Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 602.

2. Weil, M. H., Spink, W. W., *J. Lab. and Clin. Med.*, 1957, v50, 501.

3. Zahl, P. A., Hutner, S. H., Cooper, F. S., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 137.

4. Thomas, L., *Ann. Rev. Physiol.*, 1954, v16, 467.

5. Thomas, L., Good, R. A., *J. Exp. Med.*, 1952, v96, 605.

6. Witebsky, E., Neter, E., *J. Immunol.*, 1936, v30, 471.

7. Meyer, M. W., *Fed. Proc.*, 1960, v19, 102.

8. Haddy, F. J., Richards, A. C., Alden, J. L., Visscher, M. B., *Am. J. Physiol.*, 1954, v176, 355.

9. Stish, R. J., MacLean, L. D., Visscher, M. B., *J. Applied Physiol.*, 1956, v9, 297.

10. Meyer, M. W., Ph.D. Thesis, University of Minnesota, 1961.

11. Johnson, P. E., Selkurt, E. E., *Physiologist*, 1958, v1, 38.

12. Aust, J. B., Johnson, J. A., Visscher, M. B., *Surg. Forum*, Proc. 43rd Clin. Congress, 1958, v8, 8.

13. Meyer, M. W., Visscher, M. B., *Am. J. Physiol.*, 1962, v202, 913.

14. Hinshaw, L. B., Vick, J. A., Carlson, C. H., Fan, Y.-L., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 379.

15. Feldberg, W., *Histamine*, Ciba Foundation Symposium, 1956, p4-13.

Received February 26, 1962. P.S.E.B.M., 1962, v110.

Effects of Added Casein on Goitrogenic Action of Different Dietary Levels of Soybeans.* (27535)

FREDERIC W. NORDSIEK† (Introduced by W. H. Sebrell, Jr.)
Institute of Nutrition Sciences, Columbia University, New York City

In at least 14 papers, from 1933 onward, it has been reported that diets containing soybeans (*Glycine max*) or soybean products cause enlargement of the thyroid gland in the rat, chick, guinea pig, rabbit or human. Greer (1,2) has reviewed many of these findings, and has suggested that soybean diets may be goitrogenic simply because they have a low iodine content. Some of the soybean diets reported as goitrogenic contained only a small proportion of beans. Hence in these studies it seems possible that the goitrogenic effect of the beans was not manifested, with the result that the thyroid enlargement seen could have been due solely to iodine deficiency. To shed light on these questions, one of the experiments in the present study was designed to measure and compare the goitrogenic effects of low and high dietary levels of soybeans.

An observation on which there has been unanimity is that heating of soybeans reduces or obliterates their goitrogenic property(3). Moreover, the best studied of the known toxic factors of the soybean, the trypsin inhibitor, is similarly reported to be heat labile(4). This similarity raises a question as to whether the two effects might have the same basis, and has suggested the remaining experiments reported here. These experiments were designed to determine whether protein supplementation of raw soybeans, as is known to reduce or eliminate the growth-retarding effect of the soybean trypsin inhibitor(4), has an effect on the goitrogenic property of soybeans.

Materials and methods. The soybeans used were obtained from an oil processing plant (courtesy of Dr. H. W. Howard, The Borden Co.). The beans, of a small, yellow, black hilum variety, were ground in the laboratory, with care to avoid frictional heating. Soybean meal made from the same lot of beans was also obtained. This meal is the residue from fat extraction of soybeans, after which the meal is "toasted" and ground.

The diets designated 35 and 35CAS (Table I) contained 35% by weight of raw soybeans; those designated 60 and 60CAS contained 60% of raw soybeans. To equate the fat and

* This investigation was supported by grants from Nat. Inst. of Health and the Nutrition Foundation.

† Present address, Memorial Sloan-Kettering Cancer Center, New York City.

TABLE I. Effects on Thyroid Gland Parameters of Feeding Soybean Diets *ad Libitum* to Male Sprague-Dawley Rats for 7 Weeks Starting When Weaned at 3 Weeks of Age with Weights Averaging 48 g. Avg values, 10 rats/group.

Diet code	60MEAL*	35	35CAS	60	60CAS
Iodine in diet, μg/g	.005	.005	.003	.003	.003
Food consumed, g	601	460	637	603	590
Wt gained, g	194	95	208	165	187
Thyroid wt, mg/100 g					
Fresh	19.9	18.8	17.1	27.4	20.8
Stand. dev.	4.0	3.3	4.1	6.9	2.9
Dry	4.31	5.16	4.06	6.05	4.85
Stand. dev.	.95	.78	.99	1.23	.73
Iodine in pooled thyroids, % of dry wt	.0063	.0150	.0180	.0033	.0110

* Control.

crude fiber levels of these diets, 5% of corn oil (Mazola, Corn Products Refining Co.) and 6% of cellulose (Alphacel, Nutritional Biochemicals Corp.) were included in diets 35 and 35CAS.

Casein (Purified, Nutritional Biochemicals Corp.) was included in 2 of the diets; 17% in diet 35CAS and 15% in diet 60CAS. All diets included 4% of U.S.P. XIII No. 2 salt mixture (which contains no iodine salts) and 2.2% of a complete vitamin mixture triturated in glucose (Diet Fortification Mixture, Nutritional Biochemicals Corp.). The remainder of each diet was made up of corn starch (Argo, Corn Products Refining Co.).

As Table I indicates, the control diet was the diet designated 60MEAL. This diet differed from diet 60 only in that it contained, in place of 60% of raw soybeans, 49.6% of soybean meal plus 10.4% of corn oil to restore the fat level to that of whole soybeans. Extraction of this meal had subjected it to approximately 75°C for one-half hour, after which it was subjected to moist heat at 110° for 25 minutes. There is a consensus in prior publications(3,5,6) that this much heating destroys the positive goitrogenic property of soybeans. Hence diet 60MEAL may be considered to have no goitrogenic effect other than that produced by its low iodine content. To verify this assumption, an experiment (not reported here for the sake of brevity) was conducted with the same experimental design as described in the present report, comparing thyroid weights of rats fed diet 60-MEAL with those of rats fed the Remington diet(7), a standard formulation known to produce an uncomplicated iodine deficiency goiter(8). The fresh and dry thyroid weights of animals fed one or the other of these diets did not differ significantly.

All 5 diets were found to contain 0.003 or 0.005 μg of iodine per g, and hence provided less than 2% of the normal rat's iodine requirement (Table I). Iodine in diets and in thyroid glands was determined by the method of Chaney(9).

Male albino rats, descendants of the Sprague-Dawley strain (from S & K Animal Co., Jamesburg, N. J.) were weaned at 3 weeks of age when their body weights ranged from 41 to 55 g and averaged 48 g. Ten rats were assigned, by statistical randomization, to each experimental group. The animals were housed in separate metal cages with wire mesh bottoms, were provided *ad libitum* with water from an ion exchange column (Barnstead Bantam) and were fed one of the 5 diets *ad libitum* for 7 weeks, while records of individual food intake and body weights were kept. Then the animals were killed with ether and the fresh weights of their thyroid glands determined individually. Thyroid gland weights were expressed in terms of mg per 100 g of total weight (Table I) because the weight of the thyroid gland in this strain and sex of rats has been found to bear an approximately linear relationship to total weight, at weights ranging from 87 to 328 g (10).

Weight of dry matter in each gland was determined by drying to constant weight in a

convection current oven at 105°C. The thyroid glands of the 10 animals in each experimental group were finally pooled to provide enough material for iodine assays.

Results. The animals fed diet 60 (60% raw soybeans) had significantly heavier thyroid glands than those fed the control diet 60MEAL, whether judged by fresh weight of the glands ($P = 0.01$) or by dry weight ($P = 0.005$). The thyroid glands of animals fed diet 60 also had a lower iodine content than the controls.

The thyroid glands of animals fed diet 35 (35% raw soybeans) did not differ significantly from those of control animals in fresh weight, but were significantly heavier in dry weight ($P = 0.05$). Iodine assay of the thyroid glands of animals fed diet 35 gave the anomalous result of a higher iodine level than the controls.

Adding casein to diets containing 35% or 60% of raw soybeans increased the rate of body weight gain. Such additions of casein also entirely obliterated the thyroid gland effects of the soybean diets. Thus the thyroid glands of animals fed diet 60CAS were significantly lighter than those of animals fed diet 60 ($P = 0.02$, fresh or dry weights); and not significantly different from those of animals fed the control diet 60MEAL. As the effect of 35% of raw soybeans was seen only in increased dry weight of the thyroid gland of animals fed diet 35, it is not surprising to note that the sole significant effect of adding casein to a 35% soybean diet was on this parameter. Thus fresh thyroid weights of animals fed diets 35 and 35CAS did not differ significantly, whereas dry thyroid weights of animals fed diet 35CAS were significantly lighter than those of animals fed diet 35 ($P = 0.02$). Thyroid gland weights of animals fed diet 35CAS did not differ significantly from those of animals fed the control diet 60MEAL. Animals fed the diets with casein had thyroid glands containing more iodine than those of animals fed the corresponding soybean level without casein.

Discussion. The minimum proportion of raw soybeans in the diet capable of producing a goitrogenic effect might be expected to depend upon the goitrogenic potency of the particular beans used, as well as perhaps upon the species studied and the nature of the remaining dietary constituents. Sharpless *et al.* (5) reported that rats fed a 35% unprocessed soybean flour diet had thyroid glands about half as big, containing about twice as much iodine, as those fed a diet containing 75% of this soybean material. Halverson *et al.*(6) found no goitrogenic effect when a 10% raw soybean diet was fed to rats, but observed goiters when a 30% soybean diet was fed. Wilgus *et al.*(11) used chicks, considered to be more sensitive to goitrogenic influences than rats; but they fed expeller process soybean oil meal which would be expected to have low goitrogenic potency as it is heated vigorously during manufacture. These investigators could offer no explanation for their observation that 12% or 30% of meal in the ration produced equal enlargement of the thyroid gland, whereas 60% produced goiters twice as large. Their diets were reported to contain less than 0.1 μg per g of iodine. Thus it seems likely that their 12% or 30% soybean meal diets produced a simple iodine deficiency goiter, whereas 60% of soybean meal produced a positive goitrogenic effect.

These previous workers measured goitrogenesis solely by fresh weight of the thyroid gland. Hence it is of interest that in the present study the effect of 35% raw soybeans in the diet was apparent only in increased dry weight of the thyroid gland, whereas 60% of raw soybeans caused increases in both fresh and dry weights.

Casein has generally been reported to have an antigoitrogenic effect(12). This property has been held variously to be due to iodine in the casein(7) or not to be due to iodine(13). The antigoitrogenic effect of casein in soybean diets is clearly seen in the present experiments, where it appears not to be due to iodine introduced by the casein, as diets 35CAS and 60CAS were assayed to have iodine contents as low as, or lower than, diets 35 and 60.

The sole protein in diets 35 and 60 was raw soybean protein, known to have low biological value(4). Hence the addition of casein, a protein having high biological value, may have served to reduce the goitrogenic ef-

fect by providing proteins to aid humoral transport of thyroid hormones(14) or perhaps to aid in reabsorption of thyroid hormones from the gut in the enterohepatic cycle(15). These possibilities raise a question as to whether the soybean goitrogenic effect may be related to, or even identical with, the soybean antitrypsin effect.

Summary. The goitrogenic effect of raw soybeans was seen in significant increases of both fresh and dry weights of thyroid glands when a diet containing 60% of beans was fed to rats, and a significant increase in the dry weight only of the gland when a diet containing 35% of raw soybeans was fed. The goitrogenic effect of 35% or 60% of raw soybeans in rat diets was obliterated by adding casein to the diets. This result was not attributable to iodine in the casein.

1. Greer, M. A., *Physiol. Rev.,* 1950, v30, 513.
2. ———, *Borden's Rev. of Nutrition Res.,* 1960. v21, 61.
3. Block, R. J., Mandl, R., Howard, H. W., Bauer, C. D., Anderson, D. W., *Arch. Biochem. Biophys.,* 1961, v93, 15.
4. Markley, K. S., ed., *Soybeans and Soybean Products,* Interscience, N. Y., 1950, 1951.
5. Sharpless, G. R., Pearsons, J., Prato, G. S., *J. Nutrition,* 1939, v17, 545.
6. Halverson, H. W., Zepplin, M., Hart, E. B., *J. Nutrition,* 1949, v38, 115.
7. Remington, R. E., *J. Nutrition,* 1937, v13, 223.
8. Money, W. L., Rall, J. E., Rawson, R. W., *J. Clin. Endo. and Metab.,* 1952, v12, 1495.
9. Chaney, A. L., *Anal. Chem.,* 1950, v22, 939.
10. Nordsiek, F. W., Doctoral Dissertation, Columbia Univ., 1961.
11. Wilgus, H. S., Jr., Gassner, F. X., Patton, A. R., Gustavson, R. G., *J. Nutrition,* 1941, v22, 43.
12. Van Middlesworth, L., *Science,* 1955, v121, 871.
13. McCarrison, R., *Brit. Med. J.,* 1933, v2, 671.
14. Robbins, J., Rall, J. E., *Physiol. Rev.,* 1960, v40, 415.
15. Van Middlesworth, L., *Endocrinology,* 1957, v61, 570.

Received March 21, 1962. P.S.E.B.M., 1962, v110.

O-Aminophenol: A Urinary Product of Tryptophan Metabolism in the Human.* (27536)

L. V. HANKES, M. SCHMAELER AND K. RAI
Medical Research Center, Brookhaven National Laboratory, Upton, L. I., N. Y.

Recently, interest in the relationship of diseases to the metabolites of tryptophan has intensified(1). 3-Hydroxy-anthranilic acid and 3-hydroxykynurenine, known tryptophan metabolites, have demonstrated carcinogenic behavior in mice(2) and appear in elevated quantities in the urine of patients with bladder tumors, anemias and leukemias(1). More recently, *o*-aminophenol has been identified in the urine of humans(3), and appears in elevated quantities in the urine of bladder tumor patients(3). However, whether tryptophan was the precursor of this urinary aminophenol is not known. We present here evidence of the conversion of tryptophan to *o*-aminophenol (Fig. 1) in the human.

Experimental. A female patient with multiple-myeloma was given orally in a gelatin capsule 39.376 mg of DL-Tryptophan-7a-C^{14}† containing 51.3817 μc of C^{14}. The patient's respiratory $C^{14}O_2$ was collected for 12 hours. Urine was collected for a 24-hour period under toluene in a refrigerated dark bottle. Aliquots of urine were analyzed for C^{14} content (4), and 100 ml of urine was used for isolation of the *o*-aminophenol.

A column of Amberlite IR-45-ag‡ resin 22 mm × 34 cm was used for the isolation. The

* This research was supported by U. S. Atomic Energy Commission.

† This DL-Tryptophan-7a-C^{14} was synthesized in the laboratory of Dr. L. M. Henderson, Oklahoma State Univ., Stillwater.

‡ The authors wish to thank Rohm & Haas Co., Philadelphia, Pa. for a generous supply of this exchange resin.