pituitary have a reciprocal influence on the inactivation of hypertensin is under study.

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The Effect of Browning on the Essential Amino Acid Content of Soy Globulin[1]

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Soybean oil meal turns brown during heat processing; in fact, the consumer has developed a preference for a brown-colored product with a cooked, nutty flavor. Although some heat treatment seems desirable, it is probable that a large share of the soybean meal on the market has been overheated (*3*). Soybeans contain about 30% carbohydrate, which apparently has never been completely characterized. A typical solvent-process meal will contain about 0.5% free reducing sugar, and an abundance of polysaccharides which can readily yield more reducing sugar. In addition, there are probably other browning reactants, which may be broadly described as compounds containing carbonyl radicals. Hence, soybeans constitute a system which is conducive to the occurrence of nonenzymatic browning (the Maillard reaction), and this reaction is probably responsible for the brown color which develops in the heat processing.

While there are probably several mechanisms which are responsible for the so-called "heat damage" to proteins, including the proteins in soybeans, we have been struck by the apparent similarity between the results recorded by Riesen, *et al.* (*6*), in which prolonged heating apparently destroyed part of the lysine, arginine, and tryptophan, and the results we obtained in studying the effects of the nonenzymatic browning reaction upon the nutritive value of casein (*5*). Consequently, we have determined the effect on the 10 essential amino acids of heating purified soy globulin in the presence of glucose.

A sample of commercial raw soybeans was finely ground and extracted with cold hexane. Soy globulin was then isolated by peptization with 10% NaCl and precipitated by dialysis. The process was repeated twice for further purification. The globulin was then washed with alcohol followed by ether, and dried at room temperature. Browned samples were obtained by refluxing 2.0-gm portions of the soy globulin for 24 hrs in 200 ml of 5% CP glucose and removing the glucose by dialysis before hydrolysis. In an attempt to eliminate heat *per se* as a factor, the control samples were refluxed for 24 hrs in 200 ml of distilled water. For tryptophan, refluxed samples were hydrolyzed for 24 hrs in 5N NaOH. For the other amino acids 30-hr hydrolysis in 6N HCl was used. The nutritive availability of the essential amino acids in the hydrolysates was determined microbiologically by the method of Stokes, *et al.* (*7*), modified by substituting sucrose for glucose in the media to prevent further browning loss (*4*). *Lactobacillus delbrückii* LD5 was used for phenylalanine; *Streptococcus faecalis* R, for the other amino acids. The final concentration of sucrose in the media was 19 gm/liter; both organisms grew well in sucrose instead of glucose. The results are shown in Table 1. These indicate that glucose

TABLE 1

Essential Amino Acid Content of Soy Globulin Refluxed 24 Hrs

Amino acid	In water %	In 5% glucose %	Loss in glucose %
Lysine	5.84	4.24	27.4
Arginine	3.43	2.65	22.7
Tryptophan	1.44	1.22	15.3
Histidine	3.21	2.74	14.6
Methionine	1.20	1.24	−3.3
Leucine	7.62	7.53	1.2
Isoleucine	6.28	6.37	−1.4
Valine	5.41	5.23	3.3
Threonine	3.94	3.83	2.8
Phenylalanine	5.10	5.15	−1.0

interaction destroyed 27.4% of the lysine present, 22.7% of the arginine, 15.3% of the tryptophan, and 14.6% of the histidine. It is doubtful if any of the other essential amino acids were affected.

Under the conditions of this experiment, the use of a water-refluxed control turned out to be unnecessary. Apparently the destruction observed was entirely due to the glucose and not to the heat *per se*, since comparison of the total essential amino acid content of untreated and water-refluxed samples showed the presence of 43.49% and 43.47%, respectively.

The opinion has been expressed in Quartermaster Reports and elsewhere that a toxic, growth-inhibiting substance is produced as a result of the browning reaction. In such a case, one would expect to obtain decreased growth in each of the amino acid assays. Since in our experiments certain amino acid assays gave equal growth responses before and after browning, it seems unlikely that a toxic substance is involved.

Whether the amino acids are nutritionally less available but still chemically present is a disputed point.

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Our type of inactivation may be different from that of Block, Jones, and Gersdorff (*2*), since they found that heated and unheated casein yielded the same quantities of lysine after acid hydrolysis. Our conditions are certainly similar to those of Block, *et al.* (*1*), who obtained lysine inactivation in high protein cakes. Their recipe included 11% molasses as well as other browning reactants. Paper partition chromatography appears to provide evidence that we are dealing with actual amino acid destruction. In preliminary experiments we have found that an excess of glucose in a solution of the essential amino acids does not interfere with their chromatography unless the mixture has been heated. Upon chromatographing heated and unheated portions of the same glucose amino acid mixture, we have found that the amino acid spots from the heated sample have either declined in density or vanished completely. At the same time new, unidentified "spots" appear, with higher R_F values than the corresponding amino acids, which are visible by fluorescence under ultraviolet light *before heating the paper.*

Soy globulin was used instead of whole soybeans for the following reasons: There are difficulties in obtaining a true picture of the amino acid destruction in a heat-treated protein food or feed which do not seem to be universally realized. It is not possible to accomplish this with any degree of accuracy by hydrolysis in hot acid or alkali. We have found that, as hydrolysis proceeds, the carbonyl compounds present or produced from carbohydrates or other components react with the amino acids as they are released. This causes a non-specific, over-all destruction which tends to mask the true heat-processing losses. Enzymatic digestion cannot be used, because overheating is reported to decrease the amount of each of the essential amino acids liberated by enzymes (*6*). We believe this effect on enzymatic digestion is separate from the destructive effect of browning with which the present article is concerned.

We do not wish to emphasize the actual *percentages* of loss obtained; they probably apply only to the conditions of individual trials. Statistical analysis, as previously shown for casein (*5*), succeeds only in demonstrating that the precision of the microbiological assay is excellent. We do wish to point out, however, that there seems to be a pattern of destruction which is significant and would probably apply to other heat processes which cause nonenzymatic browning. Judging from experience with casein (*5*) and soy globulin, it appears that the amino acids which are attacked by browning an intact protein are chiefly those containing functional nitrogen groups unattached in peptide linkages. There seems to be little doubt that lysine is most susceptible to attack, followed by arginine, tryptophan, and histidine. Presumably the radicals involved are the epsilon amino, guanido, indole, and imidazole groups, respectively. There is no conclusive evidence that any of the other essential amino acids are significantly altered. Apparently the amino groups in peptide or other protein linkages are blocked and do not readily react until hydrolysis has liberated them. On the basis of this hypothesis, terminal amino groups should also react.

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Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoon Parasite (Toxoplasma)[1]

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The purpose of this preliminary communication is to describe a new immunity phenomenon in which dyes of certain chemical composition have been found capable of indicating the presence or absence of antibody activity. This phenomenon was discovered during the course of a search for some *in vitro* manifestation of the action of neutralizing antibody on toxoplasma, an obligate, intracellular protozoon parasite. After finding that toxoplasma in properly diluted mouse peritoneal exudate could be counted with great accuracy in a standard hemocytometer, we observed that in mixtures with immune serum the toxoplasma remained intact but lost the refractility they exhibited in mixtures with normal serum. When, after incubation at room temperature for several hours, large drops of such mixtures were allowed to dry slowly on slides overnight and then were stained with Wright's stain, large numbers of toxoplasma could be seen in the preparation from the normal serum mixture, whereas very few were found in that from the immune serum mixture. Small drops, spread thin and rapidly dried, revealed that, with few exceptions, the cytoplasm of the toxoplasma in the immune serum mixtures was distorted, poorly stained, or unstained as compared with the deep blue staining and granular structure of the cytoplasm of the toxoplasma in the mixtures with normal serum; the chromatin of the toxoplasma appeared the same in both types of mixtures. One of us (A. B. S.) had observed a number of years ago that, when alkaline methylene blue was added on a slide to a drop of peritoneal exudate containing toxoplasma, immediate deep purple staining of the parasites could be observed under the microscope. When this was done with

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