We have not been able to get graded responses with graded doses. For example, when the following doses of vitamin B_1 hydrochloride were fed, the irregular results shown in Table I were obtained.

TABLE I.

Experiment	Dose	Interval before back to original rate, days
1	3γ	4
	6γ	2.6
	9γ	3.6
	12γ	3.2
2	3γ	2.6
	6γ	6
	9γ	4.5

Anomalous results such as the following were not infrequently encountered. When the rate was 389 per minute, the rat was fed $6\ \gamma$. On the next 5 days the rates were 480, 345, 389, 476, 326.

Several variations in the technique were tried. For instance, smaller rats were used which weighed about 30 gm. when they were put on the deficient diet. Also the vitamin B_1 was given when the heart rates were from 400 to 420 per minute. Also larger doses ranging from $12\ \gamma$ to $30\ \gamma$, and from 2.5 to 7.5 I. U. were used. None of these alterations improved the results.

In all, we have studied the responses of 179 rats to single doses of vitamin B_1, and in our hands this method gave inconsistent results.

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Dietary Calcium and pH of the Lower Intestine.*

ELIZABETH CHANT ROBERTSON. (Introduced by F. F. Tisdall.)

From the Hospital for Sick Children and Department of Pediatrics, University of Toronto.†

Shortly after weaning, litters of young rats were divided into 5 groups and fed the 5 following diets for 7 to 11 weeks, when they were killed and the pH at various levels of their intestinal tract was determined, using the B.D.H. capillator. The capillator was checked

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† Under the direction of Alan Brown, M.D., F.R.C.P.(C).

with a potentiometer and in the pH range of 5.5 to 7.5, they checked very well.

All the diets contained corn starch (60.5%-64%), casein (18%), Crisco (10%), dried brewer's yeast (6%), and cod liver oil (2%). This diet with no additions was the mineral deficient diet. In the second diet, 1% K_2CO_3 was added to the mineral deficient diet; in the third, 1% $CaCO_3$ was added; in the fourth, 1% K_2CO_3 and 1% $CaCO_3$ were added; and in the fifth, 3.5% of Hawk and Oser's[1] salt mixture was added and this contains approximately as much Ca and K as 1% $CaCO_3$ and 1% K_2CO_3. The fifth diet was known as the adequate diet.

The pH readings in the stomach, duodenum and jejunum were practically the same regardless of the diet fed. In the rats fed the diets low in calcium (that is, those fed the mineral deficient or the mineral deficient diet + 1% K_2CO_3) there was a slightly more acid reaction (pH 6.5-6.6) in the lower ileum than in those fed the other diets, which were rich in calcium, where the pH was from 6.9-7.0. The former rats had abnormally large amounts of fecal material in their lower ileums.[2] The reactions in the cecums of the rats on the calcium poor diets varied from 5.9 to 6.1, as compared with 7.0 to 7.1 in the rats on the calcium rich diets. The readings in the colons were 6.0 with the calcium poor and 7.0 with the calcium rich diet. These figures are the averages of determinations made on at least 25 different rats, and in many cases (*e. g.,* those on the adequate and mineral deficient diets) 70 to 100 rats were used. These results are summarized in Table I.

TABLE I.
pH in Lower Intestines of Rats Fed Various Diets for 7-11 Weeks.

	Min. Defic. Diet	Min. Defic. Diet + 1% K_2CO_3	Min. Defic. Diet + 1% $CaCO_3$	Min. Defic. Diet + 1% K_2CO_3 + 1% $CaCO_3$	Adequate Diet
Colon	6.0	6.0	7.0	7.0	7.0
Cecum	5.9	6.1	7.0	7.0	7.0
Ileum	6.6	6.5	6.9	7.0	7.0

The standard deviations of the readings calculated according to the method of Banister[3] varied from 0.2 to 0.3. At each of these 3 levels in the intestinal tract the differences in pH between the rats fed the calcium poor and the calcium rich diets were statistically sig-

[1] Hawk, P. B., and Oser, B. L., *Science,* 1931, **74,** 369.

[2] Robertson, E. C., *Am. J. Dis. Child.,* 1937, **53,** 500.

[3] Banister, H., *Elementary Applications of Statistical Method,* Blackie, London, 1929, p. 24.

nificant. The rats fed the calcium poor diets showed marked stasis and dilatation in the large intestines, whereas those fed the calcium rich did not show this.[2]

From these results it appeared that the calcium in the diet was the factor responsible for keeping the pH of the lower intestine around 7.0. To test out this hypothesis, diets were made up which were adequate except for calcium, adequate except for potassium, and adequate except for calcium and potassium, by means of leaving out the Ca and/or K salts from the salt mixture.

Young rats were fed these diets and similar controls were fed the mineral deficient and the adequate diet. After 7 to 11 weeks, the pH determinations were made as before. The results obtained are shown in Table II, each figure being the average from at least 20 rats.

TABLE II.
pH in Lower Intestines of Rats Fed Various Diets for 7-11 Weeks.

	Min. Defic. Diet	Adeq. except K and Ca	Adeq. except Ca	Adeq. except K	Adequate Diet
Colon	5.9	6.0	6.2	7.1	7.2
Cecum	6.0	6.1	6.3	7.1	7.3
Ileum	6.7	6.7	6.8	7.1	7.0

When the rats were fed the diets low in calcium (first 3 diets) the pH values of the large intestine varied from 5.9 to 6.3 and these rats had intestinal stasis. On the other hand,when the diets were rich in calcium (last 2 diets), the pH in the large intestine varied from 7.1 to 7.3 and these rats did not have intestinal stasis.

The stasis apparently occurs only when the calcium intake is low, and the acid reaction in the large intestine occurs when there is stasis. The feces of the rats fed the adequate diet contained about 6.2% Ca, those of rats on the mineral deficient diet contained about 0.18% Ca. In a few rats on the deficient diet the excretion of calcium was trebled by means of feeding 2 grains of desiccated thyroid (Parke, Davis & Company)[4] daily, but no constant change in the reaction of the large intestine occurred.

By means of a Petroff-Hausser bacterial counter, counts were made of the bacteria in the cecums of rats fed the adequate and the mineral deficient diets. It was consistently found that there were many more organisms in the deficiently fed (14.5×10^{10} per gram, on the average), than in the adequately fed animals (average 4.7×10^{10}). Spread plates of dilutions of the cecal contents gave similar results. Possibly the greater acidity is due to the biochem-

[4] Pugsley, L. I., and Anderson, E., *Biochem. J.*, 1934, **28**, 754.

ical activity of the more numerous bacteria in the mineral deficient animals. Kjeldahl determinations showed that there was 10.2% protein in the cecal material in the deficiently fed as compared with 6.8% in the adequate controls. The higher protein might favor bacterial growth.

Conclusions. Young rats fed diets low in calcium have a pH of about 6.0 in their large intestines. Controls fed the corresponding adequate diet have a pH of about 7.0.

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Additional Sources of Androgens.

M. D. KRITZER AND B. CUNNINGHAM. (Introduced by W. A. Perlzweig.)

From the Department of Zoology, Duke University.

Stallion Urine. It is rather surprising that the occurrence of androgens in stallion urine has not been reported, or if it has that the record has not found its way into the literature of the subject, since stallion urine has been studied in relation to estrogenic content. For this reason we have made assays upon stallion (Percheron) urine and report them here. The occurrence of an androgenic substance in stallion urine was demonstrated in our laboratories by Mr. Norman Nathenson in 1936, but his assays were erratic due to massive doses. The studies were repeated by us during the fall of 1936, smaller doses being used and fairly concordant assays being obtained. The urine was acidified to Congo red with sulfuric acid and allowed to stand 2-3 days. It was then extracted with benzene, evaporated to dryness, residue taken up in ether, washed with 10% NaOH solution, dissolved in olive oil and assayed by the capon comb method. The assays showed a bird unit in (1) 548 cc., (2) 570 cc., (3) 562 cc.; an average of 560 cc. per bird unit. (A bird unit is taken herein to be the amount of each of 5 daily injections required to produce a total of 5 mm. growth in length plus height as measured on the 6th day.)

Dog Urine. Dog urine was collected from a male German police dog; the same animal serving as a source for 2 experiments with an interval of about a year intervening. The urine was extracted in the same manner as that of the stallion. The first sample was assayed on 4 birds, the latter on 2. The amount of urine necessary for a