the production of liver PN from the tryptophan in the 9% casein ration could be studied. The rations were prepared as a slurry in water and force-fed by stomach tube in 3-4 portions daily. After the 10-day force-feeding period the rats were sacrificed and liver PN concentrations determined as before(8). The experiments were repeated in their entirety, and the results of both studies with the average of the two are reported in Table II. The low PN levels in the groups without threonine† indicate that the tryptophan in a 9% casein ration is extremely limiting for PN synthesis. However, the decrease observed when threonine was included in the ration indicates that this amino acid further limits the maintenance of liver PN. The hypothesis that threonine might induce the effects of an imbalance by inhibiting the conversion of tryptophan to PN is still possible though the authors feel that a better explanation is more probable in view of other effects observed in these experiments. In the course of the force-feeding experiments it was observed that gross utilization of the 9% casein ration was greatly inhibited by threonine.

† For normal weanling rats fed a good stock ration, liver PN is about 900-1000 γ/g liver.

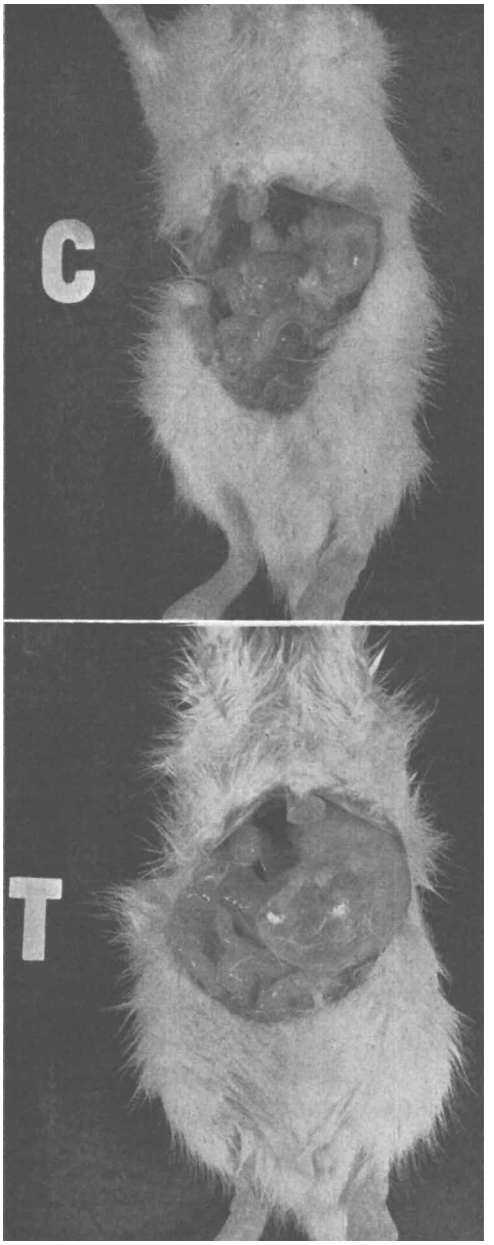

FIG. 1. Effect of excess dietary threonine on food retention in gastro-intestinal tract. Examples were chosen by different observers as the most typical of the two groups of rats. C = 9% casein control; T = 9% casein control + .180% DL-threonine.

This was demonstrated by the fact that the threonine-containing ration was only slowly digested and absorbed. When the animals were sacrificed for the PN determinations, very large amounts of the force-fed ration

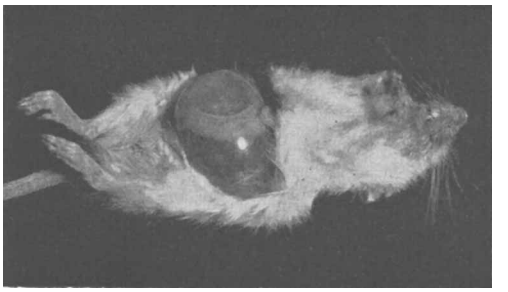

FIG. 2. A rat force-fed the 9% casein + .180% DL-threonine ration showing that the enlargement of the stomach is due mainly to unutilized food rather than to gas.

were found to be retained in the gastrointestinal tract, particularly in the stomach. This was true, however, only for the ration containing the excess threonine. Photographs of the most representative animal from each group, as chosen by a number of observers, are presented in Fig. 1. The marked retention of ration in the stomach of the threonine-fed animal can easily be observed. Another threonine-fed animal is shown in Fig. 2 in order to show that the enlargement is mainly due to food retention rather than to the presence of gas. From these results, therefore, it appears that threonine inhibits the digestive and absorptive processes when fed in excess in a 9% casein ration. The low liver PN values for this group might be explained by the decreased availability of the tryptophan in the ingested ration rather than by a threonine inhibition of the enzymatic synthesis of PN from tryptophan. This explanation appears more probable especially in view of the negative effect of threonine in the earlier experiments reported in this paper. In other results from this laboratory(10), it appears that if the protein is fed in the form of free amino acids rather than whole protein, threonine does not produce the typical amino acid imbalance syndrome in rats as measured by growth. Therefore, it appears from this evidence that excess dietary threonine induces the effects of the imbalance by interfering with the digestion and/or absorption of the intact dietary protein and thus limiting the availability of the tryptophan to the animal.

Summary. Experiments have been reported in which the function of threonine in produc-

ing an amino acid imbalance has been investigated. The hypothesis that threonine inhibits the conversion of tryptophan to tissue pyridine nucleotides via 3-hydroxyanthranilic acid is not strongly supported by our results. The results indicate, however, that excess dietary threonine markedly interferes with the digestion and absorption of the ration and that this is probably a better explanation for the action of excess threonine in producing growth inhibition in the rat.

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Gamma Globulin Passive Protection Tests in Mice Injected Intraperitoneally with MEF1 Poliomyelitis Virus.*† (19552)

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Human gamma globulin prepared from pooled adult plasma has been shown to possess substantial amounts of antibody against the Lansing strain (Type 2) of poliomyelitis virus (1). Similar observations have been made in connection with the other known types of poliomyelitis virus(2). Human gamma globulin when administered intraperitoneally to mice has been shown to afford some protection against the subsequent intracerebral inoculation of Lansing virus(3). More recently Bodian(4) demonstrated that in the case of monkeys 2.0 ml of gamma globulin per kg of body weight gave significant protection against the intramuscular inoculation of Lansing virus. The globulin and the virus were inoculated into the calf muscles of opposite legs. A protective effect could be demonstrated if the globulin was inoculated as much as 3 weeks before or 3 days after the injection of virus. Amounts of antibody sufficient to protect against the intramuscular injection of active virus did not interfere with the subsequent development of active immunity.

The recent demonstration that the suckling mouse-adapted MEF1 strain of poliomyelitis virus(5) can infect and cause the death of a significant proportion of mice aged 5-15 days when inoculated by the intraperitoneal route (6) has made available another laboratory animal which may be used for determining the efficacy of human gamma globulin in furnishing protection against the peripheral inoculation of a rodent-adapted strain of poliomyelitis virus. The present paper deals with the results of such an experiment.

Materials and methods. Two viruses belonging to the Lansing group (Type 2) were employed. The suckling mouse-adapted MEF1 strain (53rd passage material) was kindly furnished to us by Dr. Casals. The classical Lansing strain was obtained originally from Dr. Armstrong. Suckling mice of the CFW strain were used. The human gamma globulin was processed by E. R. Squibb (Lots No. 116-1 and No. 157-3) from pools of human plasma provided by the American National

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