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The Utilization of Radioactive Glycine in the Livers of Normal and Depleted Mice

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George L. Nardi, M. D.

March 14, 1949

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Installation	Copy Nos.
Argonne National Laboratory	1-10
Armed Forces Special Weapons Project	11
Atomic Energy Commission, Washington	12-13
Battelle Memorial Institute	14
Brookhaven National Laboratory	15-24
Bureau of Ships	25
Carbide & Carbon Chemicals Corporation (K-25 Plant)	26-29
Carbide & Carbon Chemicals Corporation (Y-12 Plant)	30-33
Chicago Operations Office	34-41
Columbia University (Dunning)	42
Columbia University (Failla)	43
General Electric Company, Richland	44-50
Hanford Operations Office	51-58
Iowa State College	59
Kellex Corporation	60-61
Knolls Atomic Power Laboratory	62-65
Los Alamos	66-71
Massachusetts Institute of Technology (Gaudin)	72
Massachusetts Institute of Technology (Kaufmann)	73
Mound Laboratory	74-81
National Advisory Committee for Aeronautics	82-83
National Bureau of Standards	84-85
Naval Radiological Defense Laboratory	86-87
NEPA Project	88
New York Operations Office	89-96
North American Aviation, Inc.	97-98
Oak Ridge National Laboratory	99-110
Patent Branch, Washington	111
Sandia Base	112-113
Technical Information Branch, ORE	114-128
UCLA Medical Research Laboratory (Warren)	129
University of California Radiation Laboratory	130-135
University of Rochester	136-137
Western Reserve University (Friedell)	138-141
Sylvania Electric Products, Inc.	142
Total	142

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THE UTILIZATION OF RADIOACTIVE GLYCINE IN THE LIVERS OF NORMAL AND DEPLETED MICE*

by

George L. Nardi, M. D.**

Radiation Laboratory and Division of Medical Physics,
University of California, Berkeley, California

March 14, 1949

ABSTRACT

Mice maintained for 72 hours on normal diet, glucose diet and water diet were injected with C^{14} methylene-labeled glycine and sacrificed two, four and six hours after injection. Liver biopsies showed some vacuolization in the glucose animals and marked changes in the water animals. Total liver activity was determined and an aliquot hydrolyzed. The amino acid fraction of the hydrolysate was removed by passing the latter through an ion exchange column and the activity of the glucose fraction determined. The amino acid solution was concentrated, and two-dimensional paper chromatograms made and radioautographs of the latter were then produced in an attempt to determine the radioactive products. Glucose-fed animals showed the highest percentage of both active amino acids and active glucose, with the highest rate of radiocarbon turnover. Radioglycine was present in all livers. Radioserine seemed to be formed sooner in the normal and glucose animals. The water animals showed presence of radiocystine not present in the other two groups.

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* The work described in this paper was sponsored by the Atomic Energy Commission.

** While on leave from Massachusetts General Hospital, Boston, Massachusetts: Milton Fellow, Harvard University.

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The importance of an aeequate protein intake in convalescent patients has long been known and vividly illustrated by the experiments of Cannon (1)(2)(3) who established a correlation between plasma protein levels and wound healing and antibody formation. In an attempt to supply parenteral nitrogen, Elman (4)(5)(6) and Brunschwig (7)(8) have shown that hydrolysates of casein fortified with tryptophan have been effective in maintaining depleted patients on a positive nitrogen balance when the source of the latter

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** While on leave from the Massachusetts General Hospital, Boston, Massachusetts; Milton Fellow, Harvard University.

(1) P. R.Cannon, J. Am. Med. Assoc., 135, 1048 (1948).
(2) P. R.Cannon, Proc. Inst. Med. Chicago, 17, 1 (1947).
(3) E. R. Benditt, E. M. Humphreys, R. W. Wissler, C. H. Steffee, Jr., L. E. Frazier and P. R. Cannon, J. Lab. and Clin. Med., 33, 257 (1948).
(4) R. Elman, Ann. N. Y. Acad. Sci., 47, 345 (1946).
(5) R. Elman,"Parenteral Alimentation in Surgery," Paul. B. Hober Co., New York, 1947.
(6) R. Elman, Am. J. Med., 5, 761 (1948).
(7) A. Brunschwig, R. Bigelow and S. Nichols, J. Am. Med. Assoc., 129, 441 (1945).
(8) A. Brunschwig, N. T. Ricketts and R. Bigelow, Surg., Gynecol. and Obstet., 80, 252 (1945).

was entirely parenteral. It is a general clinical practice now to use such hydrolysates in maintenance of convalescent surgical and depleted patients. In attempts to make the utilization of these compounds more effective, investigations have been carried out to determine if the enzymatic hydrolysates which contain peptides in various forms do not supply factors which are necessary to metabolism and absent from the acid hydrolysates (which contain no peptides). (9)(10) It has also been adequately demonstrated that certain caloric requirements must be met before the amino acids can be utilized to form protein, otherwise they are broken down and transformed into glycogen, thus defeating the purpose for which they are given. Elman (11) has provided convincing evidence that if minimum caloric requirements are met with about 100 g. of glucose, there will be a high rate of conversion of amino acid to protein.

It was felt that by using tagged amino acids one might follow the activity into either the protein or glucose fraction. Because of its availability glycine labeled in the methylene position with C^{14} prepared by Ostwald (12) and having an activity of 1.01×10^7 dis./min./mg. or 4.57 μc/mg. was used.* The radioactive samples were counted on end-

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(9) A. Brunschwig, P. E. Clark and N. Corbin, Ann. Surg., 15, 1091 (1942).
(10) D. P. Cuthbertson, Am. J. Med., 5, 879 (1948).
(11) R. Elman, Bull. N. Y. Acad. Med., 20, 220 (1944).
(12) R. Ostwald, J. Biol. Chem., 173, 207 (1948).

* It is realized that glycine is not an essential amino acid and that only very tentative conclusions can be based on the use of a single amino acid; nevertheless, it was felt worthwhile to attempt such an experiment if only as a guide for future work.

window counters having geometry factors of 15 to 22 dis./count.

Nine "A" strain male mice were equally divided into three groups; the first group of three was supplied with its normal diet, the second group was given only a saturated solution of glucose in water while the third group was only allowed water. This diet was continued for 72 hours. The mice were then injected with about one mg. of radioglycine each and after that one mouse was killed with ether at 2, 4 and 6-hour intervals. The livers were removed in toto and a very small piece taken for histological section. The liver was then dried for 48 hours in a vacuum desiccator. Previous experiments (13)(14) have shown that no volatile activity is present and hence none is lost in this method of drying tissues. Approximately half the liver was then combusted in a quartz tube, the active CO_2 collected as $BaCO_3$, plated and counted, and the specific and total activity of each liver determined as described by Calvin et. al. (15) The uncombusted half of liver from each animal was then hydrolyzed in 6 N HCl over a steam bath for 12-18 hours and the solution filtered. A count of total activity was made of the filtered solution. It was felt that this solution represented a mixture of the liver amino acids, glycogen (in the form of glucose) and possibly other compounds. Since this was an acid hydrolysate, the solution could be passed through a cation exchange column (Dowex 50) thus removing the amphoteric amino acids and other cations while permitting the neutral glucose to pass through. The amino acids were then eluted with 2 N HCl and passed through an anion exchange column (Duolite A3) as a check on amino

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(13) G. L. Nardi, to be published.
(14) J. C. Reid and H. B. Jones, J. Biol. Chem., 174, 427 (1948).
(15) M. Calvin, C. Heidelberger, J.C. Reid, B.M.Tolbert and P. E. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, 1949.

acid activity. One could then calculate what percent of the total injected activity was present in the liver and what fraction of this is the glucose and amino acid fractions. By plotting the specific activity per mg. carbon one could also determine the turnover rate and percent of active carbon turned over per hour in each of the livers. These results are shown in Table I.

TABLE I

Mouse	% Activity in Liver	% Activity in Glucose	% Activity in Amino Acids	Biological Half-Life	% Turnover Per Hour
Norm 2	28	1.1	27	5 hours	35
Norm 4	32	1.5	30.5		
Norm 6	20	0.9	19		
Gluc. 2	42	1.9	40	3-1/2 hours	50
Gluc. 4	24	4.1	20		
Gluc. 6	24	7	14		
Water 2	33	2.8	30	15 hours	39
Water 4	20	2.1	18		
Water 6	29	3.9	25		

It was further felt that animals in varying stages of nutritional depletion might synthesize different amino acids from the same original compound supplied them (glycine in this experiment). Therefore, the recovered amino acid solution from the livers was concentrated and chromatographed on filter paper as described by Dent (16) and Zilch (17). Phenol

(16) C. E. Dent, Biochem. J., 41, 270 (1947).
(17) R. Consden, A. H. Gordon, A.J.P. Martin, Biochem. J., 38, 224 (1944).

and butanol-propionic acid were the solvents used. After the papers had dried they were placed in contact with Eastman No-Screen X-ray film and autoradiographs made and developed. The filter papers were then sprayed with a 0.1% alcoholic solution of ninhydrin to color the amino acids. When the autoradiographs were superimposed on the sprayed chromatograms one could determine which of the many amino acids present contained radioactivity derived from the original radioglycine. (See Benson and Calvin (18)). These results are summarized in Table II.

TABLE II

Mouse	Amino Acids Present	Relative Concentration	Other Products
Normal 2	x Glycine	++++	
Normal 4	x Glycine	++++	
	o Serine	++++	
	Alanine	Trace	
	Threonine	Trace	
	Proline	Trace	
Normal 6	x Glycine	++++	
	o Serine	++	
Glucose 2	x Glycine	++++	
	o Serine	++	
Glucose 4	x Glycine	++++	Glucose
	x Serine	+++	
	Glutamic Acid	++	
Glucose 6	x Glycine	++	Glucose
	o Serine	++	
Water 2	x Glycine	++++	
Water 4	x Glycine	+++	
	o Cystine	+	
Water 6	x Glycine	+++	
	o Serine	+++	
	Cystine	+	

(18) M. Calvin and A. A. Benson, Science, 109, 140 (1949).

From the results in Table I it would seem that protein depleted animals whose caloric needs are satisfied synthesized the greatest percentage of protein from injected radioglycine. The amount of radioglucose was also highest and they seemed to have the most rapid turnover rate of C^{14} in the liver. The water fed animals had a much slower turnover rate which one might expect from the gross damage seen in the liver on the biopsy slides.

Because of the heavy loading of the chromatogram papers required to obtain sufficient activity to obtain radioautographs within a reasonable period of time, the active amino acids autographed were not too clear cut. However, it can be seen from Table II that radioactive glycine was present in all the livers and that both the normal and glucose fed animals had produced considerable amounts of radioactive serine after 4 hours. This is in accord with the experiments of Greenberg et. al.(19) on liver homogenates and radioglycine. In addition, the glucose-fed animals seemed to show traces of radioglucose which checks with the higher percent activity found in the glucose fraction of the hydrolysates (Table I). The water-fed animals showed no radioserine until the 6-hour period which is in accord with the slower turnover rate of these livers. In addition, some radiocystine seemed to be present at both 4 and 6 hours; this was not found in the other two groups of animals.

SUMMARY

Mice maintained for 72 hours on a normal diet, glucose diet and water diet were injected with C^{14} methylene-labeled glycine and sacrificed 2, 4 and 6 hours after injection. Liver biopsies showed some vacuolization

(19) T. Winnick, F. Friedberg and D. M. Greenberg, J. Biol. Chem., 175, 117 (1948).

in the glucose animals and marked changes in the water snimals. Total liver activity was determined and an aliquot hydrolyzed. The amino acid fraction of the hydrolysate was removed by passing the latter through an ion exchange column and the activity of the glucose fraction determined. The amino acid solution was concentrated, and two-dimensional paper chromatograms made and radioautographs of the latter were then produced in an attempt to determine the radioactive products. Glucose-fed animals showed the highest percentage of both active amino acids and active glucose, with the highest rate of radiocarbon turnover. Radioglycine was present in all livers. Radioserine seemed to be formed sooner in the normal and glucose animals. The water animals showed presence of radiocystine not present in other groups.

CONCLUSIONS

The presence of glucose in the diet of protein depleted animals results in a higherconversion of amino acid to protein.

Animals which are depleted and are not given glucose to satisfy caloric requirements still convert a relatively high percentage of injected radioglycine to protein, though this process is slower and may proceed by different metabolic paths.