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The Effect of Autoclaving with Dextrose [1] on the Nutritive Value of Casein

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INTRODUCTION

A number of studies on the effect of heat treating proteins by autoclaving and dry heating have been reported in recent years dealing chiefly with the effect on their growth promoting qualities. However, few attempts have been made to determine the effect of heat treatment of proteins in the presence of reducing sugars on the growth promoting qualities of the proteins or protein derivatives. Stevens and McGinnis (1) have shown that lysine autoclaved in the presence of cerelose was either destroyed or rendered unavailable for chick growth. Hill and Patton (2) found that the slight discoloration occurring during autoclaving of the media for the microbiological assay of L-tryptophan was caused by interaction with glucose, resulting in decreased growth of *Streptococcus faecalis* R. Later work by these investigators (3) showed this decrease in growth was due not to formation of growth inhibitors as products of the browning (Maillard) reaction but to actual destruction of part of the tryptophan. This destruction, however, was not limited to tryptophan, as both L-lysine and DL-methionine, upon heating in the presence of glucose, underwent similar destruction. In this paper, we wish to report the effect of heat treatment in the presence of a reducing sugar on the growth promoting value of casein.

EXPERIMENTAL

Plain casein (General Biochemicals, Inc.) was used in these studies. To this crude protein was added a dextrose solution so prepared as to contribute 0.5 ml. distilled water/g. of crude casein and 1 g. dextrose (anhydrous-Merck) for every g. of protein

[1] During preparation of this manuscript, a paper appeared, by Patton, Hill and Foreman (7), which demonstrated a decrease in the lysine, arginine, and tryptophan content of casein heated with dextrose.

(N × 6.38) present in the casein. This mixture of casein and dextrose was autoclaved for 2 hr. at 250°F., the resulting material being dark brown and quite tough and rubbery, and containing no detectable free amino groups (Table I). To determine the effect of dextrose at room temperature, a similar mixture of casein and dextrose as above was air-dried at room temperature.

As controls, to show the effects of autoclaving in the absence of dextrose, the crude casein and casein to which was added the same ratio of distilled water as contributed by the dextrose solution were autoclaved for 2 hr. at 250°F. The casein autoclaved "as is" formed a hard, brittle, buff-colored mass, whereas the casein autoclaved with water formed a tough orange-colored gel.

TABLE I

Calculation of Biological Value

Diet	Free amino N (Van Slyke)	Average initial weight	Average final weight	Average total weight change	Average weight food eaten	Protein in diets	Average total protein intake	Average gain or loss/g. protein intake	Biological value (Casein 100)
		g.	*g.*	*g.*	*g.*	*per cent*	*g.*		
A. Casein autoclaved with dextrose	0.00	55	45	−12 ± 1	123	10.8	13	−.87 ± .07	—
B. Casein and dextrose dried at room temp.	0.21	54	108	54 ± 3	216	10.7	23	2.33 ± .09	100
C. Casein autoclaved with water	0.34	56	98	42 ± 4	214	10.3	22	1.85 ± .16	79
D. Autoclaved casein	0.99	57	102	45 ± 2	239	10.4	25	1.82 ± .27	78
E. Untreated casein	0.24	55	112	57 ± 2	227	10.8	24	2.34 ± .08	100

Isocaloric diets A, B, C, D, and E were prepared, containing, in addition to their respective casein components at 10% protein level, fat as Mazola Oil (Corn Products Refining Company) to 6%, Salt Mixture No. 2 (U.S.P. XII Wyeth & Company) 4%, and sufficient starch to make the diets isocaloric. To each kg. of diet was added a vitamin supplement of choline chloride 1.0 g., α-tocopherol acetate 0.10 g., pyridoxine HCl 0.002 g., calcium pantothenate 0.005 g., riboflavin 0.037 g., and flour enrichment (Type 5-N-Richment A, Novadel-Agene Corp.) 0.9 g. Vitamins A and D were added as a daily supplement to each animal's food.

Five groups of weanling albino rats (Carworth Farms) were used for the feeding test, one group for each diet. Each group consisted of 5 male and 5 female weanling animals 28–29 days of age. The animals were so placed on test that the average weights of the animals of each sex were comparable within the groups.

The feeding test was carried out for 28 days, during which the animals were weighed at 4 day intervals. Daily observations were made for abnormalities in appearance and physiological activities. At the conclusion of the assay period, the animals were sacrificed and autopsied for gross pathology.

All animals were placed on test on the same date and were fed *ad libitum* until it was evident that the animals receiving the casein heated with dextrose (Diet A) were

eating the least. The daily average food intake of male and female animals of this group controlled the daily intake of the respective sexes of the other 4 groups until it was noticeable that Diet A animals were rapidly losing weight and would jeopardize the paired feeding technique. At this time, group A was fed *ad libitum* while groups B, C, D, and E remained on paired feeding, their food intake being controlled by the group other than A that consumed the least food.

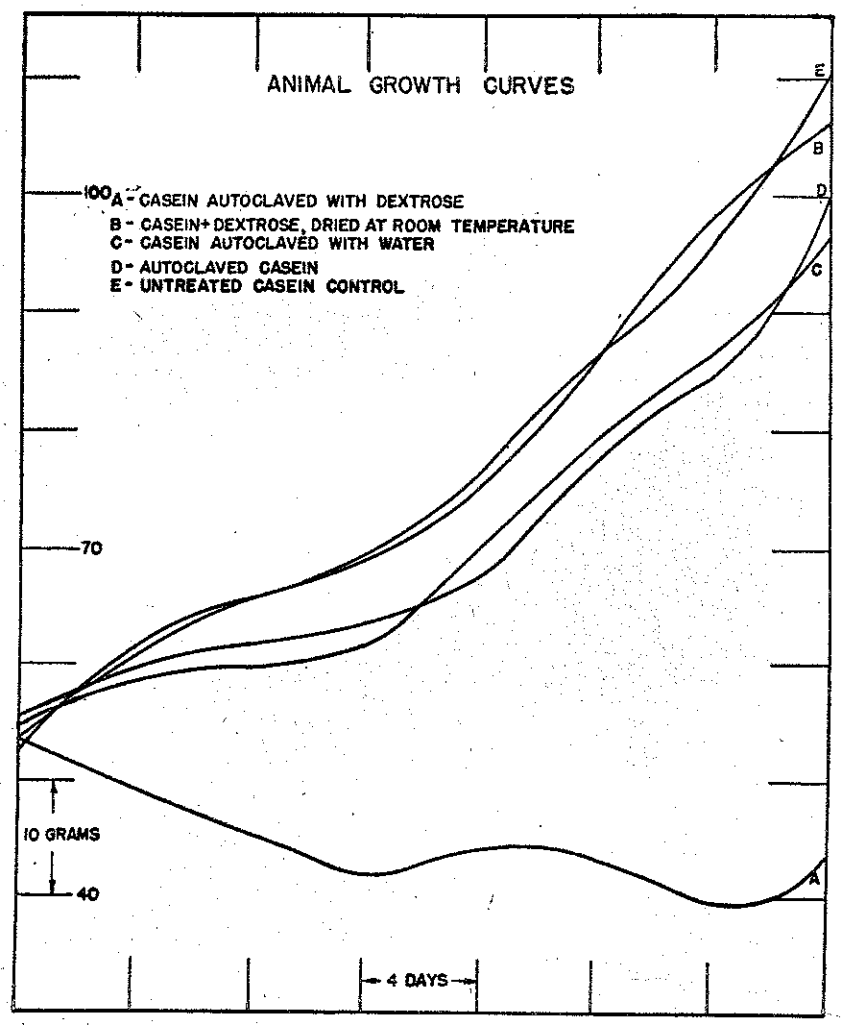

FIG. 1. Animal growth curves showing the impairment in growth on Diet A containing the autoclaved casein-dextrose.

Results and Discussion

During the 28 day test period, animals on Diet A, receiving casein autoclaved with dextrose, rapidly lost weight (Fig. 1), 2 animals succumbing on the 20th day. The animals receiving casein and dextrose dried at room temperature (Diet B), casein autoclaved with water (Diet C), autoclaved casein (Diet D), and the casein control (Diet E), grew favorably, the animals receiving Diet B growing as favorably as the control animals. In accordance with results obtained by previous investigators (4,5) on the effect of heat-treating casein, autoclaved casein and casein autoclaved with water had a lower biological value than the unheated protein (Table I). However, when the casein was autoclaved in the presence of dextrose, its biological value was lowered to a point where it would no longer promote tissue synthesis. Drying with dextrose at room temperature had no apparent effect on the growth promoting qualities of the casein, the biological value of this material (B) being comparable to the untreated casein control.

During the test, abnormalities in appearance and physiological activities were prevalent in animals fed casein autoclaved with dextrose (Diet A). Attendant with a marked loss in weight in these animals, was the development of a pasty-white appearance of ears and feet, a reddish appearance of the head, bedraggled appearance of fur and, as the test progressed, a slightly humped appearance, encrustation of the eyes, and a bloody whitish discharge from the nostrils. In the early stages of the test, the animals were slightly nervous but, as the test progressed, they became very sluggish. Two animals of this group, both exhibiting the above typical symptoms of unbalanced and inadequate amino acid intake, died on the 20th day of test. Throughout the test, the feces of animals receiving Diet A were black and sticky. Animals fed diets B, C, D, and E exhibited no typical symptoms of unbalanced and inadequate amino acid intake.

Autopsy for gross pathology showed abnormalities prevalent in animals receiving casein autoclaved with dextrose. Each animal of this group had a small light-colored liver, discolored kidneys and spleen, and slightly haemorraghic intestines. No significant prevalence of any gross pathological condition was noted in any of the other groups.

The destruction of the growth promoting qualities of casein by autoclaving with dextrose may be in part explained by reference to data of Table I, in which it will be noted that the free amino nitrogen

(Van Slyke) (6) of the casein protein was reduced to zero by heat treatment in the presence of dextrose. Presumably the aldehyde group of the reducing sugar reacted with free amino groups of the amino acids of the proteins through typical mechanisms of the Maillard (browning) reaction. Certain essential amino acids so reacted may be rendered nutritionally unavailable to the animal system, thereby disrupting the essential amino acid balance of the casein, resulting in a drastic decrease in its growth-promoting qualities. It is suspected that the unavailability of any amino acids so reacted may be due to the formation of an enzyme- and acid-resistant linkage.

The reduction in the biological value of the casein heated with water alone may be due to the reaction of some of the essential amino acids with reducing sugars inherent in the casein protein complex.

Deamination and decarboxylation are not excluded as factors in the reduction of the biological value of the heated casein.

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Summary

Casein autoclaved for a 2 hr. period at 250°F. in the presence of a large amount of dextrose (1 g./g. of protein) was nutritionally impaired to such a degree that it would not support the growth of weanling albino rats. When similarily treated in the absence of dextrose, its growth-promoting quality was only slightly impaired. Drying with dextrose at room temperature had no effect on the nutritive value of the protein.

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