factor (GPF) extracted from a transplantable mouse tumor, CE 1460. The strain differences appeared to be reflected in both the normal leukocyte range and the response to isotonic saline, parameters from which the strain-specific response to GPF could apparently be predicted. It was postulated that the active principle in GPF may be the same as that present in "leukopoietin" preparations from plasma and tissue sources. Furthermore, it was hypothesized that the greater granulocyte release promoted by GPF, as compared to that promoted by "leukopoietin"-rich preparations, might conceivably be due 1) to species or strain differences in the bioassay systems or 2) to a greater concentration of the active (granulocytosis-promoting) principle and/or a lower concentration of a theoretical granulocyte-release inhibiting substance in the partially purified GPF prepared by our method or 3) to a combination of these factors. These results indicate that genetic (strain and species) differences should be taken into consideration in evaluating the biologic activity of GPF and possibly also of "leukopoietin."

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Received January 3, 1966. P.S.E.B.M., 1966, v121.

Effects of Various Soybean Products on Flatulence in the Adult Man.* (31014)

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The potential of soybeans as a source of vegetable protein for human consumption has long been recognized(1-3). Although remarkable advances have been made in developing the uses of soybeans, there still remains a flatus-producing factor which must be identified and eventually removed so that the soybean food products available will become acceptable for human consumption. In our investigation, various soybean products were tested for their capacity to produce flatulence, the results of which are reported here.

* Work done in part under contract with U. S. Dept. of Agriculture and authorized by the Research and Marketing Act. The contract was supervised by the Northern Utilization Research and Development Division, Agri. Research Service, Peoria, Ill.

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Materials and methods. Four adult males served as subjects for the experiments. Each experiment lasted 6 days during which time the subject consumed at each meal a measured amount of muffins and mush containing known amounts of wheat flour, soybean meal, and corn meal. This diet was supplemented with specific amounts of hamburger, skim milk, and fruits in the form of orange juice, bananas, and canned pears. Noncaloric syrup was used on the muffins and soy-corn mush at each meal. Adequate vitamin supplements were also taken each day. The caloric content of the diet was 2334 calories per day, and the total amount of soybean products (Fig. 1) consumed each day was 146 g. For purposes of comparison, soybean meal was replaced in some experiments with ground navy bean meal. Previous to use, the soy-

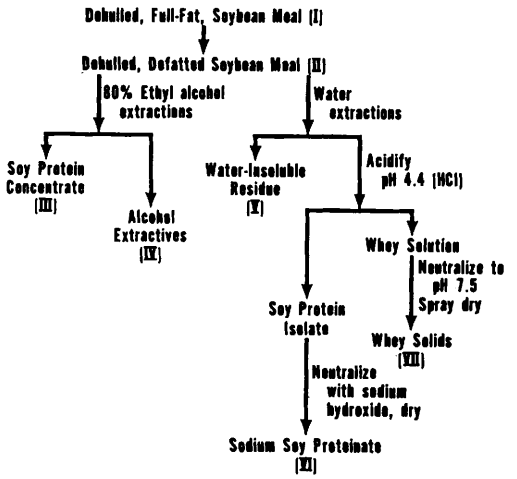

FIG. 1. Schematic outline showing preparation of soybean products used in testing for flatulence.

bean products were treated with live steam at 100°C for 40 minutes to inactivate antinutritional factors(4). This treatment with live steam is referred to as toasting.

Effects of the different soybean meal products on flatulence were usually recorded on the third and fifth days of each 6-day experimental period. The flatus was collected by inserting a well-lubricated catheter through the anus and 15 cm into the rectum. The catheter was perforated with a number of 1-cm holes for 7 cm back from the tip. The flatus was collected by attaching the open end of the catheter, with tube attachments, to previously calibrated cylinders containing a solution of saturated sodium sulfate in 5% sulfuric acid. This displaceable fluid was used to prevent the gases from going into solution.

The subjects were always in a reclining position on an adjustable cot during the collection period, which lasted for 2 hours after lunch and 2 hours after dinner; a total of 8 hours of flatus collection time was made on each subject during the experimental period.

Preliminary testing was carried out with a canned commercial product consisting of soybeans cooked in tomato sauce. The product was consumed in rather large quantities at the time of the experiment to assure detection of any flatulent-producing factor in the different individuals tested. Although this preparation did stimulate flatus production (average of 89 cc per hour), the distaste and sensation of nausea reported by all the subjects made the product, at least in the amounts consumed, practically intolerable.

To ascertain whether or not the hulls and high fat concentration of whole soybeans was in any way responsible for the distaste and nausea observed, an equivalent amount (366 g dry weight) of dehulled, defatted soybean meal was consumed in the form of a cooked muffin. Soybean meal, accounting for nearly 72% of whole soybeans, produced even more flatulence (average of 190 cc per hour) and did not relieve the undesirable symptoms of nausea, intestinal cramps, and, in some cases, vomiting. A prolonged pungent aftertaste, observed while consuming the whole soybeans, also persisted.

To continue with the program, it was necessary to decrease the daily consumption of soybean meal to 146 g and then to have that amount consumed in the form of a soybean meal-wheat flour muffin and a soy-corn meal mush. This concentration could be tolerated without complaint and served as a baseline for all future testing. The soybean products tested for flatulence production are listed and identified in Fig. 1. To determine whether or not the flatus-producing factor was in the soybean protein or in the extractives, a number of tests were made on full-fat and defatted soybean meal and other products prepared by either alcohol or water extraction from dehulled, defatted soybean meal (Fig. 1). When dehulled, defatted soybean meal was extracted several times with 80% ethyl alcohol, a soy protein concentrate (III) containing 72% protein (N $\times$ 6.25) was produced. Yields of about 20% were obtained. The alcohol extractives (IV) are a mixture of low molecular-weight constituents and contain very little, if any, protein.

The water-insoluble residue (V) was prepared by extracting dehulled, defatted soybean meal twice with water by using a water: meal ratio of 10:1 and 5:1 at pH 7.2. The residue contains approximately 75% high molecular-weight carbohydrate material and 25% protein. After centrifugation, the extract was acidified to pH 4.4, and again centrifuged; the precipitated soy protein isolate was neutralized with NaOH and dried. Com-

TABLE I. Effects of Various Soybean Products on Flatus Production in Man. Each experiment represents average data on 4 subjects.

Exp group	Soybean product	Schematic number (Fig. 1)	Amt consumed/day (dry wt, g) Amt of each product	Total	Avg flatus collected (cc/hr)
Group A	Dehulled, full-fat soybean meal	I		146	30
	Dehulled, defatted soybean meal	II		146	71
Group B—Series 1	Soy protein concentrate	III		146	36
	" " "	III	137	146	58
	Alcohol extractives	IV	9		
	Soy protein concentrate	III	128	146	138
	Alcohol extractives	IV	18		
Series 2	Sodium soy proteinate	VI		146	2
	" " "	VI	119	146	21
	Alcohol extractives	IV	27		
	Sodium soy proteinate	VI	92	146	39
	Alcohol extractives	IV	54		
	Sodium soy proteinate	VI	97	146	52
	Whey solids	VII	49		
Series 3	Water-insoluble residue	V		146	13
Group C	Basal diet (no beans)			146	14
	Navy bean meal				179

mercially, this product is referred to as sodium soy proteinate (VI), a product that is approximately 98% protein on a moisture-free, ash-free basis. The whey solution was neutralized to pH 7.5 and spray dried. The resulting whey solids (VII) contained nearly all the low molecular-weight constituents of the meal. The whey solids, obtained in yields of about 30%, had a protein content of 13%. Commercial food-grade preparations, corresponding to soybean meal, soy protein concentrate, and sodium soy proteinate, were also tested.

Experiments were also made with diets containing no soybean products to serve as a control. Also, a test of a toasted navy bean meal product as a flatulence producer was carried out for comparative purposes.

Results and discussion. As tabulated in Table I, the results obtained were divided into 3 different groups: Group A specifically pertained to the effects of dehulled soybean products with and without fat on flatus production; Group B was obtained with meal fractions prepared by both alcohol and water extraction; and Group C, which can be thought of as a control to the soybean products, was a diet in which no beans were consumed as well as one in which a toasted navy bean meal was tested for its effectiveness as a flatulence producer.

Series 1 in Group B represents the effects of the addition of different amounts of the alcohol extractives to a soy protein concentrate diet; Series 2, the effects of still greater amounts of the alcohol extractives or whey solids added to a 98% sodium soy proteinate; and Series 3, the effects of the water-insoluble residue fraction as a flatus-producing substance. It must also be pointed out that these must be considered as pilot experiments because only 4 adult male subjects were used in each experiment, and often rather wide variations occurred in the volumes of flatus collected from individual subjects. Examination of the data in Table I, Group A, shows that when 146 g of dehulled, defatted soybean meal was consumed, average flatus production (71 cc per hour) was more than twice that observed with similar amounts (by weight) of dehulled, full-fat soybean meal (30 cc per hour). (The actual percentage decrease was 43%.) As already mentioned, this same type of relation existed in the preliminary experiments performed when a comparison was made between the consumption of

366 g of dehulled, defatted soybean meal and a similar amount of whole soybeans (dry-weight basis) in tomato sauce, the calculated percentage decrease being 47%. Since the fat concentration in dehulled, full-fat soybean meal is approximately 22%, these results demonstrate rather clearly that the flatus-producing factor in soybeans does not reside in the oil or hull fraction.

As shown in Table I, when the protein content of the soy products was increased from a normal value of about 50% for dehulled, defatted soybean meal to 72% and 98% for soy protein concentrate and sodium soy proteinate, respectively, there was a progressive, but marked, decrease in flatus volume at the same level of consumption. For example, average flatus obtained with 146 g of soybean meal was 71 cc per hour. Flatus production following the consumption of soy protein concentrate and proteinate was reduced to average values of 36 cc per hour and 2 cc per hour, respectively. These results indicate that the flatulence factor is in both the alcohol extractives and whey solids fractions described in Fig. 1.

Results with alcohol extractives in diets containing soy protein concentrate are summarized in Table I (Group B, Series 1). Replacing the protein concentrate with 9 and 18 g of alcohol extractives increased flatus production 1.6- and 3.9-fold, respectively. When comparing these results with those obtained with dehulled, defatted soybean meal in Group A, 18 g of extractives produced twice as much flatus as 29 g extractives in the form of soybean meal, based on a 20% yield of extractives.

In a second series of experiments sodium soy proteinate inhibited markedly the flatulent effect of alcohol extractives (Table I, Group B, Series 2), even when consumed at both the 27- and 54-g level. The flatulent-inhibitory effect of soy proteinate becomes particularly evident when the results of Series 1 and 2 of Group B are compared. Sodium soy proteinate was also effective in inhibiting markedly the flatus-producing ability of whey solids. In other experiments not reported, whey solids produced more flatus compared with alcohol extractives on an equal weight basis. Food-grade, sodium caseinate in similar experiments inhibited soybean flatulence to the same extent as isolated soybean protein.

The water-insoluble residue fraction (Table I, Group B, Series 3) when consumed at the 146-g-per-day level caused an average flatus production of 13 cc per hour and a flatus volume that was no different from that observed when no beans were consumed in the daily diet (Group C). (This suggests that the flatus-producing factor is absent.)

The results of the experiments reported in Group B suggest two things: First, that the flatus-producing factor does not reside in either the protein (VI, Fig. 1) or in the high molecular-weight polysaccharide fraction (V) and that only the low molecular-weight meal constituents produce flatus. Secondly, upon comparing the results observed when soy protein concentrate and sodium soy proteinate are consumed, there appears a possibility that the protein might in some way have an inhibiting action on the flatus-producing mechanism. Research is now in progress to test the nature of the relationships of these factors.

For the sake of comparison, it is noteworthy that when dehulled, defatted soybean meal is consumed in amounts of 146 g per day the average flatus volume is 71 cc per hour, whereas when similar quantities of navy bean meal are consumed, the average flatus volume is 179 cc per hour (Table I, Group C). This increase indicates that navy beans contain the flatus-producing factor in significantly higher quantities than do soybeans.

Conclusions. In 4 human male subjects, kept on a carefully controlled diet to which various fractions of soybean meal were added, the flatus-producing factor in soybeans was concentrated primarily in the low molecular-weight constituents. These experiments also showed that the soybean hulls, fat, water-insoluble polysaccharides, and protein are not associated with flatulence production to any significant degree. Caseinate and soybean proteinate appear to inhibit flatulence. When equivalent amounts of navy bean meal were

consumed, flatus volumes increased 2.52 times that following the consumption of dehulled, defatted soybean meal.

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Received January 3, 1966. P.S.E.B.M., 1966, v121.

Effect of Estrogen on Mammary Gland Growth of Immature Female Rats.* (31015)

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Previous studies of mammary gland growth in the rat using deoxyribonucleic acid as an index have involved sexually mature females during pregnancy(1,2,3,5), and ovariectomized animals stimulated with estrogen and progesterone for 19 days(4). Earlier studies of mammary gland growth in immature rats were concerned with visual examination by means of whole mounts of the glands from birth to sexual maturity(6,7,8). During this period the rudimentary duct system develops extensively into the fatty pad. It has been suggested that duct growth is at first isometric, *i.e.*, the same rate as body growth; but after sexual maturity, duct growth is allometric, *i.e.*, more rapid than body growth(9-11).

The present study was designed to determine the rate of growth of the duct system of young normal female rats compared to a similar group administered the estrogenic hormone as measured by DNA.

Materials and methods. Twenty-day-old female rats were divided into 2 groups. One group, serving as controls, received 0.2 ml of sesame oil, whereas each animal of the second group received 1 μg of estradiol benzoate (EB) in 0.2 ml of sesame oil daily. Animals of each group were sacrificed 20, 40 and 60 days after the first injection. The initial mean body weights of the 2 groups were similar, about 50 g. They were maintained on Purina Lab Chow with tap water *ad libitum* in a constant environmental temperature of 78 $\pm$ 1°F. Six abdominal-inguinal mammary glands were collected on ice from each animal and the DNA was estimated by the method previously described(1,6).

Results. The mean DNA/100 g body weight (BW) of the control female mammary glands at 40 days was 2.60 $\pm$ 0.16 mg, at 60 days 3.42 $\pm$ 0.12 mg, and at 80 days 3.62 $\pm$ 0.26 mg (Table I).

The daily injection of 1 μg EB for 20 days resulted in no difference in the mean body weights of the treated and control groups. However, after 40 and 60 days of EB a decrease of 15 and 39 g in mean body weights respectively was observed, compared to the controls.

The mean DNA/100 g body weight of the treated group at 40 days was 2.81 $\pm$ 0.14 mg, significantly higher ($P < 0.001$) than its control; at 60 days 3.45 $\pm$ 0.15 mg; and at 80 days 3.0 $\pm$ 0.18 mg, about 20.6% less than the corresponding control ($0.02 > P < 0.01$).

Discussion. Based upon the visual observation of duct growth in immature female rats, it has been suggested that early duct growth is isometric in relation to body weight gain until puberty, then duct growth is stimulated at a greater rate by recurring estrous cycles(9,10). Since this strain of rats reaches puberty at between 30 and 40 days, the mean control value at 40 days would represent isometric growth. The increased growth of the duct system of the EB injected group indicates that the duct system is susceptible to allometric growth at this time. At 60 days of age, the control and experimental groups showed the same DNA/

* Contribution from Mo. Agr. Exp. Sta. Journal Series 3042. Approved by Director. This investigation was supported in part by a grant from USPHS.