Videnskab. Selskab. Biol. Med., 1937, v13, 1.

4. Halberg, F., Halberg, E., Barnum, C. P., Bittner, J. J., In *Photoperiodism and Related Phenomena in Plants and Animals*, Withrow, R. B., Ed. Publ. #55, A.A.A.S., Washington, D.C., 1959, Pp. 803-878.

5. Halberg, F., Visscher, M. B., *Endocrinology*, 1952, v51, 329.

6. Walker, H. M., Lev, J., *Statistical Inference*, New York: Henry Holt Co., 1953, p103, 510 pp.

7. Bruss, R. T., Jacobsen, E., Halberg, F., Zander, R. A., Bittner, J. J., *Fed. Proc.*, 1958, v17, 21.

8. Halberg, F., *Lancet*, 1953, v73, 20.

9. Halberg, F., Peterson, R. E., Silber, R. H., *Endocrinology*, 1959, v64, 222.

10. Halberg, F., *Acta Med. Scand.*, 1955, Suppl., 307, 117.

11. Dunn, T. B., In Homburger, F., Fishman, W. H. *The Physio-pathology of Cancer*, 1953, N. Y., P. B. Hoeber, p123.

12. Loeb, L., Kirtz, M. M., *Am. J. Cancer*, 1939, v36, 56.

13. Silberberg, R., Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 510.

14. Rashkis, H. A., *Science*, 1952, v116, 169.

15. May, R. M., *Transplantation Bull.*, 1955, v2, 62.

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Apparent Loss of Glycine on Acid Hydrolysis. (25349)

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Studies concerning loss of amino acids during acid hydrolysis of protein containing materials have been reported(1,2). For the most part, such losses resulted from outright destruction, racemization, or irreversible binding to human material. The present report is concerned with an apparent loss of glycine, as determined microbiologically, with increasing time of acid hydrolysis. This loss is due to the presence of glycine peptides in the short time hydrolysates which promote greater growth of the assay organism than a corresponding amount of free glycine. The relationship of these findings to the antagonistic effects of certain amino acids in the basal medium upon glycine was investigated.

Methods. Hydrolysates were prepared by autoclaving 1 g of material with 20 ml of 3 N HCl in a sealed tube at 15 lb 121°C for varying time. The hydrolysates were taken to dryness, resuspended in water and made up to pH 4.0 with KOH. After filtering through a sintered glass funnel, the resulting clear filtrate was adjusted to pH 6.8, made to desired volume and stored under toluene at 5°C. Glycine was estimated microbiologically with *Leuconostoc mesenteroides* strain P-60 (ATCC 8042) using a commercially prepared amino acid assay mixture.* After distributing 2 ml of assay medium, samples and standards were added and total tube volume was made to 3 ml. The tubes were autoclaved for 10 min. at 15 lb 121°C, cooled, and inoculated with one drop of a washed suspension of test organism. Incubation was carried out at 37°C for 72 hours and the resulting acidity titrated with 0.025 N NaOH.

Results. Typical results illustrating effect of acid hydrolysis upon glycine content of soybean oil meal are shown in Fig. 1. The apparent glycine reaches a maximum at one hour, and thereafter drops rapidly to 6 hours. Additional autoclaving to 24 hours results in a further slight loss in activity. In separate experiments, where glycine was added prior to hydrolysis, recovery values ranging from 97.1 to 100.8% showed that this apparent loss was not due to destruction of free glycine. In addition to decrease in glycine activity with increased time of hydrolysis, a downward drift with increasing aliquot of sample assayed was observed. These results are shown in Table I. In the various hydrolysates, decrease in assayed glycine value is less with larger aliquot of sample and more pronounced with smaller aliquot. However, as time of hydrolysis is lengthened these differences become less significant.

Therefore, it seemed expedient to examine

* H.M.Chemical Co.

TABLE I. Effect of Hydrolysis Time upon Glycine Content of Soybean Oil Meal.

Hydrolysis time, hr	Sample wt, μg	Glycine, %
1	400	4.4
	800	3.7
	1200	3.3
	1600	3.0
2	400	4.0
	800	3.7
	1200	3.3
	1600	3.0
6	400	3.5
	800	3.3
	1200	3.0
	1600	2.8
12	400	2.8
	800	2.7
	1200	2.6
	1600	2.5

in more detail the nature of glycine activity present in both short- and long-term hydrolysates. According, 2- and 12-hour hydrolysates of soybean oil meal were passed through Dowex 50 columns, previously prepared in the hydrogen form, and eluted with successive volumes of water, 1.5, 2.5, and 4. N HCl. Several distinct fractions containing glycine activity were found in the 2-hour hydrolysate (Fig. 2). Of this total activity, approximately 20% was found in fractions which came off the column at points removed from that of free glycine. A quite different picture was obtained with the 12-hour hydrolysate (Fig. 3). All glycine activity was now found in one fraction which eluted from the column in a manner similar to that of free glycine. However, paper chromatograms and bioautographs of this fraction showed presence of glycine and an unidentified component which had glycine activity, gave an initially yellow ninhydrin reaction, and had an Rf value similar to that of glycylglycine.

Discussion. The most logical explanation of above results is that the 2-hour hydrolysate contains a variety of peptides which are largely hydrolyzed when the time is extended to 12 hours. It is also quite likely that the higher glycine values obtained with short-time hydrolysis as well as with smaller aliquots are due to presence of these peptides. In support of this supposition, it has been well established with *Leuconostoc mesenteroides* and other microorganisms that under certain conditions simple peptides may allow greater growth than their component amino acids(3). For ex-

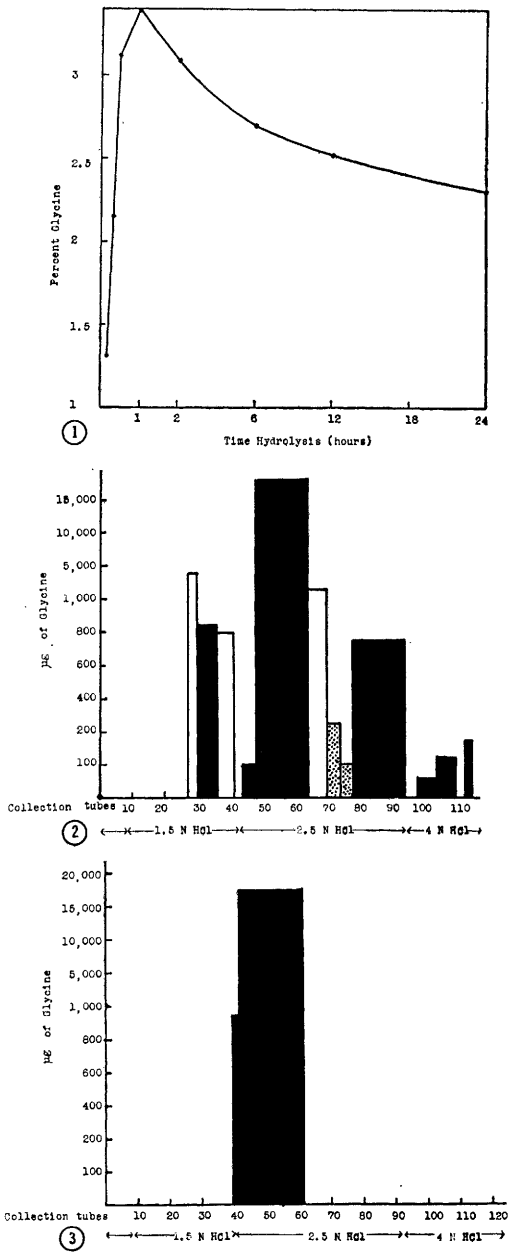

FIG. 1. Effect of time of hydrolyses on glycine activity of soybean oil meal (44% protein).

FIG. 2. Passage of a 1-g, 2-hr soybean oil meal hydrolysate through a Dowex 50 column. Black bars were ninhydrin positive. Speckled bars gave a green ninhydrin. Blank bars were ninhydrin negative.

FIG. 3. Passage of a 1-g, 12-hr soybean meal hydrolysate through a Dowex 50 column.

ample, with *L. mesenteroides,* growth at low levels of glycine is effectively inhibited by any one of several amino acids. However, the inhibitions may be overcome and growth restored by presenting glycine in the form of a simple dipeptide such as glycylglycine(4). Therefore, if the basal medium employed contained amino acids antagonistic to glycine at levels sufficiently high to inhibit growth, a greater growth promoting effect of glycine peptides as compared to free glycine would be expected. This was apparently the situation for when the alanine content of the medium was reduced to 1/20, leucine to 1/6, and the remaining amino acids by 1/2 of the original concentration, the short- and long-term hydrolysates, as well as various aliquots, gave equal growth (Table II). Therefore, in short-term hydrolysates, particularly, and somewhat even in the 12-hour hydrolysate, the peptide-bound glycine apparently allowed the assay organism to give greater growth in presence of antagonistic levels of various amino acids than an equivalent amount of free glycine. Comparison of such tubes to the standard glycine curve would necessarily give greater values. In the smaller aliquot, the effect of peptides is more striking than with the larger aliquot, because as quantity of glycine increases, inhibitory action of the antagonistic amino acids in the medium becomes less effective.

Although only data from experiments with soybean oil meal have been presented, similar results were obtained with casein, alfalfa, peanut meal, corn, wheat, blood meal, and fish meal. Using a medium modified as described above and a hydrolysis time of 2 hours, a number of feedstuffs have been assayed for glycine. The results of these assays are shown in Table III.

TABLE II. Effect of Media Change upon the Assay of Glycine in Soybean Oil Meal.

Media	Wt of sample, μg	% glycine Time of hydrolysis 1 hr	6 hr	12 hr
Prepared	400	4.4	3.5	2.8
	800	3.7	3.3	2.7
	1200	3.3	3.0	2.6
	1600	3.0	2.8	2.5
Modified	200	2.05	2.05	2.0
	400	2.0	2.02	1.97
	800	2.1	2.05	2.05

TABLE III. Glycine Content of Various Feedstuffs.*

Feed	Protein, %	Glycine, %
Soybean oil meal	43.3	2.1
Alfalfa	20.6	1.2
Peanut meal	44.2	2.4
Corn	8.7	.4
Wheat	13.6	.6
Blood meal	84.5	3.4
Fish "	57.3	4.2
Casein	80.0	1.8
Cottonseed meal	39.2	2.0
Dried milk	32.3	.7
Meat scraps	52.9	7.5
Oats	12.3	.6

* Air dried.

Summary. An apparent loss of glycine with increasing time of acid hydrolysis has been described. Short-term hydrolysates contain glycine peptides which largely disappeared as time of hydrolysis was extended. Lowering the concentration of amino acids in the basal medium resulted in disappearance of this apparent loss of glycine. With such a modified medium, a number of feedstuffs have been assayed for glycine.

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1. Schweigert, B. S., Snell, E. E., *Nutrition Abst. and Rev.,* 1946-1947, v16, 497.

2. Horn, M. J., Blum, A. E., Gersdorff, C. E. F., Warren, H. W., *Cereal Chem.,* 1955, v32, No. 1, 64.

3. Kihara, H., Snell, E. E., *J. Biol. Chem.,* 1952, v197, 791.

4. O'Barr, T. P., Levin, H., Reynolds, H., *J. Bact.,* 1958, v75, No. 4, 429.

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