Is Protein Catabolism Nonrandom?

In an earlier study[1] it was observed that protein loss induced by underfeeding or starvation of mice was accompanied by greater specific activity of the lysine-labeled protein compared with normally fed controls. The total counts per minute (cpm) of the protein was the same in both groups, however. Among the several possible explanations, it was suggested that 'nonrandomized breakdown in protein occurs, i.e., "older" (unlabeled) protein is preferentially broken down.' However, this was rejected since there was no known basis for this.

In a study on protein metabolism in aging mice[2], an observation was made which once more raises this possibility. 54 male mice uniformly distributed in age from 320 to 750 days were available for the experiment. At approximately 320 days, growth had ceased and the carcass protein content had reached the maximum value for the total life span[3]. They were injected with 0.1 μC of ^{14}C-lysine and were killed after 48 and 120 h. It had been determined that the log-linear phase had been reached by 48 h. Only 2 time periods could be used because of the scarcity of old animals. It was observed that although carcass protein decreased by 12.4% during this interval (320 to 750 days), $t^1/_2$ increased progressively to more than 30% greater than the value at 320 days (from 146 to 194 h)[2]. During the $t^1/_2$ interval for 320 day old animals, they exhibited a net loss of 0.94 mg of protein while the oldest lost 1.28 mg of protein. The specific activity (SA) increased respectively by 0.054 cpm/mg of protein and 0.072 cpm/mg of protein during the $t^1/_2$ intervals. The total cpm of the protein extrapolated to zero time (injection time) was practically independent of age (12,600 cpm for the 320-day-old animals to 12,950 cpm for the oldest).

Results and discussion. The data were recalculated to show the net decrease in carcass protein nitrogen ($-\triangle$N) compared with the maximum value at 320 days (623.4 mg) as a function of age, and SA and cpm as a function of $-\triangle$N (Table). It is apparent that an extremely high correlation exists between the net loss of protein nitrogen and increase in SA and cpm 48 h and 120 h after injection of the ^{14}C-lysine. This is not inconsistent with the possibility that 'older' protein is broken down preferentially.

SHIMKE and DOYLE[4] commented on the possibility that nonrandom breakdown of enzymes occurs. A theory of 'aging' of protein molecules as a signal for degradation seems excluded generally since the degradation of specific proteins wherever studied in animal tissue follows first order kinetics. However, SHIMKE and DOYLE point out that protein molecules exist in a number of different thermodynamic states. A protein might be subject to degradation only when molecules assume certain conformations. The microheterogeneity of proteins is well known[5]. That of albumin has been studied extensively[6]. One aspect of the heterogeneity is associated with the nonintegral sulfhydryl content and includes nonmercaptoalbumin components. Could the latter relate to albumin which has been modified as a result of its longer history in the body? Can this material be 'recognized' and catabolized in preference to mercaptoalbumin?

With regard to the problem of aging, if this hypothesis is correct, the data suggest the following: with increasing age there is a greater net loss of protein during the $t^1/_2$ interval. Since cpm in the total carcass protein extrapolated to zero time did not change with age and SA after 48 and 120 h actually increased with age[2], more 'older' protein must have been broken down during this interval. This suggests that there is no defect in the 'recognition' mechanism with age but an increase in the rate that unlabeled ('older') protein becomes modified for recognition. This is not inconsistent with the increase in inactive enzyme molecules reported in aging nematodes[7] and the thesis of OERIU and OERIU[8] that disorders of redox control occurs in aging resulting in enhanced oxidation of functional -SH groups of proteins with an accumulation of -S-S- groups.

Zusammenfassung. Injektion von ^{14}C-Lysin in 320 bis 750 Tage alten Mäusen deutet darauf hin, dass während des Alterns alte Proteinmoleküle infolge ihrer Strukturänderung rascher katabolisiert werden als neue.

Correlation of net loss of carcass protein nitrogen ($-\Delta$N)[a] with age and of specific activity (SA) and total counts per minute (cpm) with $-\Delta$N.

Regressions[b]	No[c]	r[d]	P
$-\Delta N = 51.18 - 0.16A$[e]	54	0.31	0.02
SA_{48}[f] $= 16.211 - 0.049\ (-\Delta N)$	24	0.84	<0.001
$SA_{120} = 11.520 - 0.037\ (-\Delta N)$	30	0.87	<0.001
$cpm_{48} = 9922.1 - 11.7\ (-\Delta N)$	24	0.55	<0.01
$cpm_{120} = 7071.7 - 7.0\ (-\Delta N)$	30	0.87	<0.001

[a] $-\Delta N$ in mg = N — 623.4 (observed protein nitrogen content of skinned eviscerated carcass (N) compared with the value for 320-day-old mice). [b] Regression were calculated with a Control Data 6400 Computer. [c] No. = number of animals in each group. [d] r = correlation coefficient, [e] Age in days (from 320 to 750 days). [f] 48 and 120 refers to time in h after injection of ^{14}C-lysine.

H. SOBEL and R. BOWMAN[9]

Aging Research Laboratory, Department of Biochemistry, Colorado State University, Fort Collins (Colorado 80521, USA), 5 April 1971.

[1] H. SOBEL, Proc. Soc. exp. Biol. Med. *132*, 314 (1969).
[2] H. SOBEL and R. BOWMAN, J. Geront., in press (1971).
[3] H. SOBEL, H. E. HRUBANT and M. J. HEWLETT, J. Geront. *23*, 387 (1968).
[4] R. T. SHIMKE and D. DOYLE, Rev. Biochem. *39*, 929 (1970).
[5] J. R. COLVIN, D. G. SMITH and W. H. COOK, Chem. Rev. *54*, 687 (1954).
[6] L. J. KAPLAN and J. F. FOSTER, *Biochemistry 10*, 630 (1971).
[7] H. GERSHON and D. GERSHON, Nature Lond. *222*, 1214 (1970).
[8] S. OERIU and I. OERIU, Rev. Roum. Physiol. *5*, 273 (1968).
[9] Acknowledgment. This work was supported by NIH Grant No. AM1 4051 and Navy Contract No. NOO14-67-A-0299-0012.

Lettuce Seedling Growth: Antagonism Between Gibberellic Acid and Niacin

Niacin, a member of 'B'-complex vitamins, has been found to suppress germination of seeds and seedling growth of certain monocotyledonous plants when such seeds are presoaked with the aqueous solution of this vitamin[1]. Gibberellic acid antagonism to the action of many growth substances is also well known[2,3]. There appears to be no reference in the published literature to gibberellic acidniacin interaction in seedling growth. The present report summarizes observations on the effect of gibberellic acid and niacin, alone and in combination, on lettuce seedling growth.

Material and method. Lettuce seeds (*Laetuca sativa* var. Cabbage) were allowed to germinate in sterilized petridishes lined with filter paper and thoroughly moistened with each of the following solutions: 10 ml of niacin (500 ppm) combined with 10, 25, 50, 100 and 250 ppm of gibberellic acid (GA_3). For controls, separate petri dishes were used with filter papers moistened with niacin or GA_3 solutions in concentrations mentioned above or distilled water. Experiments were conducted in light (fluorescent tube light) at a temperature of 25 °C ± 2 °C.

Results and discussion. The effect of niacin (500 ppm) alone on growth of lettuce seedling is shown in the Table. It will be seen that the chemical, at the concentration used, strongly inhibited root growth[4]. GA_3 at concentrations between 25 to 100 ppm greatly increased the hypocotyl length, while the root growth was observed to be normal and similar to that of the seedlings grown in distilled water.

The combined effects of niacin (500 ppm) and GA_3 on root and hypocotyl growth have been recored in the Table. It is clear that GA_3, at the concentrations used (100 ppm and 250 ppm), greatly reversed the niacin-induced root inhibition. As a result the seedlings treated with GA_3-niacin combination indicated stimulation of root growth to the extent of 50% compared with those treated with niacin alone. It is, however, important to note that even an increase of GA_3 concentration up to 250 ppm in the presence of niacin (500 ppm) did not appear to affect hypocotyl growth to any significant degree. This would perhaps be of interest, since GA_3 is known to cause marked elongation in hypocotyl of lettuce seedlings[5,6].

The way in which niacin influences root growth is not clear. It is known, however, that niacin might disrupt the endogenous auxin levels by competing for tryptophan in the substrate induced biosynthesis[7,8]. On the other hand GA_3 is known to stimulate endogenous levels of auxins in many plant systems[9]. It would, therefore, appear possible that GA_3 reversed the inhibitory effect of niacin by affecting the endogenous auxin levels. This, however, remains to be tested in further experiments.

Zusammenfassung. Gibberellin und Niacin sind Antagonisten, Niacin hemmt das Wurzelwachstum von *Lactuca sativa*, während Gibberellin diese Wirkung teilweise aufhob.

PRAKASH MULLICK and U. N. CHATTERJI

Laboratory of Tissue Culture and Biochemistry, Department of Botany, University of Jodhpur, Jodhpur (India). 4 March 1971.

Combined effect of 500 ppm niacin and GA_3 on the growth[a] of radical and hypocotyl as compared with that of niacin and distilled water

Treatment	Length in cm	
	Root	Hypocotyl
Distilled water control	4.3 ± 0.3	2.6 ± 0.2
Niacin (500 ppm)	1.0 ± 0.2	2.4 ± 0.1
Niacin + GA_3		
500 + 10 ppm	1.1 ± 0.1	3.1 ± 0.1
500 + 25 ppm	1.0 ± 0.2	2.9 ± 0.2
500 + 50 ppm	1.3 ± 0.1	3.4 ± 0.1
500 + 100 ppm	2.5 ± 0.2	3.5 ± 0.2
500 + 250 ppm	2.4 ± 0.2	3.4 ± 0.1

[a] Results indicate growth of four days old seedlings.

1 PRAKASH MULLICK and U. N. CHATTERJI, Curr. Sci. *38*, 423 (1969).
2 M. M. LALORAYA and S. A. NAQVI, Science *133*, 1357 (1961).
3 M. M. LALORAYA and V. K. RAI, Ind. Plant Physiol. *5*, 218 (1962).
4 PRAKASH MULLICK and U. N. CHATTERJI, Curr. Sci. *40*, 40 (1971).
5 P. FRANKLAND and P. W. WAREING, Nature *185*, 255 (1960).
6 B. B. STOWE and T. YAMAKI, A. Rev. Plant. Physiol. *8*, 181 (1957).
7 A. NASON, Am. J. Bot. *37*, 612 (1950).
8 K. HASSE, *Encyclopedia of Plant Physiology* (Ed. W. RUHLAND, Springer-Verlag, Berlin 1958), vol. 9, p. 177.
9 S. KURAISHI and R. M. MUIR, Plant Physiol. *38*, 19 (1963).

Pantothenic Acid Distribution and Protein Synthesis in the Particulate Fractions of Rat Liver

It was reported in earlier communications[1,2] published from this laboratory that pantothenic acid exerted a marked effect on the growth of rats and that it stimulated the synthesis of blood proteins.

The present paper gives an account of the distribution of pantothenic acid in vaious particulate fractions of liver of rats receiving pantothenic acid supplements in the diet.

Materials and methods. It was observed from the experiments conducted earlier[1] in respect of ad libitum feeding, that the food consumption of the animals fed on a pantothenic acid deficient diet was much less than than those maintained on the adequate diet, and their growth-rate was also lower. In order to eliminate the effect of inantion from that of panthothenic acid deficiency, pair-feeding experiments were carried out. 4–5 week-old male albino rats, weighing 35–40 g, were distributed according to the body weight and litter mates into 6 pairs. The control rat in each pair was fed on the pantothenic acid deficient diet as described in the previous paper[2] and its pair-fed experimental mate was fed on the diet supplemented with calcium pantothenate (20 mg/kg diet). The actual amount of deficient diet consumed by the control rat was determined daily and an equal amount of experimental diet was fed to its pair-fed mate on the following day. Both the diets were equicaloric. The pair-fed mates consumed the entire amount of food given to them.

After the 10th week, when the typical symptoms of pantothenic acid deficiency, such as bloody whiskers and reddening of paws, were developed in the control group, the animals in both groups were sacrificed by decapitation and allowed to bleed profusely. The livers were quickly excized and placed in an ice-cold normal saline. They were blotted between the filter papers and weighed. The homogenates (10%) were prepared with isotonic sucrose (0.25 *M*) + $CaCl_2$ (0.0018 *M*) solution in Potter and Elveh-

1 V. P. SHINDE and S. D. AMBEGAOKAR, Ind. J. exp. Biol. *5*, 48 (1967).
2 V. P. SHINDE and S. D. AMBEGAOKAR, J. Nutr. Dietet. *4*, 189 (1967).