Decreasing "Amino Acid Hunger" of Human Muscle with Age.* (24499)

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Recently we reported that the extent to which various tissues of the rat concentrate the non-metabolizable amino acid α-aminoisobutyric acid (AIB) steadily decreases with age (1,2). For example, at 50 g body weight the level estimated for muscle cell water averages over 4.5 times the serum level, whereas at 350 g the ratio is only about 2.5 to 1. AIB was selected for these experiments because it does not enter catabolism or protein synthesis, thereby permitting isolation for study of the transport process, which it enters in typical manner(3,4). The present communication reports a very similar observation in man.

Methods. Male patients without apparent disturbances of general metabolism, hydration or circulation were selected, who were also candidates for surgical procedures in which muscle samples probably could be obtained with minimal harm. A sterile solution of 1.43 microcuries (143 μg) kg body-weight of C_1^{14}-labeled α-aminoisobutyric acid in 0.5 to 1.2 ml 0.9% NaCl was injected into the deltoid muscle the day before surgery. This dose represents 100 microcuries of C^{14} (10 mg AIB) for a 70-kilo man, in a form with roughly a one-day biologic half-life and no detectible incorporation into body structures. Whenever surgery permitted, usually from 0.5 to 1.0 g of muscle was removed at 19 ± 1 hrs after injection. (To minimize the radioactive dose necessary and for technical reasons we allowed 19 hrs for the amino acid to be distributed, rather than the 39 hrs allowed in the rat.) A blood sample was taken from an antecubital vein 2 or 3 minutes later. The muscle sample was divided at once into 3 samples, placed in tared weighing bottles. One sample was used for water determination by drying at 110°. The second sample was extracted with 0.75 *M* nitric acid and the chloride determined; by comparison with serum chloride, the content of extracellular and cellular water could be estimated(5). The third portion of muscle was homogenized by the Potter-Elvehjem method, water and 0.1 *N* acetic acid being added to total 10 volumes/g of muscle. The mixture was held at 100° ten minutes, then cooled and filtered to yield an extract of pH about 5. 0.2 ml portions of this extract, or of blood serum, were plated in triplicate on stainless steel planchets and dried, and the disintegrations counted in a Geiger-Müller thin-window counter. Corrections for self-absorption were determined for the tissues used. In a few of the later experiments where muscle was not analyzed, the plasma proteins were precipitated by heating plasma diluted 1:4 at pH 5 and removed before counting. In this case 0.8 ml of extract were plated by applying first 0.4 ml, then taking to dryness before applying a second 0.4 ml portion. The counts per min were referred in each case to one gram of cellular or plasma water.

Results. Table I shows that for 3 men in their early fifties, the level of AIB calculated for muscle cell water was about *2* to *4 times* the level for plasma water. In contrast, muscle cells of 2 boys, 2.2 and 3.5 years old, appeared to concentrate the amino acid 11 *times* with respect to the extracellular phase; for a

TABLE I. Distribution of AIB between Serum and Muscle-Cell Water 19 Hours after Injection.

Age in yr	Serum (cpm/g H_2O)	Muscle (cpm/g H_2O)	Ratio
2.2	82	903	11.0
3.5	121	1385	11.6
9.0*	102	630	6.2
6 children, 2–9	117 (82–144)		
50	395	1500	3.8
54	244	961	3.9
54	636	1480	2.3
6 men, 34–54	331 (244–636)		

* Girl, elective leg amputation.

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9-year-old girl AIB was concentrated 6.2 times.

The subjects actually showed no great difference in their *muscle* levels of AIB, a finding which is not strange because they all received the same dose on a weight basis and muscle probably is the largest single site of retained amino acid. Instead the age difference is expressed almost entirely in the plasma level necessary to "back-up" the given tissue level. Extensive experimentation with various cells and tissues has established the concept that an ability to retain a given tissue level in the face of a lower extracellular level represents stronger concentrative activity(6-8).

A combination of 2 factors, an infrequency of patients who met the above criteria on the one hand, and reticence on the other, led us to take advantage of the observed striking difference in plasma levels and therefore to omit surgical removal of muscle tissue in the remaining subjects (Table I). These were normal persons or convalescent surgical patients. A mean 19-hr AIB level for plasma water of 117 counts/min/g for the 6 children contrasts with a mean of 331 counts/min/g for 6 men.

Two men excreted during the first 3 hrs 18.2 and 15.6% of the dose, during the second 3 hrs, 6.7 and 8.4%. Total excretion in the first 24 hrs of the first subject was 45.6%, for the second subject 41.7% in the first 19 hrs. A 4-year-old boy L.S. excreted 37.0% of the dose in 19 hrs.

The following technic is proposed for examining partition of AIB in various endocrinologic disturbances: After intramuscular injection all urine is collected for the standard interval of, say, 19 hrs. At this point a plasma sample is taken and counted. The *apparent volume of distribution* is then calculated, *i.e.*, the volume required to contain the AIB not yet excreted if concentration were uniformly that found in plasma. This procedure gave a volume of distribution at 19 hrs 2.6 times the estimated body water for the 34-year-old subject M.S., and 5.7 times the estimated body water for the 4-year-old L.S.

Fig. 1 shows the apparent volume of distribution of residual AIB, compared to calculated total body water content for an 80-kg man who received 190 microcuries of AIB

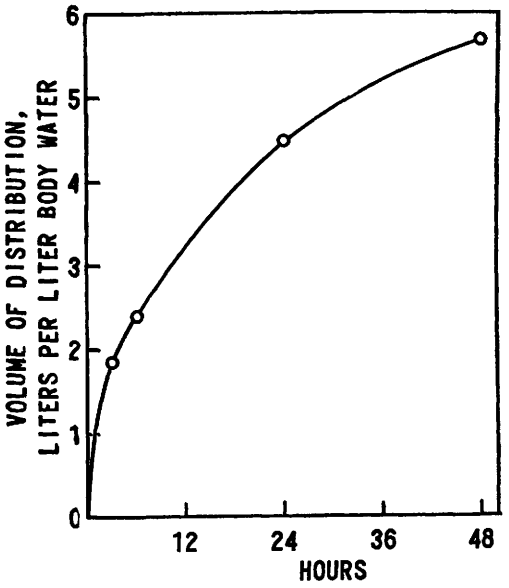

FIG. 1. Gradual increase of apparent volume of distribution of residual AIB remaining in 80 kg man (H.N.C.). Body water was taken to be 68% of body wt. Data on excretion rate appear in text.

intramuscularly. Average concentration in body water is about 4.5 times the plasma level at 24 hours. The further increase of the apparent volume in the second day may represent gradual entrance into less accessible compartments; it arises at least partly, however, from our neglect of minor routes of AIB loss from the body and therefore an increasing overestimate of the AIB left in the body. Comparison of plasma and urine levels during the second day yields a renal plasma clearance of AIB of 56 ml per minute, indicating that transfer out of the renal tubule back into the plasma, whether mediated or passive, is slower than for normal amino acids and insufficient to establish a gradient in favor of the plasma.

The respiratory carbon dioxide was collected as lithium carbonate (4 moles) for the first 6 hours of experiment. A slurry in methanol of the finely ground lithium carbonate was plated to infinite thickness on a planchet and counted for nearly an hour. The count was indistinguishable from background. If we take the maximum count to be one/minute above background, and the plate to represent effectively 25 mg of precipitate/cm^2 with a self-absorption of 86%(9), less than 2200 counts/min (or 0.002%) were collected from

the 111 million counts/minute injected. This result compares with a less sensitive earlier estimate of maximum catabolism of AIB in the rat of 0.02% (1).

Discussion. Although the test amino acid actually was present at very low concentrations, we believe that we should take the effective extracellular level entering the concentration process for most cells to be roughly 2 millimolar, representing the sum of the various neutral amino acids of plasma, since the concentrative process distinguishes only weakly among the neutral amino acids.

The foregoing effect of age was shown for a number of tissues in the rat, and undoubtedly is not limited to muscle in man. Upon stunting rats by calorie-restriction extent of tissue concentration associated itself more closely with body weight than with age(2). According to our interpretation, tissue "amino-acid hunger" is quantitatively associated with potentiality for growth or protein synthesis.

Summary. 1. Injected $C_1{}^{11}\alpha$-aminoisobutyric acid was much more strongly concentrated by skeletal muscle in children than in men. The greater general uptake by tissues could be recognized by the resultant consistently lower plasma level in the young. 2. The absence of catabolism of this amino acid was shown by observation of less than 0.002% of the injected C^{14} in expired air during 6 hours, counting respiratory CO_2 in the form of Li_2CO_3. After 24 hours average concentration in the body water was about 4 times that in the plasma water. A renal clearance of 56 ml of plasma per min was observed.

1. Noall, M. W., Riggs, T. R., Walker, L. M., Christensen, H. N., *Science,* 1957, v126, 1002.

2. Riggs, T. R., Walker, L. M., *J. Biol. Chem.,* 1958, v233, 132.

3. Christensen, H. N., Riggs, T. R., *ibid.,* 1956, v220, 265.

4. Christensen, H. N., Parker, H. M., Riggs, T. R., *ibid.,* 1958, v233, 1485.

5. Lowry, O. H., Hastings, A. B., *ibid.,* 1942, v143, 257.

6. Christensen, H. N., Streicher, J. A., Elbinger, R., *ibid.,* 1948, v172, 515.

7. Christensen, H. N., Cushing, M. K., Streicher, J. A., *Arch. Biochem.,* 1949, v23, 96.

8. Christensen, H. N., in *Amino Acid Metabolism,* W. D. McElroy, B. Glass, Eds., Johns Hopkins Press, Baltimore, 1955.

9. Hevesy, G., *Radioactive Indicators,* Interscience, N. Y., 1948, p49.

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Changes in Protein and Nucleic Acid Content on HeLa Cells Infected with Equine Abortion Virus.*† (24500)

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Study of the effects of equine abortion virus (EAV) on hamster liver has been reported (1,2). In this host, a rapidly lethal hepatitis usually results in death of the animal within 18 hours and is characterized by very numerous conspicuous intranuclear inclusions. Sequential studies of the infection have shown increased RNA and protein in infected nuclei isolated in citric acid, DNA remaining constant. A search for a better defined, easily attainable and readily susceptible system with which to investigate further the nature of chemical changes induced in hamster liver cells infected with EAV led to the use of HeLa cultures and this adaptation has been reported recently(3). Investigators have utilized tissue culture to demonstrate certain changes in host cell due to virus infection. Kovacs(4) demonstrated marked changes in activities of 9 enzyme systems of poliomyelitis-infected Rhesus monkey kidney epithelium. Maassab,

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