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Presence of a Growth Inhibiting Substance in Raw Soybeans.

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The low nutritional value of raw soybean proteins and the marked improvement on heating have been recognized for some time;[1,2,3] however, the fundamental mechanism by which this change takes place has not been satisfactorily explained. The experimentally demonstrated effect of heating is to make the methionine of the protein nutritionally available. Johnson, Parsons, and Steenbock[4] have shown that this effect is not brought about by increased absorption of the sulfur-containing amino acids but that heating apparently increases the utilization of these amino acids after absorption. Ham and Sandstedt[5] and Bowman[6] have demonstrated the presence of a trypsin inhibitor in the pH 4.2-soluble fraction of unheated soybean flakes, and Ham and Sandstedt suggested its possible significance in relation to the poor biological value of the raw soybean protein. They were not able at that time to demonstrate any growth-inhibiting effect of the fraction containing the trypsin inhibitor.

Recently, while this paper was in the process of review, a report by Ham, Sandstedt, and Mussehl[7] appeared which extended their observations and established a growth-retarding effect on chicks of the pH 4.2-soluble, acetone-insoluble fraction of soybeans. We had obtained similar results with rats, and these results are presented in this report.

In connection with work in progress at the Northern Regional Research Laboratory, dealing with the effects of processing on the food value of soybean proteins, we have been investigating the effects of heating on the growth-supporting properties of the separated acid-soluble (pH 4.2) and acid-insoluble proteins. In this investigation it was found that the crude pH 4.2-insoluble protein still responded to heat treatment by increased biological value. However, when the unheated acid-soluble fraction was added back to the heated acid-insoluble protein, the growth-promoting property was again reduced to that of unheated soybean proteins. It is obvious, therefore, that a growth-inhibiting substance is present in unheated soybean proteins which explains partially at least the nutritional inadequacy of raw soybean proteins.

Litters of albino rats (Sprague Dawley strain) were maintained on a stock diet of Purina Dog Chow Checkers supplemented with cod-liver oil and liver until they were approximately 40 days of age and 90 g per rat in weight. Each litter was then divided equally as far as possible among the experimental groups of 10 rats each and fed the corresponding diets and water *ad libitum*.

The soybean protein fractions were prepared in the following manner: ground hexane-extracted soybean flakes (No. 113) were stirred in a large volume (10 cc per g of flakes) of distilled water, and the mixture was adjusted to a pH of 6.7 with dilute sodium hydroxide. The resulting extract was pressed out in a small cider press and then

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[1] Daniels, A. L., and Nichols, N. B., *J. Biol. Chem.*, 1917, **32**, 91.

[2] Hayward, J. W., Steenbock, H., and Bohstedt, G., *J. Nutrition*, 1936, **11**, 219; **12**, 275.

[3] Hayward, J. W., Halpin, J. G., Holmes, C. E., Bohstedt, G., and Hart, E. B., *Poultry Science*, 1937, **16**, 3.

[4] Johnson, L. M., Parsons, H. T., and Steenbock, H., *J. Nutrition*, 1939, **18**, 423.

[5] Ham, W. E., and Sandstedt, R. M., *J. Biol. Chem.*, 1944, **154**, 505.

[6] Bowman, D. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 139.

[7] Ham, W. E., Sandstedt, R. M., and Mussehl, F. E., *J. Biol. Chem.*, 1945, **161**, 635.

TABLE I.
Effect of Various Soybean Protein Supplements on Average Gain in Weight of Young Rats. (5 Males + 5 Females per Group).

Diet No.	Protein supplement equivalent to 14.6% crude protein	Experiment No. I 35-day test: Avg gain g/rat/day	I 35-day test: Feed-consumption, g/rat/day	I 35-day test: Gain per g protein eaten, g	II 28-day test: Avg gain g/rat/day	II 28-day test: Feed-consumption, g/rat/day	II 28-day test: Gain per g protein eaten, g	III 18-day test: Avg gain g/rat/day	III 18-day test: Feed-consumption, g/rat/day	III 18-day test: Gain per g protein eaten, g
1	Hexane-extracted soybean flakes No. 113	1.06	10.8	0.65	1.19	11.7	0.67			
2	No. 113 steamed	2.20	12.0	1.21	2.80	12.7	1.45			
3	pH 4.2 insoluble soybean protein No. 113A	0.82	9.8	0.55	1.27	11.4	0.73			
4	No. 113A steamed	2.05	12.0	1.13	1.82	11.8	1.02	2.41	12.3	1.29
5	No. 113A " 90% pH 4.2 soluble soybean protein— No. 113B 10%	1.12*	10.8	0.68	0.84	10.7	0.52	1.16	10.6	0.72
6	No. 113A steamed 95 No. 113B 5							1.70	11.7	0.96
7	No. 113A " 90 No. 113B " 10	2.06*	11.3	1.20	2.24	11.5	1.28			
8	No. 113A " 90 No. 113A 10							2.71	12.5	1.42
9	Casein, commercial†	2.93	12.9	1.49	3.22	13.2	1.60	3.57	11.5	2.04
10	Casein + No. 113B (1.5% crude protein)†							2.56	10.4	1.45
11	Casein + No. 113B steamed (1.5% crude protein)†							4.19	11.6	2.12

* Diet 7 was fed for 5 days to the group of rats which received Diet 5 for the first 30 days of the test.
† The data for 9, 10, and 11 (Exp. 3) are comparable, but not comparable with the rest of the data. Younger rats were used in these 3 groups.

centrifuged in a Sharples centrifuge to remove suspended insoluble material. A second extraction of the press cake was made in a similar fashion. The combined extracts were adjusted to pH 4.2 with dilute (1*N*) hydrochloric acid. In Exp. I the pH 4.2-insoluble material (No. 113A) was centrifuged relatively free of filtrate and then dried *in vacuo* from the frozen state. In Exp. II the pH 4.2-insoluble material was redissolved by addition of alkali and reprecipitated at pH 4.2 4 times, after which it was also dried by lyophilization. Sufficient ammonium sulfate was added to the combined supernatant filtrates from the centrifugation to bring the concentration to 2.7 *M*. The resulting precipitate (No. 113B) was separated by centrifugation, dialyzed in Visking casings against distilled water at 1°C until free of ammonium sulfate, and then dried *in vacuo* from the frozen state. An optimum heat treatment of the soybean protein was accomplished by steaming in an autoclave for 30 minutes at atmospheric pressure.

All diets were composed of 50% basal mixture, a percentage of protein supplement equivalent to 14.6% crude protein, and enough of a 50-50 mixture of starch and sucrose to make 100%. Crude protein percentage was calculated by using 6.38 × % *N* for casein and 5.71 × % *N* for the soybean proteins. The basal mixture, making up 50% of each diet, contained (as percent of the complete diet) cottonseed (Wesson) oil 5, U. S. P. cod-liver oil 2,34% tocopherol vegetable oil concentrate 0.05, salt mixture (McCollum's No. 185 + trace elements) 4, corn starch 18.5, sucrose 18.5, brewers' yeast 2, and (as mg per 100 g of the complete diet) choline chloride 50, thiamin chloride 0.2, riboflavin 0.5, pyridoxin 0.2, calcium pantothenate 2.5, and nicotinic acid 1.0.

Table I presents the results obtained on feeding the acid-soluble (pH 4.2) and acid-insoluble proteins in various combinations. The marked growth-retarding effect of the unheated, pH 4.2-soluble, 2.7 *M* ammonium sulfate-insoluble fraction when allowed to replace 10% of the heated pH 4.2-insoluble fraction and the dissipation of the effect on heating, is at once apparent. It is also obvious that the growth inhibitor is concentrated in the 10% of pH 4.2-soluble protein, since the effect of the inhibitor contained in 10% of soluble protein, when introduced in the diet, is greater than that of the inhibitor contained in 10 times the amount of pH 4.2-insoluble protein (compare 3, 4, and 5, Exp. II); also, the inclusion of 10% of unheated pH 4.2-insoluble protein results in no inhibition as compared to that produced by 10% of unheated pH 4.2-soluble protein (compare 4 and 8, Exp. III). The effect of the growth inhibitor is also demonstrated when included in a diet in which casein was the protein component (9, 10, and 11, Exp. III).

The question of the identity or non-identity of the trypsin inhibitor with the growth inhibitor cannot be answered from the present data. The trypsin inhibitor is present in the acid-soluble fraction of soybean proteins, and the 2 may therefore be identical. Since the tryptic inhibitor has recently been isolated in crystalline form by Kunitz,[8] the question can best be answered by inclusion of the isolated substance in the diet. Such studies, together with further characterization of the growth-inhibiting substance, are under way and will be reported at a later date.

Summary. A substance which inhibits the growth-promoting properties of proteins in rats is present in raw soybeans. The substance is non-dialyzable, precipitated by salt, and inactivated by heat. It appears, therefore, to be a protein, which has been shown to be concentrated in the acid-soluble (pH 4.2) fraction of the soybean protein. These results are in agreement with those presented by Ham, Sandstedt, and Mussehl[7] for the chick.

[8] Kunitz, M., *Science*, 1945, **101**, 668.