JOURNAL OF NUCLEAR MEDICINE 6:131-135, 1965

Determination of the Amino Acid Active Transport Into Tissue Cells[1,2]

Walter Henderson, B.S., Everett Bowser, M.S. and Gerald Williams, M.D.

Chicago, Illinois

The active transport of amino acids into tissue cells is an essential step in protein synthesis, and the rate and magnitude of this transport is often used as an indirect measurement of protein synthesis (1-4). Alpha-aminoisobutyric acid (AIB) is transported into cells similarly to naturally occurring amino acids, but being nonmetabolizable, it is neither degraded nor incorporated into protein (2). It is therefore possible, by utilizing this amino acid, to isolate and study the active transport process in different tissue cells, and to study the effect of various hormonal and other stimuli on this process (1, 2, 3, 5, 6).

In order to accurately determine the presence and magnitude of active transport, the concentration of ^{14}C-labeled AIB (AIB-^{14}C) should be determined in the intracellular and extracellular water of the tissue, and the data expressed as the AIB-^{14}C concentration ratio between these two compartments. Because of the variability of tissue water compartments among different body tissues, and the possible variation within the same tissue under different experimental conditions, it is advisable to determine the intracellular and extracellular spaces for each specimen of tissue analyzed for AIB-^{14}C.

The important problems to be solved in the development of the present methods were: 1) the accurate weighing of very small tissue specimens in the wet and dry states; 2) the desiccation and handling of these tissue specimens without partial or total loss; 3) use of a tracer of a substance, which is limited as much as possible to the extracellular compartment, preferably naturally occurring, and whose radioactive form may be detected in the presence of ^{14}C; 4) the most satisfactory method for counting two radioisotopes at low concentrations in the same sample; and 5) the construction of suitable formulae for calculation.

The method to be described allows all of these necessary techniques and determinations to be performed on the same very small tissue specimen.

---

[1]From the Radioisotope Service and Medical Service, Veterans Administration West Side Hospital; and the Department of Medicine, University of Illinois, College of Medicine, Chicago.

[2]Presented at the 11th Annual Meeting of The Society of Nuclear Medicine, Berkeley, California, June 17-20, 1964.

METHODS

As an example of the methods developed, a procedure is presented for the determination of active transport of AIB into the parathyroid and thyroid cells of rats which had been fed a normal or a low calcium diet.

Eight-week old, female Sprague-Dawley rats were divided into two groups. The control animals received a normal diet containing 1.7 per cent calcium and 0.8 per cent phosphorus, and the experimental animals received a diet containing no discernible calcium and 2½ per cent phosphorus. After three weeks, each rat received 15μc AIB-^{14}C (S.A. = 10 mc per millimole) intraperitoneally 20 hours before sacrifice and 7μc Na^{36}Cl (S.A. = 0.4 to 7.0 mc per gm) intraperitoneally two hours before sacrifice. (^{36}Chloride was used to allow determination of extracellular space. Chloride is almost entirely confined to the extracellular compartment, and chloride space, determined chemically, has often been used as a measure of extracellular water (7). In addition, the 0.714 mev β particles from ^{36}Cl can easily be distinguished from the 0.155 mev β particles from ^{14}C by liquid scintillation counting).

At the time of sacrifice, serum was collected and the thyroid and parathyroid glands were quickly removed under dissecting microscope observation. Each tissue specimen was quickly inserted into a previously weighed, specially constructed weighing envelope; and the wet weight determination, drying procedure, and dry weight determination were carried out by a previously described technique (8). The envelope, with its dry tissue contents, was placed in a standard liquid scintillation counting vial and the tissue was digested with 0.5 ml Hyamine hydroxide. Twenty ml of a Toluene PPO-POPOP, scintillation solution, were added, and the ^{14}C and ^{36}Cl activity determined, using a two-channel liquid scintillation counter (Tri-Carb Model 314EX)[1]. As the detected activity of some samples was only 20 to 100 cpm above background, they were counted several hours to reduce counting error to less than 2 per cent.

Two different methods of measuring the ^{14}C and ^{36}Cl activity were investigated. The simultaneous two-channel counting method (Table I) permits the counting of both radioisotopes at the same time. For the specific instrument and sample composition utilized in this study, the settings indicated in the table were found to give optimum counting efficiencies. However, there was some drift in the second channel at low gain over the long counting times required for low activity samples.

The second method, the consecutive single-channel counting method (Table II), requires two separate cycles of the samples through the counting chamber at different high voltage settings but constant window and gain settings. The samples were initially counted at 825 volts and then at 600 volts. Using this method eliminated the problem of amplifier drift and, in addition, the ^{36}Cl counting efficiency was increased from 52 to 74 per cent, accompanied by a reduction in background from 17 to 20 cpm. This consecutive single-channel counting method

[1]Packard Instrument Co, Inc., La Grange, Illinois.

was used in most of the studies. Neither method required the solution of complete simultaneous equations as the ^{14}C contribution when ^{36}Cl is counted can be neglected as less than 0.5 per cent.

When very small samples of tissue were counted, such as the rat parathyroid (approximately 0.3 mg) corrections for quenching were not necessary. When larger samples of tissues were counted, internal standards for both ^{14}C and ^{36}Cl were used to determine and allow correction for quenching.

Intracellular water (ICW) was then determined by subtracting the extracellular water content from the total water content, previously determined by weighing. The AIB-^{14}C concentration ratio was then determined in the following way:

$$\frac{ICWC}{ECWC} \quad \frac{TA - (ECW \bullet ECWC)}{\dfrac{TW - ECW}{ECWC}}$$

TA = Total AIB-^{14}C tissue activity.
ECW = Extracellular water volume.
ICWC = Intracellular water AIB-^{14}C concentration.
ECWC = Extracellular water AIB-^{14}C concentration. (Assumed to be equal to serum water AIB-^{14}C concentration)
TW = Total tissue water volume.

After determination of the disintegrations per minute (dpm) of ^{14}C and ^{36}Cl for each tissue and serum sample, the following formula was used to determine the extracellular water (ECW) of the tissue:

$$ECW = \frac{\text{Tissue } ^{36}Cl \text{ activity}}{\dfrac{\text{Serum } ^{36}Cl \text{ concentration}}{0.93 \bullet 0.95}}$$

0.93 = Correction for the per cent protein in serum.
0.95 = Correction for Gibbs-Donnan effect on chloride concentration. (7)

The above procedures and calculations were performed, using the data from the study of rats on low calcium and normal calcium diets, with the results shown in Tables III and IV. As shown in Table III, the low calcium diet had no effect on the active transport of AIB into thyroid cells as shown by the AIB-^{14}C concentration ratio, but did have a significant effect both on the parathyroid weight (2-fold) and on the active transport of AIB into parathyroid cells (3-fold). These results have been published in more detail elsewhere (4). The tissue water data from which these results were obtained are shown in Table IV. Although there was no significant difference in the per cent total water of either tissue between the control and low calcium diet groups, there was a significant difference between the two different tissues. There was also a significant difference in the per cent extracellular water between the control and low calcium diet

TABLE I

SIMULTANEOUS TWO-CHANNEL COUNTING METHOD

	First Channel	*Second Channel*
Voltage	825 volts	825 volts
Window	100–550	550–∞
Gain	1000	320
^{14}C efficiency	60%	<0.5%
^{36}Cl efficiency	11%	52%
Background	20 cpm	17 cpm

TABLE II

CONSECUTIVE SINGLE-CHANNEL COUNTING METHOD

	825 volts	*600 volts*
Window	100–550	100–550
Gain	1000	1000
^{14}C efficiency	60%	<0.5%
^{36}Cl efficiency	11%	74%
Background	20 cpm	12 cpm

TABLE III

EFFECT OF DIETARY CALCIUM INTAKE ON PARAMETERS OF PARATHYROID AND THYROID FUNCTION

	Parathyroid		*Thyroid*
	Wt (mg)	AIB-^{14}C Concentration Ratio	AIB-^{14}C Concentration Ratio
Control n = 7	0.295 ± 0.087*	5.9 ± 1.0	1.8 ± 0.25
Low Ca n = 7	0.636 ± 0.126	17.5 ± 4.1	2.1 ± 0.41

*Mean ± S.D.

groups for the parathyroid. These differences would not have been known nor taken into consideration if only a standard value for the per cent extracellular water had been used, or if water compartments had been disregarded.

TABLE IV

EFFECT OF DIETARY CALCIUM INTAKE ON TISSUE WATER

	Parathyroid		*Thyroid*	
	Total Tissue Water %	Extracellular Water %	Total Tissue Water %	Extracellular Water %
Control n = 7	80.8 ± 0.64*	36.8 ± 3.4	73.4 ± 0.40	34.5 ± 1.4
Low Ca n = 7	80.9 ± 0.85	53.5 ± 6.0	74.4 ± 0.79	44.4 ± 6.0

*Mean ± S.D.

SUMMARY

The described procedure allows the determination of the volume of the intracellular and extracellular water compartments on the same tissue sample as that used to determine the activity of the transported substance (in this case AIB—^{14}C). It therefore permits a more accurate measurement of active transport than by using separate tissue specimens for these determinations. This procedure has also solved many of the technical problems involved in working with very small specimens of fresh tissue. With this procedure it has been possible to measure the active cellular transport of AIB-^{14}C in specimens as small as 0.2 mg.

REFERENCES

1. NOALL, M. W., RIGGS, T. R., WALKER, L. M., CHRISTENSEN, H. N.: Endocrine Control of Amino Acid Transfer, *Science* **126**:1002, 1957.

2. KOSTYO, J. L., HOTCHKISS, J., KNOBIL, E.: Stimulation of Amino Acid Transport in Isolated Diaphragm by Growth Hormone Added in Vitro. *Science* **130**:1653, 1959.

3. RIGGS, T. R., WALKER, L. M.: Growth Hormone Stimulation of Amino Acid Transport into Rat Tissues in Vivo. *J. Biol. Chem.* **235**:3603, 1960.

4. WILLIAMS, G. A., HENDERSON, W. J., BOWSER, E. N.: Amino Acid Transport by Rat Parathyroid Glands in vivo: Effect of Low Calcium Diet. *Proc. Soc. Exp. Biol. and Med.* **116**: 651, 1964.

5. KIPNIS, D. M., NOALL, M. W.: Stimulation of Amino Acid Transport by Insulin in the Isolated Rat Diaphragm. *Biochim. Biophys. Acta.* **28**:226, 1958.

6. NOALL, M. W., ALLEN, W. M.: Early Stimulation by Estradiol of Amino Acid Penetration in Rabbit Uterus. *J. Biol. Chem.* **236**:2987, 1961.

7. MANERY, J. F.: Water and Electrolyte Metabolism, *Physiol. Rev.* **34**:334, 1954.

8. BOWSER, E. N., HENDERSON, W. J., WILLIAMS, G. A.: A Technique for the Determination of Weight and Water Content of Minute Tissue Specimens, *Microchem. J.* **7**:442, 1963.