teraction indicates that large variations occur in the concentration of bound ions in the system with comparatively small pH changes. Because may proteins interact with phospholipids through similar reversible, electrostatic combinations as does calcium(10,20,21), some of these protein-phospholipid interactions should have a similar pH sensitivity in the physiological range. It is suggested that a sensitive equilibrium exists between protein, phospholipids and calcium under appropriate conditions. The large effect of pH on these ionic interactions in the physiological range and the ubiquitous distribution of phospholipids invite further speculation on mechanistic models concerning ionic equilibria in cells, active transport, macromolecular transformations of biosystems and other regulating mechanisms of tissue activity, all controlled at least partially by small pH variations affecting binding interactions.

Summary. The binding of calcium in phospholipid mixtures is very dependent on the pH, it being a quantitative reflection of the types of dissociable groups in the phospholipids present. As the pH increases from 6.5 to 8.5, there is a considerable increase in Ca-binding if triphosphoinositide, phosphatidylserine, or phosphatidylethanolamine is present. This effect may be of some consequence in certain physiological processes.

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Received July 25, 1966. P.S.E.B.M., 1967, v124.

Gastrointestinal Gas Production in Rats Fed Raw and Heated Navy Beans With or Without Added Antibiotics.* (31985)

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An increased production of gastrointestinal gases due to the ingestion of beans has been reported recently in rats(1,2) and humans (3,4). However, few attempts have been made so far to decrease or prevent the increased gastrointestinal gas production in animals fed the bean diet(2). Preliminary experiments indicated that rats fed the heated navy beans produced less gas than was produced by the raw navy beans. The present

* Published with approval of the Director as paper 1963, Journal Series, Nebraska Agri. Exp. Station. Supported in part by USPHS Research Grant no. A-2018.

TABLE I. Effect of Feeding Raw and Heated Navy Beans With or Without Antibiotics Supplementation on Gastrointestinal Gas Production in Rats.

Group No.	Diet	Body wt, g	Food intake, g	Total vol, ml	Total vol in ml per 100 g body wt per g food intake
	Part I—Unadapted rats				
1	Casein	157.0	6.0	.80	.08 ± .01
2	Raw beans	156.4	2.0	1.04	.35 ± .03
3	Heated beans	163.6	6.8	1.36	.12 ± .01
4	Raw beans + antibiotics	164.1	2.0	1.07	.33 ± .04
5	Heated beans + antibiotics	161.5	6.7	1.33	.13 ± .01
	Part II—Adapted rats				
1	Casein	176.1	5.5	1.23	.12 ± .06
2	Raw beans	139.2	3.8	3.72	.70 ± .11
3	Heated beans	164.3	6.2	2.73	.27 ± .01
4	Raw beans + antibiotics	151.1	4.7	3.06	.43 ± .05
5	Heated beans + antibiotics	172.7	5.0	2.41	.28 ± .03

Values represent average of 6 rats. ± Standard error of mean.

report confirms the findings of preliminary experiments and also deals with an additional experiment involving the addition of antibiotics to the raw and heated navy bean diets to examine its effects, if any, on the gastrointestinal gas production in rats.

Experimental. The preparation of raw and heated navy bean diets was the same as described before(5) except that salt mixture, H.M.W.†(6) was used instead of Hegsted salt mixture. In brief, the percentage composition of the basal diet was: sucrose, 30; corn oil, 6; salt mixture, H.M.W., 4; vitamin diet fortification mixture,‡ 2. Navy beans (protein content 24%) either raw or heated and casein was incorporated into the basal diet to furnish a level of 10% protein (N × 6.25). Corn starch was added to make the total to 100. The antibiotics, procaine penicillin and streptomycin sulfate, were added to the diet each at 0.1% level at the expense of corn starch.

Female rats of Holtzman strain weighing about 150 g were used in the present studies. They were trained to eat the maximum amount of food at one time by a restricted feeding technique. The details of technique have been given elsewhere(7). After the initial period of training of 8-10 days the rats are given the experimental diets in the morning for one hour and then killed by ether 4 hours after the feeding period. The rats were dissected and the gastrointestinal tract was exposed. The ligatures were made above the stomach and close to the anus. The whole tract was then excised and placed in the 125 ml Erlenmeyer flask containing 21% HCl. The details of the method for measuring the total volume of gas are given by Hedin and Adachi(1).

In another experiment the trained rats were given the experimental diets for a week and these rats were designated as adapted rats. At the end of the adaptation period, the rats were given the experimental diets for one hour in the morning and then killed 4 hours after the feeding time. Gas production measurements were made in these rats as described above.

Results and discussion. Table I shows the gastrointestinal gas production in rats fed casein, and the raw and heated navy bean diet with or without antibiotics supplementation. The gas producing efficiency (GPE) of the diet was evaluated by expressing the values per 100 g body wt per g food intake. This was necessary mainly because rats fed raw navy

† Purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.

‡ Purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Supplies the following vitamins in mg/100 g of diet: Vit. A concentrate (200,000 units per g), 9.0; vit. D concentrate (400,000 units per g), 0.5; alpha tocopherol, 10.0; ascorbic acid, 90.0; inositol, 10.0; choline Cl, 150.0; menadione, 4.5; P aminobenzoic acid, 10.0; niacin, 9.0; riboflavin, 2.0; pyridoxine HCl, 2.0; thiamine HCl, 2.0; Ca pantothenate, 6.0 biotin, 0.14; folic acid, 0.18; vit. B_{12}, .0027.

beans consumed less food and lost weight during the adaptation period.

The results presented in Table I indicate that GPE of raw navy beans is 4 to 6 times greater than that of casein while heated navy bean diet produced only one and one-half to 2 times as much flatus as the casein diet. In other words, heating reduced the GPE of raw navy beans to almost one-third although this value for heated navy bean diet was significantly greater than that of casein diet ($P<0.05$). The addition of antibiotics to the diets containing raw or heated navy beans did not affect the gas production in unadapted rats when compared to the rats receiving no antibiotics. However a significant reduction in gas production was observed in adapted rats fed raw navy beans with added antibiotics (Table I, II, part A groups 2 and 4, $P<0.05$). No appreciable changes were observed in the gas production in adapted rats fed heated navy beans with and without antibiotics supplementation. These results are in contrast to those of Hedin(2) who did not observe the beneficial effect of antibiotics supplementation (neomycin sulfate-nystatin mixture) to the red bean diet. This could be due to the use of different antibiotics in the diets. In the present investigation a mixture of penicillin and streptomycin sulfate was selected on the basis of previous observation that these antibiotics prevented the weight losses in rats fed raw navy beans(5). It is likely that the selected antibiotics may or may not be good choices. In view of this criticism the effectiveness of other antibiotic with specific or broad spectrum activity on the intestinal microflora in preventing or reducing the flatus production should be determined.

In the light of above results it is tempting to postulate presence of heat labile flatus producing factor(s) in navy beans. The work of Steggerda *et al*(3) indicates that a flatus producing factor is a low molecular weight compound and heat stable. It appears therefore that the increased flatus production in the animals fed the bean diet is a complex problem involving more than one factor." It is possible that the presence of trypsin inhibitor and hemagglutinins in navy beans(8) may have something to do with the increase of flatus production in animals fed raw navy beans. For example, it was reported that the action of soybean trypsin inhibitor is to stimulate the pancreas to produce excessive secretions(9,10). It is reasonable to assume that under these circumstance excessive bicarbonate secretion of the pancreas may produce excessive CO_2. Indeed, Hedin(2) found that CO_2 comprised 90% of the total flatus when rats were given the red bean diet. Steggerda and Dimmick(4) also found CO_2 to be a major component of flatus. However, no analysis on the composition of gastrointestinal gases was made in the present investigation. Since trypsin inhibitor(11) and hemagglutinins(12) interfered with the absorption of the nutrients it is likely that these factors may also impair the absorption and/or diffusion of the gases from the intestinal wall to the blood stream.

The beneficial effect of antibiotics supplementation in lowering the gas production in rats fed raw navy beans could be explained in various ways. It may be that antibiotics exert their action on deleterious intestinal microflora responsible for CO_2 production or by stimulating the growth of specific type of microflora capable of metabolizing CO_2. It is also possible that antibiotics facilitate the absorption or diffusion of gases by thinning the intestinal cell wall linings(13).

The high fiber content(14) and increased peristalsis(15) have been suggested as causative factors for increased gas production in animals fed vegetable diets. This would perhaps explain why the heated navy bean diet produced more gas as compared to casein diet.

It is interesting that all adapted rats produced more flatus than was produced by the unadapted rats. This increased flatus production in adapted rats may reflect an alteration in the microflora.

Finally it should be pointed out that although the Hedin and Adachi method(1) is a valuable tool for assessing the magnitude of gas production of various types of foods it appears to have certain limitations. For example, the method may overestimate the gas production to the extent of the mas of bicarbonate in the gut. Moreover it is possible that the food or the supplementation (in the

present case antibiotics) may change the specific gravity of the tissues in addition to the change in gas volume.

Summary. Gas producing efficiency of raw and heated navy beans with or without antibiotic supplementation was evaluated and compared with that of casein diet. Raw navy beans produced 4 to 6 times as much gas as that produced by casein. On the other hand heated navy beans produced only one and one-half to 2 times more gas than that obtained on casein diet. Addition of antibiotics to the raw navy bean diet significantly reduced the flatus production in adapted rats but was without effect in unadapted rats fed raw or heated beans. The possible mechanism of increased gas production in the animals fed raw navy beans and the beneficial effect of supplementary antibiotics in reducing the gas production have been discussed.

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Received September 6, 1966. P.S.E.B.M., 1967, v124.

MSH Activity in Rat Pituitaries After Pinealectomy.*† (31986)

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The pineal gland of mammals produces melatonin, a substance which is the most active agent known to lighten amphibian melanocytes *in vitro*(1) and *in vivo*(2). It has been recently suggested that administration of melatonin may cause a decrease in the pituitary content of melanocyte-stimulating hormone (MSH) in albino rats(3).

Removal of the pineal gland, therefore, might be expected to result in an increased level of MSH in the pituitary. Accordingly, the following experiments were performed to determine the effect of pinealectomy upon rat pituitary MSH content.

* Supported in part by USPHS grant 07467.
† Presented in part at Annual Meeting of the Southern Section of the American Federation for Clinical Research, New Orleans, Jan. 1967.

Materials and methods. Female Sprague-Dawley rats (Cheek-Jones Co., Houston) approximately 3 weeks old (50 g) and 9 weeks old (200 g) were pinealectomized by the method of Hoffman and Reiter(4). Rats serving as controls were subjected to an identical operation except that the pineal was left intact. Three days, 1 week, 4 weeks, or 8 weeks following operation the rats were decapitated and the whole pituitaries were removed, weighed, and stored at −20°C. At the time of assay, pituitaries from each group of 3-4 rats were pooled, homogenized in 0.1 N HCl-0.9% aqueous NaCl-0.1% bovine serum albumin, and kept on ice throughout the study. MSH activity was measured by an *in vivo* assay method involving injection of the test material into the dorsal lymph sac of