

altered in rats exhibiting subnormal plasma albumin levels, these organs were analyzed for total protein, and the data are presented in Table 2. It is apparent that liver size and water content were unchanged by the drug, and despite a slight decrease in percentage of protein the total protein content of the liver was normal. Since the animals fed the drug lagged in growth rate the liver protein in relation to body weight was significantly increased and in proportion to the amount of drug fed. This is interesting, in that the slower growth rate with reduced food intake would seem more likely to be associated with a smaller liver. Reduction of food intake will rapidly decrease liver size and protein level (4). 2-Acetylaminofluorene can therefore significantly increase liver protein to body weight ratios without altering plasma protein concentrations, but a decrease in plasma albumin can be induced if drug concentration is high.

References

1. COOK, H. A., GRIFFIN, A. C., and LUCK, J. M. *J. biol. Chem.*, 1949, **177**, 373.
2. ELSON, L. A. and HARRIS, R. J. C. *Brit. J. Cancer*, 1947, **1**, 327.
3. GRIFFIN, A. C. *et al. J. biol. Chem.*, 1948, **176**, 1225.
4. KOSTERLITZ, H. W. and CRAMB, I. D. *J. Physiol.*, 1943, **102**, 83.
5. LEATHEM, J. H. *Endocrinology*, 1945, **36**, 98.
6. OPIE, E. L. *J. exp. Med.*, 1946, **84**, 91.
7. PRICE, J. M., MILLER, E. C., and MILLER, J. A. *J. biol. Chem.*, 1948, **173**, 345.
8. STASNEY, J. *et al. Cancer Res.*, 1947, **7**, 356.
9. WILSON, R. H., DEEDS, F., and COX, A. J., JR. *Cancer Res.*, 1941, **1**, 595.

Correlation of Browning, Fluorescence, and Amino Nitrogen Change with Destruction of Methionine by Autoclaving with Glucose¹

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The destruction of lysine by autoclaving in the presence of glucose was demonstrated by Stevens and McGinnis (8). These workers observed that the autoclaved mixture of glucose and lysine was dark brown in color. Patton and Hill (6) reported that tryptophane, in a bacteriological culture medium containing glucose, was destroyed to a certain extent by autoclaving. This destruction was not observed when sucrose was substituted as a source of carbohydrate. Hsu, McGinnis, and Graham (4) found that methionine was destroyed or rendered unavailable to chicks by autoclaving in the presence of either glucose or lactose, and to a certain extent in the presence of sucrose. In contrast, methionine was unaffected by heating in the presence of starch or dextrin. Acid hydrolysis of the autoclaved mixture of glucose and me-

thionine failed to liberate the amino acid for support of chick growth. They also found that autoclaving a soybean protein with glucose damaged the protein to the extent that supplementary methionine, lysine, and tryptophane failed to restore its nutritive value for chicks. These observations indicate that other amino acids, in addition to the three named, were destroyed or rendered unavailable. Evans and McGinnis (2) showed that cystine in soybean oil meal was destroyed during autoclaving. In contrast, the same autoclaving treatment failed

TABLE 1
CORRELATION OF BROWNING, FLUORESCENCE, AND AMINO NITROGEN CHANGE WITH DESTRUCTION OF METHIONINE BY AUTOCLAVING WITH GLUCOSE

Methio- nine mg/culture	Dry wt of mold in mg			Brown color	Fluores- cence equiv- alents	α-Amino nitrogen
				optical density at 380 mμ (× 100)	mg quinine sulfate/g methionine	% of theoret- ical value
0.1*	11.1	11.1	11.1§	0	0	102.2
0.2	18.5	18.5	18.5			
0.4	32.0	34.4	33.2			
0.6	55.2	53.2	54.2			
0.8	64.9	61.9	63.4			
1.0	69.7	68.9	69.3			
0.1†	11.4	11.2	11.3	5.6	.965	93.7
0.2	18.3	18.3	18.3			
0.4	31.8	34.9	33.4			
0.6	50.2	52.4	51.3			
0.8	58.9	62.9	60.9			
1.0	67.0	63.1	65.1			
0.1‡	..	3.5	3.5	180	14.25	15.1
0.2	3.8	3.8	3.8			
0.4	5.8	5.8	5.8			
0.6	6.7	6.2	6.5			
0.8	8.2	8.2	8.2			
1.0	9.5	9.6	9.6			

* Methionine autoclaved alone for 2 hr at 120° C.

† Methionine (2 g) dissolved in 100 ml of 8% glucose solution and autoclaved 2 hr at 120° C.

‡ Methionine (2 g) mixed with glucose (8 g) and autoclaved 2 hr at 120° C.

§ Average of duplicates.

to alter the methionine content. Patton, Hill, and Foreman (7) reported that lysine, arginine, tryptophane, and histidine were destroyed when an intact soybean protein was refluxed in a 5% glucose solution for 24 hr. In view of the results reported by Hsu, Graham, and McGinnis (5) showing that the concentration of amino acids or crude protein in an aqueous solution of glucose markedly influenced the amount of browning and destruction of amino acids, it should be pointed out that the concentration of protein used by Patton, Hill, and Foreman (7) was only 1%.

Chen, Medler, and Harte (1) found that highly fluorescent substances were formed from certain amino acids when they were heated with Denigès reagent (paraformaldehyde and sulfuric acid). A similar observation

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had previously been made in this laboratory when glucose was used to furnish the aldehyde group instead of Denigès reagent. All of the amino acids tested (approximately 15) formed fluorescing substances when mixed with glucose and autoclaved at 125° C for 1 hr.

The experiments described in this paper were conducted to determine whether a relationship exists between the degree of browning, the production of fluorescing substances, changes in amino nitrogen, and the ability of methionine autoclaved in the presence and absence of glucose to support growth of a methionineless mutant of *Neurospora crassa* No. 38706.

The methionine used in these experiments was treated as indicated in footnotes to Table 1. These differently treated samples of methionine were appropriately diluted for the determination of optical density or brown color concentration, fluorescence, amino nitrogen, and methionine content as evidenced by the ability of the material to support growth of *Neurospora crassa* No. 38706.³ The results obtained are summarized in Table 1. From these

³ Samples were diluted in 0.1 M phosphate solution pH 4.8. Fluorescence was determined in the Coleman Photofluorometer, using the B₁ filter. The instrument was standardized at 100 with quinine sulfate solution (0.1 mg/1 0.1 N H₂SO₄). Brown color was measured as optical density of the phosphate solution at 380 mμ in the Coleman Universal Spectrophotometer. Amino nitrogen was determined by the method of Van Slyke (9). The methionine assay was conducted as outlined by Horowitz (3).

data, it is evident that brown color formation, fluorescence, and decreased amino nitrogen are associated with destruction of methionine brought about by autoclaving in the presence of glucose, but in the absence of an excessive amount of water. Under the experimental conditions in this laboratory, autoclaving methionine in an 8% solution of glucose failed to bring about any marked destruction or changes in brown color, fluorescence, and amino nitrogen.

The data clearly show the important influence of water on the destruction of methionine autoclaved in the presence of glucose, on the loss of amino nitrogen, and on the formation of brown color and fluorescing substances.

References

1. CHEN, J. L., MEDLER, J. D., and HARTE, R. A. *J. Amer. chem. Soc.*, 1948, **70**, 3145.
2. EVANS, R. J. and MCGINNIS, JAMES. *J. Nutrition*, 1948, **35**, 447.
3. HOROWITZ, N. H. *J. biol. Chem.*, 1947, **171**, 255.
4. HSU, P. T., MCGINNIS, J., and GRAHAM, W. D. *Poultry Sci.*, 1948, **27**, 668.
5. ———. *Abstracts of papers*, Amer. Chemical Society, 114th meeting, Portland, Oregon, 1948, 67 C.
6. PATTON, A. R. and HILL, E. G. *Science*, 1948, **107**, 68.
7. PATTON, A. R., HILL, E. G., and FOREMAN, E. M. *Science*, 1948, **107**, 623.
8. STEVENS, J. M. and MCGINNIS, JAMES. *J. biol. Chem.*, 1947, **171**, 431.
9. VAN SLYKE, D. D. *J. biol. Chem.*, 1911, **9**, 185; 1912, **12**, 275; 1913–14, **16**, 121.

Comments and Communications

Characteristics of Some Disease-free Ornamental Plants

Modern medicine has learned that people in good health are worth study. It may be hoped, then, that plant pathologists will learn to give special consideration to those plants which have few diseases or pests. As a step in this direction, information has been assembled in this paper regarding a considerable group of ornamental plants in which, in the eastern United States at least, no important diseases or pests are known.

Dodge and Rickett include in *Diseases and pests of ornamental plants* (1943) the names of approximately 75 plants which are practically disease-free. This is almost unique in such a book. In the introduction, the scope and purpose of the authors are carefully defined. "We have tried to select those [diseases and pests] which have been most injurious in the eastern United States; most of these are also the most troublesome in other parts of the country and of the world. . . . Our selection of cultivated plants is based upon the 3,000 species and varieties grown at the New York Botanical Garden." The book thus serves admirably as a check list of diseases and insect pests of ornamental plants in the north-eastern United States; over 500 genera are included.

In studying 75 disease-free plants, answers to three questions were sought. What are their characteristics? What, if any, characteristics do they have in common? Is it possible that these common characteristics have any bearing on their disease-free status in the eastern United States? (Much of the work of compiling the information on which this paper is based was done by members of a class in the epidemiology of plant diseases.)

The plants are not taxonomically related. They are scattered through 38 families; the largest number in any one family being nine in the Compositae. They show diverse growth habits. There is one tree (Ginkgo), one woody vine (Wisteria), eight shrubs, and the rest are herbs. The plants which have no important diseases or insect pests are predominantly seed-propagated and are either annuals or very short-lived. Only one is reproduced by bulbs, 17 are reproduced chiefly by division; the rest either may be, or must be, grown from seed.

They are also predominantly of foreign origin. Only 15 (20%) seem to be natives of the continental United States. Eurasia has furnished 33; others come from Africa, Australia, South America, and various tropical localities. An introduced plant (all but 15 of these 75 plants were introduced into the United States) which has