STUDIES ON SOYBEAN FACTORS WHICH PRODUCED GOITER IN RATS

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Summary The goitrogenicities of various preparations obtained from soybean were studied using rats. Thyroid weight, $^{131}$I uptake and the ratio of $^{131}$I-monoiodotyrosine to $^{131}$I-diiodotyrosine of the gland in rats fed the preparation was measured to determine the activity of these preparations. It was resulted that the group of rats receiving the curd had the most hypertrophied thyroid, the enlargement being equal to that in the group of these receiving soybean in the 8 weeks' feeding experiment. No difference on the content of iodine were found with the various diet preparations. The proteolytic digestion of soybean curd did not eliminate the goitrogenic property, thus, the goitrogen seems not to be a protein or peptide-like substance.

Keywords thyroid function, soybean element, goitrogen, goitrogenic factor, thyroid enlargement

Enlargement of the thyroid in rats fed soybeans was discovered by McCARRISON (1), who found that raw soybeans produced goiter. Subsequently, SHARPLESS et al. (2) showed that rats fed soybean meal required two times more dietary iodine than control rats to prevent goiter.

VAN MIDDLESWORTH (3) found that soybean flour caused high thyroxine excretion rates which were 2 to 20 times greater than with a control diet. Furthermore, the increase in fecal thyroxine excretion by soybean flour diet was confirmed by BECK (4). He concluded that the increased fecal thyroxine might be due to some upset of the absorption process, perhaps, of accelerated transport or a specific effect of a soybean flour factor on mucosal function. The goitrogenic factor, however, has not yet been indentified.

KOYANAGI and OIKAWA (5) reported that the congealed soybean curd, which comprises the majority of the water-soluble protein, affected the thyroid more severely than the original soybean. The present work was designed to study the

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MATERIALS AND METHODS

Experimental animals. Female albino rats of Wistar strain weighing about 60–70 g were used. The rats were then divided into groups of six animals each and fed the experimental diets for 8 weeks. Distilled water and the experimental rations were fed ad libitum. The composition of the iodine-deficient basal diet is shown in Table 1. The products made from soybeans were added in place of starch in the basal diet and the animals were fed a variety of diets.

Heading measurements. The heading measurements were as follows: thyroid gland weight mg per 100 g body weight; uptake of radioactive sodium iodide; and the distribution of radioactive iodoamino acids in thyroid, in particular the ratio of radioactive monoiodotyrosine to diiodotyrosine. For the determination of the uptake and the distribution, 10 μCi of 131I-Na was injected intraperitoneally into the experimental animals and after 24 hours the rats were killed by exsanguination under anesthesia with ether. The thyroids were then removed and weighed. The values of 131I-uptake were obtained by comparison with an appropriate standard in a well-type scintillation counter. Thyroids were digested with protease and the hydrolysate was separated by means of thin layer chromatography (6); the chromatogram was exposed to X-ray film for the radioautography. The ratio of monoiodotyrosine to diiodotyrosine is a good indicator of thyroid enlargement or function (7, 8). For histological observation, an enlarged thyroid was mounted in paraffin, stained with hematoxylin-eosin and examined microscopically.

Preparation of curd, whey and water-insoluble residue from soybean. In a previous communication (5), it was suggested that the goitrogenic factors were concentrated in the water-soluble fraction. Thus, in this experiment, fractionation of the water-soluble fraction of soybeans was attempted. The fractionating method is shown in Fig. 1. Two and a half kg of raw soybeans were soaked in

| Table 1. Composition of low iodine basal diet. |
|-----------------------------------------------|
| Wheat gluten          | 30 g/100 g diet |
| Potato starch         | 61.1           |
| Soybean oil           | 2              |
| Salt mixture<sup>a</sup> | 4              |
| Vitamin mixture<sup>b</sup> | 1 (water-soluble) |
| (fat-soluble)         |                |

<sup>a</sup> Harper's salt mixture (excluding iodide).
<sup>b</sup> Harper's vitamin mixture.
water overnight at room temperature, ground with a mixer and heated to boiling. The boiling was continued for 10 min, then the mixture was filtered through a cheese cloth in a large Buchner funnel. The water-extractable materials were separated as soybean milk from the residue. The residue was washed with hot water and freeze dried. Yield: 330 g.

Precipitation of curd from the soybean milk was then accomplished by adding a small amount of calcium chloride solution. The curd was separated from the whey by filtration on cheese cloth and then pressed to remove any residual whey. The cake was frozen at -40°C and freeze dried. Yield: 1,850 g.

The whey protein fraction was prepared by concentrating the filtrate with a flash evaporater. The resulting thick liquid contained 220 g of solid.

The final materials were added to the basal diet and were fed to rats for 8 weeks. Furthermore, for the purpose of observation of the preventive effect of goiter, iodine was added stepwise in experimental diets.

Digestion of soybean curd with proteolytic enzyme. The water-soluble fraction of soybean was prepared by the same method as described above. The pH was adjusted to 7.0 with hydrochloric acid and protease (pronase-p, Kaken Kagaku Co.) was added. The digestion was conducted at 30°C. After 24 hours the hydrolysate was separated by vacuum distillation and dried in a vacuum desiccator.

RESULTS

The distribution of goitrogenic factor among curd, whey and water-insoluble residue prepared from soybeans

In this study gluten was used routinely as a standard for comparison. Since the protein contents of the fractions were unequal, adjustments were made by adding gluten to bring the protein content of the diets of all groups to 21 percent.
Table 2. Effect of various fractions of soybeans on the thyroid weight and $^{131}$I uptake.

| Groups                  | Final body wt. | Thyroid mg/100 g body wt. | $^{131}$I uptake for 24 hr |
|-------------------------|----------------|---------------------------|---------------------------|
| 30% gluten              | 223 g          | 10.7±0.7                  | 67%                       |
| 40% soybean             | 198            | 26.3±4.0*                 | 48                        |
| 40% curd                | 214            | 26.9±2.9*                 | 40                        |
| 7% residue + gluten     | 216            | 16.9±1.9                  | 39                        |
| 5% whey + gluten        | 198            | 9.5±0.9                   | 52                        |
| 30% gluten + iodine supplement | 206  | 6.7±1.1                | 30                        |

Each value is the mean ±SE of six animals.
* Significantly different from 30% gluten group at $p<0.05$.

From the results of the 8 weeks' feeding experiment summarized in Table 2, the group of rats receiving the curd had the most hypertrophied thyroid, the enlargement being equal to that in the group of rats receiving soybeans. The rats fed residue showed a slightly hypertrophied thyroid, whereas those fed whey showed much less enlargement. The natural content of iodine in the experimental diets was about 6–7 μg per kg diet. No difference among the diets could be shown.

*The effects of iodine supplements in diets*

Goiter was prevented relatively easily when iodine was added to the diet. Iodine was added stepwise and the effect of prevention was examined. The results of these experiments are summarized in Table 3. When 20 μg of iodine per 100 g diet were added, goiter in the rats of the gluten and whey groups was completely prevented. However, the development of goiter in the rats fed soybean, soybean curd or residue was not completely prevented by such an addition (Photos 1, 2, 3).

Photo 1. Histology of the thyroid of a rat fed the usual commercial diet for 2 months. H. & E. 10×10.

Photo 2. Histology of the enlarged thyroid of a rat fed soybean curd under iodine-deficient conditions for 2 months. Note marked increase of follicular and parafollicular elements in hyperplastic tissues of the gland. H. & E. 10×10.

Photo 3. Histology of the thyroid, which was not enlarged, of a rat fed soybean curd supplemented with iodine (20 μg/100 g diet) for 2 months. The size and distribution of the follicles appeared normal, however considerable increase of the parafollicular elements was still noted. H. & E. 10×10.
Table 3. Effect of iodine supplements in diets.

| Groups         | Iodine supplement μg/100 g diet | Thyroid mg/100 g body wt. |
|----------------|---------------------------------|--------------------------|
| 30% gluten     | 0.0                             | 10.7 ± 0.7               |
|                | 5.0                             | 8.4 ± 0.5                |
|                | 10.0                            | 6.5 ± 0.5                |
|                | 20.0                            | 6.7 ± 1.1                |
| 40% soybean    | 0.0                             | 26.4 ± 4.0               |
|                | 5.0                             | 12.7 ± 1.3               |
|                | 10.0                            | 9.4 ± 0.5                |
|                | 20.0                            | 8.9 ± 0.3                |
| 40% curd       | 0.0                             | 26.9 ± 2.9               |
|                | 5.0                             | 10.3 ± 0.7               |
|                | 10.0                            | 8.6 ± 0.3                |
|                | 20.0                            | 9.3 ± 0.8                |
| 7% residue + gluten | 0.0                   | 16.9 ± 1.9               |
|                | 5.0                             | 9.3 ± 0.4                |
|                | 10.0                            | 8.3 ± 0.5                |
|                | 20.0                            | 9.3 ± 0.8                |
| 5% whey + gluten| 0.0                             | 9.5 ± 0.9                |
|                | 5.0                             | 9.1 ± 0.5                |
|                | 10.0                            | 8.3 ± 0.7                |
|                | 20.0                            | 7.2 ± 0.7                |

Each value is the mean ± SE of six animals.

Table 4. Effect of hydrolysate of soybean curd on the thyroid weight, MIT/DIT ratio and $^{131}$I uptake.

| Groups                   | Final body wt. | Thyroid mg/100 g body wt. | MIT/DIT ratio | $^{131}$I uptake for 24 hr |
|--------------------------|----------------|---------------------------|---------------|--------------------------|
| Gluten                   | 131 g          | 21.9 ± 1.7                | 1.9           | 39%                      |
| Soybean curd             | 161            | 27.7 ± 2.9                | 2.7           | 24                       |
| Digested soybean curd    | 169            | 28.5 ± 3.3                | 2.6           | 21                       |

MIT, monoiototyrosine; DIT, diiodotyrosine.
Each value is the mean ± SE of six animals.

**Digestion of soybean curd with proteolytic enzymes**

The water-soluble fraction of soybeans was digested with protease. The animals were fed an experimental diet to which the hydrolysate was added for 8 weeks. The results of these experiments are presented in Table 4. As shown in this table, no reduction of thyroid weight was seen by digestion. The ratio of monoiototyrosine to diiodotyrosine increased more markedly when the rats were fed soybeans and the water-soluble fraction. This ratio did not change following proteolytic digestion.
DISCUSSION

This report supports SHARPLESS' result(2) that rats fed soybeans required twice the dietary iodine to prevent goiter than the control animals. Histological changes in the glands of rats fed soybeans were not always, however, prevented when twice the amount of iodine was added to the diet.

The 24-hour value of $^{131}$I-uptake in the rats of the experimental group was higher than that in the rats fed a normal diet, but the uptake of $^{131}$I in the rats fed soybeans and fractions from soybeans was lower than the control values. In general, the uptake is increased by iodine deficiency, but this is not always the case in severe deficiency, because, in such a case, the value of $^{131}$I-uptake changes with the passage of time and the peak uptake occurs within 24 hours(8).

The results of this experiment support the previous report(5) that the goitrogenic factor is concentrated in the curd. Furthermore, it was shown that the soybean curd did not loose its goitrogenic power due to proteolytic digestion.

It was observed that the thyroid enlargement, $^{131}$I-uptake and the ratio of monoiiodotyrosine to diiodotyrosine in the gland of rats fed soybean curd treated with proteolytic enzymes were similar to the values found in rats fed soybean curd. This fact indicates that the goitrogenic factors of soybean curd are not protein or peptide-like substances and may remain closely associated with the proteins during the isolation procedure.

A further, detailed study is in progress concerning the influence of soybeans on the thyroid of rats.

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