Amino Acid Transport by the Hepatic Cell in Cloudy Swelling. (27629)

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Hepatic cloudy swelling, a form of cell degeneration easily obtainable by various procedures(1), is characterized by an increase in cell volume partially due to an increase in cell protein content(2). A faster rate of incorporation of labelled glycine into protein suggests that this increase may be the result of an enhanced protein synthesis(3), which, in turn, may be primary or secondary to an accelerated transfer of amino acid across the cell membrane. The latter possibility is supported by the observation of a slight decrease of cellular K(2), a result believed to be related to increased amino acid transport(4). The purpose of this work was to study the amino acid transfer across the membrane of normal and swollen liver cells. α-Aminoisobutyric acid (AIB), a non-utilizable amino acid analogue which seems to be transported in a manner similar to that of natural amino acids(5), has been selected as a model for the present study. Evidence suggesting that the transfer of AIB into swollen liver cells is faster than its transfer into normal cells will be presented.

Methods. Male Wistar rats, weighing 160-180 g, were injected with *S. typhimurium* toxin to produce cloudy swelling(1). After a 12-hour fast, control and injected animals were decapitated and their livers removed.

AIB transfer. About 100 mg of liver slices were incubated in open 25-ml flasks in a Dubnoff metabolic shaker at 38°C. The incubation medium had the following composition: NaCl, 1.28×10^{-1}M; KCl, 1.35×10^{-2}M; $CaCl_2$, 1.94×10^{-3}M; $MgSO_4$, 6.8×10^{-4}M; Na phosphate buffer, 1×10^{-2}M (pH 7.4); when present, inulin had a concentration of 0.5 mg/ml of medium. AIB-1-C^{14} (specific activity 0.33 mc/mmole) was added after 10 minutes of equilibration at a final concentration of 1×10^{-3}M. The final salt concentration of the medium was adjusted to an osmolarity of 0.305M. Routine procedure consisted in duplicate determinations of wet weight, tissue and medium radioactivity, extracellular space and total water of the samples from each liver at appropriate intervals throughout the incubation period. AIB extraction was carried out with boiling water (6,7) after careful blotting of the slices on acid-hardened Whatman filter paper. Extractions with 8mM acetic acid(8), 1% TCA (9) and saturated solution of picric acid(10) gave essentially the same result. Medium and extraction fluid samples were transferred to stainless steel planchets painted with shellac to bind the residue, evaporated under a lamp and counted beneath a mica window G.M. counter. Counting error was less than 2%. Extracellular space was calculated from values of inulin distribution. Inulin was determined using the method of Roe *et al.*(11) according to Del Monte(12) and Swan *et al.* (13). Total water was determined by the difference between the weight of the fresh tissue after blotting and the weight after drying at 100°C for 24 hours.

Liver cellularity. The number of nuclei per gram of wet tissue was determined in homogenates using a hemocytometer(14); nuclear populations were investigated histologically according to Abercrombie and Harkness (15). The incidence of binucleate parenchymal cells was estimated microscopically on smears after isolation of the cells with a modified Anderson technic(16).

Results and discussion. Fig. 1 shows the kinetics of inulin distribution into the extracellular space. It will be noted that inulin space increases very slowly after the first 10 minutes of incubation, reaching values of about 0.2 ml/g of normal liver tissue in about 45 minutes. The value of the extracellular space at 0 time may be obtained by extrapolation. These values agree well with those reported by Swan *et al.*(13). Intracellular water values during the incubation are presented in Table I. The slight decrease of the intracellular water with time, shows that no cell imbibition occurs during the incuba-

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TABLE I. Intracellular Water of the Liver during Incubation. % of wet wt.

Incubation time (min)	Normal liver	Swollen liver
0	51.8*	59.0
10	51.8	59.0
20	50.7	59.1
30	50.6	59.0
45	49.0	59.0
60	49.4	58.8
90	49.0	58.8

* Each figure is the mean of 8 experiments. Values are calculated by difference between total water and extracellular water.

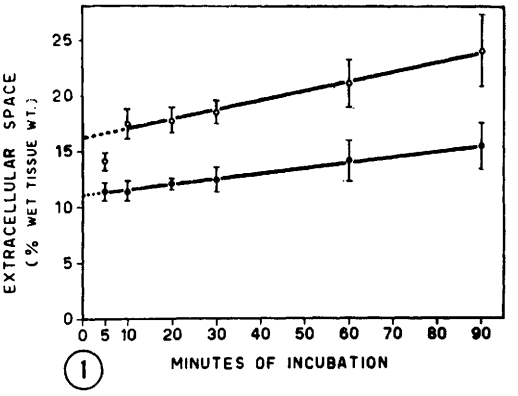
tion of normal and swollen liver. Fig. 2 provides data from experiments of AIB accumulation in the intracellular water as a function of the incubation period. A two parameters chi square test(17) applied to these data shows that the intracellular concentration of AIB in normal and swollen liver approaches a constant value exponentially according to an equation of the type:

$$y = K(1-e^{-kt})$$

where K is the asymptotic value of the curve, k has the dimension of a time constant and t is the time in minutes. The degree of confidence is greater than 75%. Actually a steady state was not reached in the 90 minutes incubation period and the theoretical asymptotic values (K best) were extrapolated using the chi square test ($t = \infty$, Fig. 2).

The curves show that swelling enhances the rate of AIB transfer and doubles the value of its final distribution in the intracellular water. The maximum intracellular concentration of AIB in normal hepatic cells is not greater than its concentration in the medium showing that, under our experimental conditions, AIB does not penetrate normal cells against a concentration gradient, while a net concentrative transfer occurs in the swollen liver. A concentrative transfer using extracellular concentrations of AIB lower than 10^{-3}M was reported for kidney(18) and diaphragm(8).

An attempt was made to calculate the absolute amount of AIB present in normal and swollen hepatocytes at the steady state. From data shown in Table II, it may be calculated that one gram of normal liver contains approximately 80.8×10^6 hepatocytes, while one gram of swollen liver contains only 70.6×10^6 cells. Correcting for the volume occupied by non-parenchymal cells (roughly 5% and 6-7% of the wet weight in normal and swollen liver, respectively(19)) and for the average extracellular water volume at 45 minutes, one finds that 80.8×10^6 normal hepatocytes weigh 0.75 g and that 70.6×10^6 swollen hepatocytes weigh 0.8 g. It follows



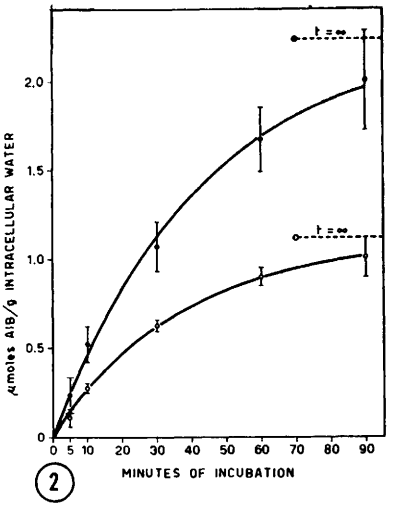


FIG. 1. Extracellular space of normal (○—○) and swollen (●—●) rat liver, estimated by inulin distribution. Each point represents the mean of 8 experiments ± S.E. Curves derived by the method of least squares. Dotted lines extrapolate the curves to 0 time.

FIG. 2. Accumulation of AIB in the intracellular water of normal (○—○) and swollen (●—●) rat liver. Each point is the mean of 3 to 8 experiments ± S.E. Initial concentration of AIB in the incubation medium was 1×10^{-3}M. Curves were drawn according to the exponential equation given in the text. Dotted lines ($t = \infty$) represent the asymptotic values. Normal liver: $K = 1.12 \pm 0.04$ μmoles/g intracellular water; $k = 0.027 \pm 0.002 \times \text{min}^{-1}$. Swollen liver: $K = 2.23 \pm 0.15$ μmoles/g intracellular water; $k = 0.023 \pm 0.003 \times \text{min}^{-1}$.

TABLE II. Cellularity and Water Content of the Liver.

	No. of nuclei per g of wet wt ($\times 10^6$)*	Parenchymal nuclei (% of total cell population)*	Parenchymal binucleated cells (% incidence)	Water (% of wet wt)* Total	Intracellular
Normal liver	169 ± 32†	61.0 ± 3.9‡	27.6§	69.2 ± .6‖	49.0
Swollen "	171 ± 28	52.9 ± 3.6	28.2	72.2 ± .4	59.0

* Measurements refer to samples of liver slices incubated for 45 min. Values of intracellular water are calculated by difference btween total water and extracellular water after 45 min incubation.

† Mean of 8 experiments ± S.D. Triplicate determinations were averaged.

‡ Mean of 8 experiments ± S.E. In each experiment number of nuclei was counted in 10 microscopic fields (0.01 mm²).

§ 4000 isolated cells were examined.

‖ Mean of 8 experiments ± S.E.

that the volume of the average normal and swollen hepatocyte, expressed in g, will be approximately 9.3×10^{-9} g and 11.3×10^{-9} g, respectively.

Since intracellular water represents 49% of normal and 59% of swollen liver, disregarding the small error introduced by the presence of non-parenchymal cells, the absolute amount of water of the average hepatocyte will be 4.6×10^{-9} g in the normal and 6.7×10^{-9} g in the swollen liver. At the steady state, the concentration of AIB is 1.12 μmoles per g of intracellular water in the normal and 2.23 μmoles per g of intracellular water in the swollen liver (Fig. 2). Hence the absolute amount of AIB in the average normal and swollen hepatocyte will be 5.2×10^{-9} μmoles and 14.9×10^{-9} μmoles respectively. This means that the amount of AIB transported across the hepatocyte membrane in swollen liver is about 3 times as great as that transported into the normal cell. Since cell surface increases less than cell volume as swelling takes place, it may be suggested that in the normal liver and at the external concentration used in our experiments, amino acid transport is submaximal and that the transport sites(20) on the cell surface are not saturated.

With the basic assumption that AIB is transported in the same fashion as the naturally occurring amino acids, the accelerated rate of amino acid transfer across the membrane of the swollen liver cell could readily explain the increase of the amino acid incorporation into protein found in such condition. Indeed, the reported 2-fold increase of the rate of AIB transfer closely parallels the increase in incorporation rate of glycine(3) and leucine into protein of swollen liver (unpublished experiments). However, 2 further assumptions are needed to accept this interpretation: 1) that the intracellular amino acid pool has the same size in normal and swollen liver since a smaller pool would lead to a smaller dilution of the entering labelled amino acid and, consequently, the protein would be labelled faster even if the rate of synthesis remained the same; 2) that the intracellular amino acid pool is functionally homogeneous with respect to protein synthesis. This assumption has been questioned as a result of recent studies on bacteria(21), yeast(22), molds(23) and mammalian cells(24), which seem to indicate either a structural or chemical compartmentalization of the intracellular amino acid pool. Experiments showing the existence of a labile lipid-bound form of amino acid, perhaps restricted to a non-polar compartment of the cell are in progress in our laboratory.

These conflicting observations suggest that a careful examination of the various factors, such as amino acid penetration rate, intracellular pool size and kinetics of amino acid incorporation is necessary when protein synthesis by surviving tissues is being studied with the aid of labelled amino acids.

Summary. Amino acid transport in normal rat livers and in livers with toxin-induced cloudy swelling has been studied using α-aminoisobutyric acid (AIB), an amino acid analogue which undergoes concentrative transfer similar to that of the naturally occurring

amino acids. Under the experimental conditions used in this work, the intracellular concentration of AIB approaches equilibrium exponentially in both types of tissue. Swelling markedly enhances the rate of AIB transfer across the cell membrane and doubles the value of its final distribution in the intracellular water. This increase is still higher if expressed in terms of individual cells. The significance of these observations with respect to the increased incorporation rate of labelled amino acids into protein found in livers with cloudy swelling is discussed.

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Distribution of Chromatin Bodies in an XX/XY True Hermaphrodite.* (27630)

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The discovery by Barr and Bertram(1) of the chromatin body in the nerve cells of female cats set the stage for the development of nuclear sexing as an important clinical tool. However, the origin of the chromatin body is still not clear although a number of theories have been proposed to explain this feulgen-positive structure found in intermitotic nuclei of most female cells. Barr originally suggested that the chromatin body was due to somatic pairing of the heterochromatic parts of 2 X chromosomes. The observation in domestic chickens(2), in which the female is the heterogametic sex, that the female is chromatin positive and the male chromatin negative was contradictory to Barr's hypothesis, and also raised the possibility that the chromatin body might be a sex influenced character subject to extracelluar factors. Several observations on different intersexual conditions, however, indicate that the chromatin body is more likely cell autonomous. For example, in the testicular feminizing syndrome, where XY cells have been subjected to a female hormonal environment, the cells are chromatin

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