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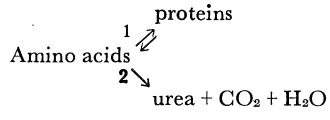
Endocrine Control of Amino Acid Transfer

Distribution of an Unmetabolizable Amino Acid

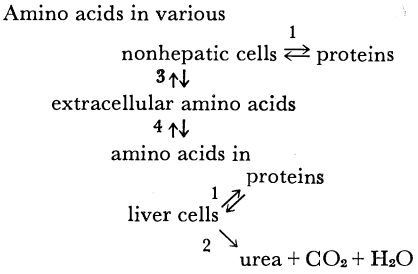
Matthew W. Noall, Thomas R. Riggs,
Lois M. Walker, Halvor N. Christensen

We can greatly oversimplify a description of amino acid metabolism by writing



The remarkable features which dominate amino acid metabolism in the higher animal are (i) the large shift from fate 1 (anabolic) to fate 2 (catabolic) which takes place when the animal passes from fetal life and infancy to adulthood and (ii) the large shifts which can be induced experimentally, either by administering a hormone or by producing conditions which stimulate secretion of hormones.

The hypophyseal growth hormone and certain androgenic steroids are recognized to have strong anabolic effects, and some of the adrenal cortical steroids, strong catabolic effects. In addition, other hormones, notably the estrogens, produce growth of particular tissues. Before trying to explain these effects, we must complicate the above scheme by remembering that anabolism and catabolism do not take place from a single homogeneous pool. We can write instead, as a second approximation



This scheme reflects the conclusion that most of the *net* degradation of amino acids occurs in the liver, whereas only hepatic proteins and some of the circulatory proteins are formed in the liver.

Steps 3 and 4 are *concentrative transfers* of the amino acids into the cells—that is, transport against concentration gradients, discovered in 1913 by Van Slyke and Meyer (*1*). The process has been extensively studied (*2, 3*), and may well be a restrained form of similar activities found in lower forms of life, which, however, have not yet been shown to be definitely concentrative.

Accordingly, concentrative transfer undoubtedly was developed before hormonal control; hormonal *restraint* appears, instead, to have been superimposed upon a primitive activity. In some microorganisms concentrative transfer may be substrate-induced (*4*). Concentrative transfer occurs across many cell barriers, such as the placental barrier (*5*), the renal tubular cells, and the intestinal mucosa, but also occurs across the cell barrier of most other cells so far studied (see Gale, *6*). It is a common step through which every amino acid must pass before it can be utilized. We have delayed calling the activity a "transportase" or "concentrase" (or, as one colleague has suggested, a "here-to-there-ase") until the enzymatic portions of the process have been more clearly demonstrated. A more objectionable term, *permease,* suggests incorrectly that we are dealing with the breakdown of barriers to diffusion, and should be rejected.

Paradoxically, we have observed many times that the free amino acid levels are higher in the more rapidly growing tissues, where they ought instead, if anything, to be depleted. This result has been observed in fetal life (*5*), in hepatic regeneration (*7*) and in neoplasia (*8*). Might an estrogen, for example, stimulate growth of the uterus, by increasing the extent to which that tissue concentrates amino acids? Might growth hormone increase the extent to which various tissues capture amino acids? Might the catabolic steroids increase particularly the hepatic capture of amino acids, thereby exposing them to accelerated destruction?

Such questions were asked (*9*), and the latter one tentatively answered in the affirmative, in 1948 (*7*), when hepatic amino acid levels were found to be increased in the rat after laparotomy. In

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the human patient, plasma amino acids are lowered by trauma or surgery or febrile illness; this effect might also arise from increased hepatic uptake.

Procedure and Analysis

Unequivocal demonstration of the regulation of amino acid metabolism at this common transport step has not been possible heretofore, although many observations have been in agreement with such regulation. The difficulty has been to distinguish possible effects upon anabolic and catabolic reactions themselves from effects upon transport. This has now been accomplished by the use of an unmetabolizable amino acid, α-aminoisobutyric acid (AIB), which undergoes concentrative transfer in an apparently normal fashion, being strongly concentrated by cells, and therefore only slowly excreted from the animal. The synthesis is described elsewhere (*10*).

The earlier observations of nonmetabolizability made at high dosage levels (*11*) were confirmed at tracer doses. At 1 milligram per kilogram of body weight, not over 0.02 per cent of the radioactivity was excreted as CO_2, nor could radioactivity be detected in tissue proteins.

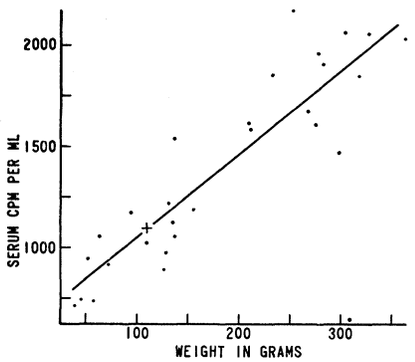

Fig. 1. Rising serum levels reached after administration of a standard dose of AIB, with increasing body size. One milligram of AIB per kilogram of body weight was injected 39 hours before analysis. The correlation coefficient is 0.883.

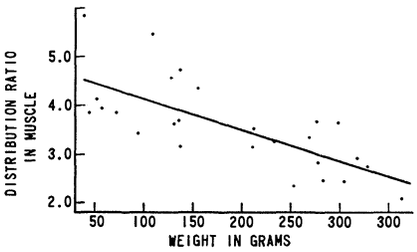

Fig. 2. Decreasing "amino acid hunger" of a tissue with increasing size of the rat. Same animals as in Fig. 1. The distribution ratio is the ratio of the tissue level of AIB to the serum level, both expressed in counts per kilogram of water. The correlation coefficient for the line is − 0.696.

Furthermore, AIB was excreted in the urine entirely in its unchanged form. Only one radioactive spot was detected on paper chromatograms [$R_f = 0.64$ in n-propanol–0.1N aqueous ammonia (1/1) and 0.83 in n-butanol–water–acetic acid (5/5/1), the amino acid fed giving the same R_f values]. Under these conditions even 1 percent of the dose excreted in another form might have been detected. Fecal excretion was very low. Appreciable levels of the amino acid were found present in the rat after 3 days, showing that a generous period was available for study of endocrine effects upon its distribution. We have waited either 20 hours (estradiol), 30 hours (epinephrine and growth-hormone) or 39 hours (all other experiments) after injection of AIB before observing its distribution. In all cases the animals were fasted about 15 hours before they were sacrificed.

For analysis, tissues were homogenized with 5 parts of very dilute acetic acid (to give a pH of about 5), and the extracts were heated to deproteinize them. Serum and urine were counted directly. A thin-windowed gas-flow counter was used throughout, correcting for self-absorption.

Effect of age or body weight on the distribution of the amino acid. Figure 1 shows that progressively higher serum levels resulted from the standard dose as the rat grew larger. This rising serum level was mainly caused by the declining activity of the tissues in concentrating the amino acid from the extracellular fluid, which is illustrated in Figure 2 for the skeletal muscle. The ordinate shows the "distribution ratio"—that is, the number of times that the amino acid appeared to have been concentrated by the cells; levels for the cellular and extracellular water were calculated by using available figures for the fluid compartment relationships.

The same result was obtained for a number of other tissues. The younger tissues showed a stronger "amino acid hunger," and the plasma level was thereby lowered; the older tissues lived in a richer medium and yet captured relatively less of the amino acid. This metabolically useless amino acid reflects the decreasing "amino acid hunger" because it corresponds structurally to the rather modest requirements for transport.

Adulthood is not as distinct an entity in the rodent as it is in man; it will be interesting to see whether this change in avidity of tissues for amino acids occurs more abruptly at maturity in the human.

Effect of epinephrine. Epinephrine has long been known to depress the level of the plasma amino acids, although in an unknown way. Figure 3 demonstrates that almost all tissues had increased levels of AIB 2 hours after 0.1 milligram of epinephrine per kilogram of body weight had been injected. With 12 animals (adrenalectomized females, 180 to 220 grams) in each group, the changes were statistically significant ($P < 0.02$) in the case of heart and kidney. The amino acid had been injected 30 hours earlier.

Pituitary growth hormone. Intramuscular injection of 1 unit of pituitary growth hormone in 200-gram female rats likewise intensified in 2 hours the concentration of AIB for all tissues studied except the heart (Fig. 4). Each group included nine animals. The changes for the liver, kidneys, duodenum, and the combined ovaries plus uterus were highly significant by statistical test.

Influence of hydrocortisone. The action of adrenal steroids was first discovered as an indirect effect of vitamin B_6-deficiency. This deficiency decreased the intensity with which various tissues concentrated AIB (*12*), supporting the role of pyridoxal in amino acid transport. The liver was a prominent exception, however, capturing if anything more AIB than usual in the deficiency; but when the animals first had been adrenalectomized, the behavior of the liver was no longer disparate.

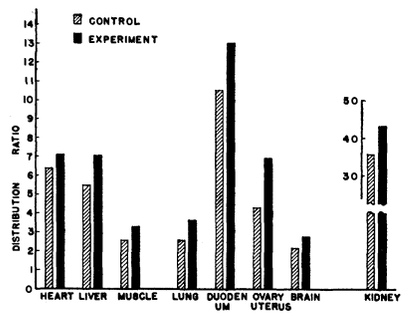

Fig. 3. Effect of epinephrine on the accumulation of AIB by adrenalectomized rats. The dose was 0.1 milligram per kilogram of body weight. Other values are averages for 12 animals.

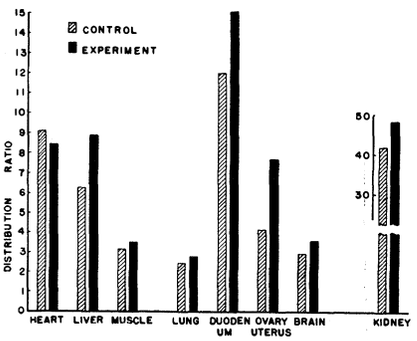

Fig. 4. Effect of growth hormone on the accumulation of AIB by rats. The dose was 1 unit per animal of body weight about 200 grams.

Figure 5 shows the normal relationship between serum AIB and liver AIB levels. The latter remained 8 or 9 times the concentration of the former. This is the kind of relationship that was found for glycine in the intact guinea pig in 1948 (*13*) and that has since been shown repeatedly with isolated cells and tissues.

Table 1 shows the disturbance in this relationship with the hepatic AIB capture increased by about 60 percent 2 hours after injection of 2 milligrams of hydrocortisone subcutaneously into the 135-g rat. The test amino acid (1 mg/kg) had been injected 39 hours earlier. This response in 2 hours is more rapid than the effect obtained by Engel upon urea formation (*14*). The prompt and vigorous action cannot be attributed to acceleration of amino acid catabolism: there *was no catabolism* of the model amino acid.

A question often discussed is whether the catabolic phase following surgery or trauma ought, if possible, to be eliminated or whether it serves a protective purpose, perhaps by flooding the injured site with building stones for repair. Such an effect is not to be expected, however, if catabolism is induced by intensified hepatic capture of amino acids. The tissue wastage appears to be initiated by a *pull* rather than a push, and therefore the catabolic phase probably does not flood the injured site with repair materials. A relatively slight intensification of the concentration of AIB by nonhepatic tissues was possibly also produced by hydrocortisone; but with a metabolizable amino acid this could scarcely compensate for the intensified destruction of the amino acid.

Referring again to the second scheme, we see that the liver not only destroys amino acids but also synthesizes them into a number of proteins. Interestingly, hydrocortisone accelerates the synthesis of plasma albumin (*15*) and of a number of liver proteins. Such an acceleration might be anticipated under the present thesis of the mode of steroid action. Hepatic gluconeogenesis from amino acids should also be stimulated. Furthermore, the retarding influence of administered sugar on amino acid catabolism (*16*) is readily understood.

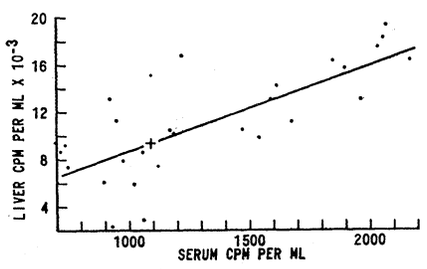

Fig. 5. Relationship between the serum and liver concentration of AIB in normal rats. The correlation coefficient is 0.744.

Table 1. Effect of administering hydrocortisone on AIB distribution. All values are thousands of counts per minute, per milliliter, followed by the standard error. The only statistically significant change is the intensification of the liver concentration. Although the hepatic levels could be seen by inspection to be clearly elevated, the following conservative method of calculation was used: The control levels for rats of the body weight taken (137 grams, standard deviation 13.5 grams) were obtained from regression lines versus body weight based upon 29 normal animals. The serum levels of the eight experimental animals averaged 1440 counts per minute, per milliliter; therefore one can predict the tissue values in the second column, based upon regression lines like those of Fig. 5.

Tissue	Normal distribution for a 137-g rat	Normal distribution for a 137-g rat, adjusted to a serum level of 1440 count/min ml	Found 2 hr after administration of hydrocortisone
Serum	1.21 ± 0.04	1.44	1.44 ± 0.10
Muscle	4.73 ± 0.31	5.78 ± 0.63	5.72 ± 0.29
Liver	10.17 ± 0.57	11.06 ± 1.71	17.50 ± 0.93
Kidney	45.4 ± 2.2	55.7 ± 4.7	64.2 ± 4.8
Heart	8.62 ± 0.66	9.87 ± 0.99	10.6 ± 0.39
Duodenum	18.9 ± 0.7	20.72 ± 1.45	18.54 ± 0.23

Estradiol. Four 21-day-old female rats were injected intraperitoneally with the tracer dose of AIB and subcutaneously with 0.1 microgram of estradiol in 0.1 milliliter of sesame oil. Four control animals received, instead, AIB and pure sesame oil. After 20 hours the animals were sacrificed, and samples of plasma, uterus, and liver were analyzed (Table 2). At the time of sacrifice the uterus had entered a phase of rapid growth under the influence of the estrogen (*17*). For the liver, no intensification of AIB concentration was observed, but now the uterus concentrated the amino acid by 280 percent of the original value.

Discussion

Several endocrine agents of widely different character are thus seen to influence the ability of cells to capture amino acids from the surrounding medium, in directions consistent with their growth-promoting and catabolic actions. The changes observed with AIB are consistent with many of the observations already made of hormone action on the distribution of the ordinary amino acids, indicating that this amino acid responds like other amino acids to humoral influence. The following effects, for example, have been shown: (i) decreased plasma amino acids accompanied by increased total free amino acids in the dog receiving growth hormone (*18*); (ii) decreased plasma amino acids after stress in man, increased liver amino acids after surgery in rats (*7*), and decreased plasma amino acids in rats receiving hydrocortisone (*19*); and (iii) decreased plasma amino acids after epinephrine injection.

For all of these changes there has always remained the possibility that the influences were at other points than the transfer step; but for AIB, possibilities other than the transfer reaction appear to be eliminated.

Probably we should be oversimplifying matters were we to imply that all cellular reactions which use amino acids are necessarily accelerated when a cell concentrates amino acids more strongly. Quantitative aspects, such as the extent and duration of action, must be considered. Furthermore, increased capture at a given site may be deprived of net effect if the amino acids are drawn away by simultaneously increased hepatic capture and destruction.

Finally there is the important question whether the amino acid-using reactions may already be saturated with respect to the various amino acids, so that no stimulation would result from elevated levels. Clearly, this is not the case for catabolic breakdown in the liver; we all know how readily this rate adjusts itself to the level of protein intake; therefore one may confidently expect accelerated amino acid breakdown to follow intensified amino acid concentration by the liver.

Table 2. Change in the accumulation of AIB by immature uterus upon administration of estrogen. Each value represents results obtained from one animal. The concentration by the livers of the same animals was not detectably altered.

Distribution ratio, uterus/serum	
Control	Experimental
5.55	16.6
4.23	13.6
5.01	14.7
4.83	9.62
Mean 4.90	Mean 13.6

For reactions of protein synthesis, the question has not been answered as completely. Broad ranges have been observed over which the rates of incorporation of an amino acid or of the net synthesis of protein *in vitro* do vary with the amino acid level; but such ranges are not yet adequately defined for the various tissues *in situ*. In addition, mutual supplementation experiments among the amino acids have shown how readily one amino acid may fall to levels suboptimal for growth.

Furthermore, the finding should be considered that amino acid levels are rather uniformly high where protein synthesis or growth is accelerated. Before we can imply a causal relationship in this association, we must consider one alternative explanation. Protein synthesis undoubtedly proceeds through activated amino acid intermediates; by spontaneous dissociation, these intermediates (or the proteins themselves) might release amino acids at high levels. At high synthetic rates such intermediates might be more abundant, and the free amino acids could therefore reach higher levels. (Such a hypothesis must also assume that the precursor amino acids are taken from one compartment—for example, the cell exterior—and that the intermediates are dissociated into another—for example, the cell interior). For example, when the immature uterus entered an estrogen-stimulated growth phase, perhaps the high amino acid levels were a secondary expression of the high rate of protein synthesis.

Such an explanation becomes highly improbable, however, for the present observations. α-Aminoisobutyric acid is not incorporated into protein, and it is unlikely that it proceeds even to intermediate stages of that synthesis. For example, no stimulation by it of the exchange of radioactive phosphorus between pyrophosphate and adenosine triphosphate has been detected (*20*). Accordingly, the reaction which was stimulated upon administration of estrogen was most likely the transfer reaction itself.

Note that estradiol intensifies AIB concentration by the uterus and not by the liver, whereas hydrocortisone intensifies mainly the hepatic capture. If we assume that the same amino acid carrier operates in these two tissues, there must be local factors which make the transport more susceptible to an endocrine agent in one tissue than in the other. For example, a particular hormone molecule might gain access to the transport apparatus more readily in one than in the other.

Apparently we have in nitrogen metabolism additional instances of endocrine control operating on transfer reactions. Certain other steroids are already recognized to influence the transfer of Na^+ and K^+ between cells and surrounding fluids, and insulin has been shown to increase the access of sugars to the cell interior (*21*). The fate of the amino acids appears to be collectively modified by changing the extent to which various cells concentrate amino acids from the extracellular environment.

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22. The experimental work described in this article was supported in part by a grant (C-2645) from the National Cancer Institute, U.S. Public Health Service. One of us (M. W. N.) held a fellowship of the American Cancer Society during the major part of this work.

Ward Vinton Evans, Physical Chemist

On 2 August 1957, Ward Vinton Evans died in Rawlinsville, Pennsylvania, where he had been born 77 years before.

His professional activities had made him well known within his own scientific group, but his part on the world stage did not come until 1954 when, well into his 70's, he served on the special three-man security board which reviewed charges against J. R. Oppenheimer. Before this, he had served on a number of review boards dealing with security clearances in the Chicago area. His dissent from the majority finding against Oppenheimer attracted world-wide attention. As the *Washington Post* said in an editorial on his death, "his pungent dissent from the majority finding against Dr. Oppenheimer, written in earthy language, stands as a model of clarity and common sense. The failure to clear Dr. Oppenheimer, he noted, will be a 'black mark on the escutcheon of the country.' Conservative in his politics and personal views, Dr. Evans had a refreshing tolerance for disagreement and idiosyncrasy once basic loyalty was established. He knew that a narrow conformity in thought and action produces stereotyped minds. He followed his own philosophy of tolerance, and those who were exposed to it will not soon forget him."

"Doc" (he was so universally known as "Doc" that, even here, it seems inappropriate to refer to him in any other way) entered his chosen profession somewhat late. He received his bachelor's degree from Franklin and Marshall in 1907 (and an honorary D.Sc. in 1932). Following graduation he taught high school for 6 years in Pennsylvania and New York before commencing graduate work at Columbia University. He was 36 when he was awarded the Ph.D., in 1916. He remained at Columbia for a year as Harriman fellow and then joined the faculty of the department of chemistry at Northwestern University, where