puppies, were spaced at irregular intervals, never less than 10 days apart.

In vitro studies were made on "Straub heart" or strips of intestine or stomach, using the usual recording methods for these preparations. All chemicals used in the kymographic studies were made up in frog saline solution.

A series of 14 American newts were injected intraperitoneally with 0.05 ml of horse serum in an attempt to sensitize them. At the same time four other newts were set aside uninjected as controls and were held under conditions comparable to those of the test group. All of the test animals were reinjected, using 0.10 ml of horse serum; two each at intervals of 18, 23, 28, 42, 48, 55, and 65 days after the initial injection. A control animal was injected with the same dose of antigen along with the first four pairs of test animals injected. No demonstrable evidence of hypersensitivity was observed. Similarly, the injections of the control animals produced no results. Two animals died subsequent to injection, but autopsy revealed evidence of trauma that could account for the deaths. Three animals received three injections each at irregular intervals, without reaction.

The injection of test and control animals with 5 γ of histamine phosphate produced no reaction, but 10 γ produced a transitory reaction of obvious distress.

Muscle preparations using a section of the duodenal end of the intestine were disappointing, because the small size of the material rendered kymographic studies difficult. However, it appeared that in three muscle preparations studied, histamine 10 γ, epinephrine 10 γ, and horse serum 0.2 ml produced no demonstrable effect. Barium chloride, 2% solution, produced significant contractions of the gut, showing the ability of the muscle to respond. Heart and stomach preparations were not studied in the newt because of the technical difficulties involved. In view of the results of the organ studies, it was felt that the reaction *in vivo* with 10 γ histamine was an artifact caused by the relatively large size of the dose administered.

Of five mudpuppies obtained, two were sacrificed, without previous attempts to sensitize them, for kymographic studies of heart, stomach, and intestine; and three were injected intraperitoneally with 0.2 ml of horse serum. As this produced no evident reaction, the animals were reinjected four days later, using 0.25 ml antigen in an effort to build up the allergic state. Again there was no immediate reaction, but one animal died two days later; autopsy showed blood in the abdomen, the cause of which was probably the trauma of injection. The remaining two animals were injected again 18 days after the first sensitizing dose and showed no symptoms of shock. Five days later these animals were sacrificed for study of isolated organ preparations.

In kymographic studies of Straub heart preparations of both normal and serum-injected animals, histamine in doses as high as 10 γ and horse serum in 0.2-ml doses failed to exert any effect on the contractility of the heart. Similar doses of histamine and horse serum failed to produce demonstrable responses when administered to stomach or intestinal preparations. One γ of epinephrine produced a marked increase in the amplitude of the myocardial contractions, while the same amount of acetylcholine depressed the contractility of the heart. These reactions were in agreement with those observed using fish Straub heart preparations (*2*) and our unpublished experiments with frog preparations.

As a result of these data it can be concluded that, while teleost fish have the ability to become sensitized to protein antigens, are able to show histamine shock, and can show smooth muscle contractions in the preparations *in vitro* treated with histamine, the American newt and the mudpuppy have none of these characteristics. Acetylcholine depresses the activity of frog, salamander, and fish heart, but seems to play no role in hypersensitization. It is suggested that these data be used as additional evidence of the role of histamine in anaphylaxis.

References

1. ARLOING, F. and LANGERON, L. *Compt. rend. Soc. biol.*, 1922, **87**, 634.
2. DREYER, N. B. *Arch. internat. de pharmacodyn. et therap.*, 1946, **72**, 440.
3. DREYER, N. B. and KING, J. W. *J. Immunol.*, 1948, **60**, 277.
4. FRIEDBERGER, E. and MITA, S. *Z. Immunitätforsch. u. exp. therap.*, 1911, **9**, 362.
5. FRIEDE, K. A. and EBERT, M. K. *Z. Immunitätforsch. u. exp. therap.*, 1926, **49**, 329.
6. GOODNER, K. *J. Immunol.*, 1926, **11**, 335.
7. KRITCHEVSKY, I. L. and BIRGER, O. G. *J. Immunol.*, 1924, **9**, 339.

Inactivation of Amino Acids by Autoclaving[1]

Robert John Evans and Helen A. Butts

Department of Agricultural Chemistry, Michigan State College, East Lansing

Recent work has shown that cystine is the only amino acid partially destroyed by autoclaving casein for 20 hr at 15 lb pressure (*3*). Lysine, arginine, and tryptophan suffer partial destruction when casein (*5*) or soy globulin (*6*) is refluxed for 24 hr in a glucose solution, or when soybean oil meal is autoclaved for 4 hr (*7*). The amounts of aspartic acid, isoleucine, lysine, methionine, and threonine liberated by enzymatic digestion from casein *in vitro* are decreased after autoclaving (*3*), as are all of the amino acids in soybean oil meal (*7*).

Evans and Butts (*1*) found that autoclaving causes two types of inactivation of lysine, one a reaction of the lysine with sucrose to destroy it and the other a reaction with protein to render it unavailable after enzymatic digestion *in vitro*. Moreover, methionine reacts with sucrose or glucose to form a linkage not hydrolyzed by enzymes *in vitro* (*2*).

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1007 (new series).

The present investigation was carried out to study the mechanism of amino acid inactivation by autoclaving. Soybean protein,[2] and a mixture of 8 g of soybean protein and 2 g of sucrose, were autoclaved for 4 hr at 15 lb pressure. Unautoclaved protein was used as a control. Total and available amino acid contents of the proteins were determined by microbiological assay after either acid or enzymatic digestion *in vitro* as described previously (*1, 2*). *Leuconostoc mesenterioides* P–60 was used for lysine, methionine, cystine, and aspartic acid assays; *Lactobacillus arabinosus* 17–5 for leucine, isoleucine, valine, threonine, phenylalanine, and glutamic acid; *Streptococcus faecalis* R for histidine; and *Lactobacillus casei* for arginine. The media of Sauberlich and Baumann (*3*) were used for all assays except methionine and cystine for which the oxidized peptone medium of Lyman, *et al.* (*4*) was used.

The results are summarized in Table 1. None of the amino acids was significantly destroyed by autoclaving soybean protein alone; but when the protein was mixed with sucrose prior to autoclaving, over 40% of the diamino acids, lysine and arginine, was destroyed. This destruction was caused, apparently, by a reaction of the free amino groups with sucrose, because more than 45% of each of the free amino acids used, except cystine, was destroyed when added to the protein-sucrose mixture before autoclaving (Table 2). Protein-bound cystine was also partially destroyed (22%) by the treatment.

Autoclaving the mixture of soybean protein and sucrose, decreased the amounts of all amino acids liberated by enzymatic digestion *in vitro*, some to a greater extent than others. Aspartic and glutamic acids were

TABLE 1

AMINO ACID INACTIVATION CAUSED BY AUTOCLAVING SOYBEAN PROTEIN ALONE OR WITH SUCROSE

	Acid hydrolysis			Enzyme hydrolysis		
	Total amino acid content %	% Lost on autoclaving	% Lost on autoclaving with sucrose	Available amino acid content %	% Lost on autoclaving	% Lost on autoclaving with sucrose
Arginine	6.91	3	42	6.62	8	55
Lysine	4.65	3	47	3.07	30	84
Histidine	2.29	8	10	1.97	16	42
Aspartic acid	5.75	7	6	1.04	37	34
Glutamic acid	17.10	2	3	4.74	24	50
Cystine	0.27	9	22	0.21	14	86
Methionine	1.12	3	2	0.62	6	41
Phenylalanine	5.17	0	0	4.00	9	14
Threonine	3.28	4	1	2.14	8	15
Leucine	7.31	2	0	6.14	8	13
Isoleucine	5.83	0	0	4.92	8	8
Valine	6.32	2	0	5.19	8	8

TABLE 2

INACTIVATION OF FREE AMINO ACIDS CAUSED BY AUTOCLAVING WITH SOYBEAN PROTEIN OR WITH SOYBEAN PROTEIN AND SUCROSE

	Percentage of added amino acid destroyed*		Percentage of added amino acid inactivated†		Percentage of added amino acid inactivated but not destroyed	
	Autoclaved with soybean protein	Autoclaved with soybean protein + sucrose	Autoclaved with soybean protein	Autoclaved with soybean protein + sucrose	Autoclaved with soybean protein	Autoclaved with soybean protein + sucrose
Arginine	7	45	2	50	0	5
Lysine	7	56	31	75	24	19
Aspartic Acid	22	57	33	56	11	0
Cystine	8	20	30	50	22	30
Methionine	2	79	7	74	5	0
Phenylalanine	15	59	21	70	6	21
Valine	14	46	25	52	11	6

* Percentage of added amino acid destroyed was calculated from the difference in recovery of amino acid from acid digests of the autoclaved and unautoclaved materials.

† Percentage of added amino acid inactivated was calculated from the difference in recovery of amino acid from enzyme digests *in vitro* of the autoclaved and unautoclaved materials.

[2] "Alpha" protein furnished by The Glidden Company, Chicago.

also partially inactivated when the protein was autoclaved in the absence of sucrose, possibly by a reaction of their free carboxyl groups with the free amino group of lysine to give a linkage resistant to enzymatic digestion. Cystine, methionine, and histidine inactivation was primarily caused by a reaction with sucrose to form an enzyme-resistant linkage. As only small amounts of phenylalanine, threonine, leucine, isoleucine, and valine were inactivated, it appears that the amino acids which are inactivated are those with free amino or carboxyl groups, or with other active groups such as the sulfur of cystine and methionine, or the imidazole of histidine.

Representative free amino acids were added to samples of soybean protein and the protein-sucrose mixture before autoclaving. The results are presented in Table 2. No relation between the behavior of free and protein-bound amino acids is apparent. Except for lysine, cystine, and phenylalanine, destruction accounted for practically all of the inactivation. The important point is that, except for cystine which is very insoluble under the conditions of autoclaving, over 45% of each of the free amino acids was destroyed when autoclaved with a mixture of soybean protein and sucrose.

From the results of this investigation it appears that at least three types of reaction are involved in the inactivation of amino acids by prolonged autoclaving of a sucrose containing food or feed, such as soybean oil meal. Lysine, aspartic, and glutamic acids combine with some constituents of the protein, probably the free carboxyl with the free amino groups, to form enzyme-resistant linkages. The amino acids with free amino groups react with sucrose to destroy the amino acids. Protein-bound methionine, cystine, and histidine with sucrose form linkages resistant to enzymatic hydrolysis *in vitro*.

References

1. EVANS, R. J., and BUTTS, H. A. *J. biol. Chem.*, 1948. **175**, 15.
2. *Ibid.* In press.
3. HANKES, L. V., *et al.* *J. biol. Chem.*, 1948, **176**, 467.
4. LYMAN, C. M., *et al.* *Arch. Biochem.*, 1946, **10**, 427.
5. PATTON, A. R., HILL, E. G., and FOREMAN, E. M. *Science*, 1948, **107**, 623.
6. *Ibid.* 1948, **108**, 659.
7. RIESEN, W. H., *et al.* *J. biol. Chem.*, 1947, **167**, 143.
8. SAUBERLICH, H. E., and BAUMANN, C. A. *J. biol. Chem.*, 1946, **166**, 417.

Improved Apparatus for Radiobiological Syntheses[1]

Clyde A. Dubbs

University of Southern California Medical School, and Los Angeles County Hospital[2]

Radioactive natural compounds, invaluable tracers for studying intermediary metabolism, are most conveniently prepared by radiobiological methods. Livingston and Medes (*4*) demonstrated that a detached leaf can efficiently photosynthesize $C^{13}O_2$ into C^{13} carbohydrates. Aronoff, Benson, Hassid, and Calvin (*1*) first reported a preparation of radioactive C^{14} glucose; and Putnam, Hassid, Krotkov, and Barker (*6*) have given detailed instructions for preparation. However, the apparatus designs employed by these pioneer workers warrant considerable improvement. A simplified photosynthesis apparatus and procedure, used to prepare C^{14} glucose for poliomyelitis studies, is described in this paper.

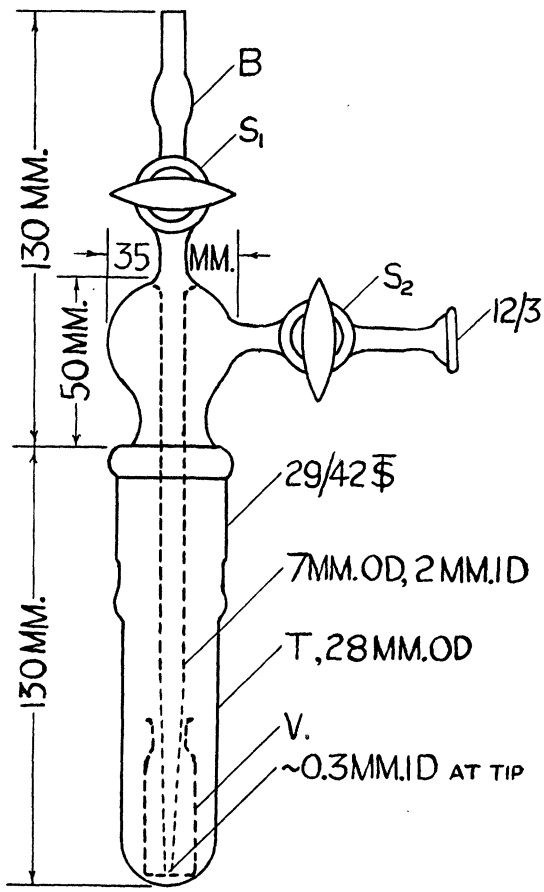

FIG. 1. Photosynthesis vessel.

Tube (T) (Fig. 1) is rinsed with water and left moist. A mature sweet potato leaf (petiole detached, fresh weight about 350 mg), which was removed from a plant in the light about 18 hr earlier, wet thoroughly, wrapped in wax paper and stored in the dark, is now arranged (under side facing in) about the inner surface of the tube. A 6-ml vial (V), containing 50 mg of $BaC^{14}O_3$, moistened to avoid possible scattering, is placed at the bottom of the tube; the standard taper joint lubricated; and the photosynthesis vessel assembled as shown. The vessel is evacuated through stopcock (S_2), 0.5 ml of 3N $HClO_4$ is introduced into bulb (B) by means of a narrow-tipped dropper, and stopcock (S_1) is carefully opened to admit the acid to the carbonate. When the

[1] This work was aided by a grant from the National Foundation for Infantile Paralysis.

[2] Present address: General Medical Research Laboratory, Veterans Administration Center, Los Angeles 25, California.