TABLE IV.
Effect of Salmonella Endotoxin and Insulin on the Glucose Uptake of the Rat Diaphragm.

No. of exp.	Normal, mg/g	+Insulin 1 unit/ml, mg/g	+Toxin 0.1 mg/ml, mg/g	+Toxin-+insulin, mg/g
1	3.44	4.4	2.1	4.0
2	2.91	3.92	1.9	3.1
3	4.89	5.1	3.1	4.6
4	3.94	4.6	0.1	3.7
5	4.95	5.4	3.2	4.4
6	2.81	4.21	2.0	2.6
7	2.5	3.40	0.6	1.6
8	4.7	6.1	2.2	4.8
9	3.9	5.1	1.9	2.8

Glucose uptake is expressed in terms of mg glucose per g of tissue utilized in 2 hours.

similarity does not mean that the mode of action of diphtheria toxin is necessarily the same as that of the endotoxins. It was recently found that the phosphorylation of glucose was inhibited by meningococcal endotoxin.[11] This inhibition, together with the inhibition of the succinoxidase[1] might serve as an explanation of the inhibition of glycogen synthesis.

Summary. Intraperitoneal injection of Salmonella endotoxin completely inhibited *in vivo* glycogen synthesis from glucose in the liver of rats. In liver slices of rabbits, there was less glycogen formed *in vitro* from glucose and pyruvate in the presence of meningococcal endotoxin. The glucose uptake of rat diaphragm *in vitro* was decreased by Salmonella endotoxin and this inhibition of glucose utilization could be diminished by the addition of insulin.

[11] Kun, E., *J. Biol. Chem.*, 1948, **174**, 761.

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Influence of Crude Trypsin Inhibitor on Utilization of Hydrolyzed Protein.

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As a result of the reports of Ham and Sandstedt[1,2] on the presence of a trypsin inhibitor in raw soybeans, there has been considerable interest regarding the possible role that this trypsin inhibitor plays in altering the nutritive value of proteins. A relationship between the well known phenomenon of increased nutritive value of soybean protein resulting from heat treatment and the destruction of trypsin inhibitor has been suggested by several investigators. In fact, an excellent correlation between nutritive value and trypsin inhibitor content was observed in soybean preparations heated under varying conditions by Westfall and Hauge.[3] Melnick, Oser, and Weiss[4] observed that the relative rates of liberation of methionine, lysine and leucine as a result of tryptic digestion were different in raw soybeans and in soybeans in which the trypsin inhibitor had been inactivated by heat. They concluded from this study that the influence of the trypsin inhibitor on nutritive value of the soybean protein was due to an alteration in the time factors of availability of essential amino acids, particularly methionine, in the intestinal tract. Desikachar and De[5] have provided some evidence that does not support the conclusions of Melnick and his associates. They compared the digestibility and biological value of unmodified raw soybeans with papain-digested soybeans to which a water extract of soybeans containing

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

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

[3] Westfall, R. J., and Hauge, S. M., *J. Nutrition*, 1948, **35**, 379.

[4] Melnick, D., Oser, B. L., and Weiss, S., *Science*, 1946, **103**, 326.

[5] Desikachar, H. S. R., and De, S. S., *Science*, 1947, **106**, 422.

the trypsin inhibitor had been added so that the trypsin inhibitor content of both the digested and undigested samples was approximately the same. Both preparations showed the same digestibility and biological value and Desikachar and De concluded that there must be factors separate from the proteolytic inhibitor that affect the nutritive value of the soybean protein. In the work of these investigators the nutritive value of the papain digested soybean before supplementation with trypsin inhibitor was not measured. Therefore, the possibility remains that the addition of trypsin inhibitor to the digested protein did not alter the biological value of the protein. Since this important control was not included in this study it was considered advisable to reinvestigate the effect that soybean trypsin inhibitor has on enzymatically digested protein.

Methods. The hydrolyzed protein chosen for this study was Protolysate.* This hydrolysate represents the pancreatic enzyme digest of casein and other animal proteins. The extent of digestion is not complete since the free amino nitrogen represents approximately 65% of the total nitrogen. However, it is basically a tryptic digest in which hydrolysis has been carried farther by the action of naturally occurring exopeptidases. The trypsin inhibitor preparation was made according to the general directions described by Klose, Hill, and Fevold,[6] and essentially is an ammonium sulfate precipitate of an acid extract of raw soybeans. The trypsin inhibitor activity of this extract was determined by a modification of the method of Charney and Tomarelli[7] and the extract was added to either casein or Protolysate in such amounts that the trypsin inhibitor potency was approximately the same as that of raw soybeans. As an additional control the trypsin inhibitor extract was inactivated by autoclaving at 15 pounds pressure for 15 minutes and was added in the same

TABLE I.
Growth Response and Protein Efficiency Ratio (Grams Gain in Weight per Gram Protein Ingested) of Mice Receiving an Extract of Raw Soybeans Containing Trypsin Inhibitor.

Protein source	% prot. in diet (N × 6.25)	Avg initial wt, g	Avg final wt, g	Avg wt gain, g	Avg food consumed g/10 days	Avg protein consumed g/10 days	Protein efficiency	Relative protein efficiency
Casein	10.29	7.50	12.86	5.36	21.73	2.24	2.39	100
Casein + 1% inhibitor	11.54	7.57	11.46	3.89	21.10	2.43	1.60	67
Protolysate	10.84	7.30	12.01	4.71	20.83	2.26	2.08	87
Protolysate + inhibitor (1%)	11.30	7.26	9.81	2.56	21.51	2.43	1.05	44
Protolysate + inhibitor (1%) autoclaved	11.29	7.47	12.96	5.49	24.90	2.81	1.95	82

* Trademark of a protein hydrolysate preparation purchased from Mead, Johnson and Company.

[6] Klose, A. A., Hill, B., and Fevold, H. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 10.

[7] Charney, J., and Tomarelli, R. M., *J. Biol. Chem.*, 1947, **171**, 501.

amount to the Protolysate.

Diets containing casein or Protolysate as the source of protein were fed *ad libitum* to groups of 7 male albino mice. General details of this type of feeding experiment including diet composition have been described by Bosshardt *et al.*[8] Growth rate and food consumption were measured for 10 days. The addition of the active or autoclaved extract as 1% of the diet increased the protein content of the diets as is shown in Table I.

Results. The results in Table I show that addition of the trypsin inhibitor extract to casein markedly reduced growth and the protein efficiency ratio. This confirms the observations of various investigators.[2,6] The addition of the active extract to the hydrolyzed protein also resulted in a decrease in growth rate and protein efficiency ratio. However, the autoclaved extract was without appreciable effect. It is interesting to note that the harmful effects of the active soybean extract were not associated with a decrease in food consumption. This is contrary to many observations with raw soybeans.

These results confirm and extend the observations of Desikachar and De. They do not support the conclusion of Melnick *et al.* that the activity of the trypsin inhibitor in lowering the nutritive value of proteins is due to an alteration in the intestinal digestion of protein. The general conclusion can be drawn that an extract of raw soybeans containing active trypsin inhibitor is deleterious to the growth rate and protein utilization of mice receiving as a sole source of protein in the diet a well hydrolyzed protein. Since the harmful effect of the extract was destroyed by autoclaving, it can be concluded further that the deleterious factor, whether it be trypsin inhibitor or some other inhibitory substance of soybeans, is heat labile. The fact that the soybean extract containing trypsin inhibitor decreased the utilization of a hydrolyzed protein cannot in itself be used as evidence that the trypsin inhibitor is not the factor responsible for the poor growth response and utilization of intact protein. There are many possibilities of indirect mechanisms whereby an inhibitor of proteolytic activity could exert a deleterious effect in the intestinal tract or in the animal body aside from the inhibition of tryptic digestion of dietary protein within the intestinal tract.

Summary. The addition of an extract of raw soybeans containing active trypsin inhibitor to an enzymatic protein digest (Protolysate) caused a decrease in growth rate and protein utilization. Autoclaving, which inactivated the trypsin inhibitor, abolished the harmful effects of the extract. It has been concluded that the deleterious effects resulting from the ingestion of an extract of raw soybeans are not necessarily due to an interference with the intestinal digestion of dietary protein.

[8] Bosshardt, D. K., Ydse, L. C., Ayres, M. M., and Barnes, R. H., *J. Nutrition*, 1946, **31**, 23.