# Free Amino Acids of Cytoplasmic Fractions of Liver.* (20067)

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Information on the concentrations of the free amino acids in mitochondrial preparations and other cytoplasmic fractions of liver is of interest in connection with the problem of the biosynthesis and dissimilation of proteins as well as of other problems of cellular metabolism. Such information is lacking in print. In the present investigation the concentrations of 13 amino acids have been determined by microbiological assay in mitochondrial fractions prepared in two ways and on the supernatant fluid containing microsomes from the centrifuged mitochondria. The effect of a 2-hour incubation period on the free amino acid concentrations also was determined.

*Experimental. Preparation of cytoplasmic liver fractions.* The livers of fed, stock rats were employed. All steps of the isolation procedures were performed in the cold at 0-5°C. Centrifugation was done in an International refrigerated centrifuge for low speeds and in a Spinco preparative ultracentrifuge for high speeds. The incubations were carried out in a constant temperature bath at 37-38°C in rotating tubes. *Buffer mitochondrial preparation ($M_B$).* This was prepared according to the procedure of Peterson and Greenberg (1) employing a buffer solution composed of 0.12 M KCl-0.04 M $KHCO_3$ saturated with 95% $O_2$-5% $CO_2$. *Sucrose mitochondrial preparation ($M_s$).* The isolation procedure was a modification of the 0.25 M sucrose method of Schneider and Hogeboom(2) designed to cut down the preparation time because of the lability of the mitochondria in amino acid incorporation experiments. The essence of the procedure was one centrifugation at 2500 r.p.m. (1100 x g) for 3 minutes to remove the nuclear fraction, two centrifugations of the supernatant fluid at 1600 r.p.m. (450 x g) for 3 minutes each, and two centrifugations of the mitochondria sediment suspended in the 0.25 M sucrose in the Spinco ultracentrifuge at 10,000 r.p.m. (6,590 x g) for 20 minutes each. The sediment, after the final centrifugation, was diluted with an equal volume of the KCl-$KHCO_3$ buffer and aerated with the 95% $O_2$-5% $CO_2$ gas mixture in preparation for incubation. The ratio of ribose nucleic acid to nitrogen of mitochondria prepared in this manner became constant

TABLE I. Free Amino Acid Contents of Cytoplasmic Fractions.

| | Supernatant | | Buffer-mitochondria | | Sucrose-mitochondria | |
|---|---|---|---|---|---|---|
| | B.I.* | A.I.* | B.I. | A.I. | B.I. | A.I. |
| Nitrogen (γ/ml) | 2.3 ± 0 | 2.4 ± .11 | 2.1 ± .04 | 2.2 ± .13 | 1.4 ± .04 | 1.4 ± .08 |
| Amino acids (γ/ml) | | | | | | |
| Alanine | 6.5 | 11.3 | 1.4 | 5.5 | 1.6 | 4.6 |
| Arginine | 1.2 | .7 | 1.4 | 2.1 | .4 | .7 |
| Aspartic acid | 4.1 | 1.9 | 4.2 | 8.8 | 1.3 | 4.8 |
| Glutamic acid | 38.9 | 58.2 | 13.6 | 25.9 | 2.6 | 1.9 |
| Glycine | 10.0 | 12.1 | 5.0 | 13.3 | 4.7 | 10.1 |
| Isoleucine | 2.0 | 2.0 | 2.2 | 10.8 | — | — |
| Leucine | 2.4 | 2.5 | 2.8 | 11.4 | 6.4 | 8.7 |
| Lysine | 3.0 | 4.6 | 2.1 | 8.4 | 1.3 | 2.3 |
| Phenylalanine | 1.0 | 1.1 | 1.2 | 6.8 | 2.4 | 4.1 |
| Proline | 1.5 | 2.3 | 1.7 | 3.0 | 3.1 | 2.6 |
| Tryptophan | .3 | .1 | .0 | .2 | .2 | .6 |
| Tyrosine | 1.1 | .4 | .7 | 3.8 | 2.2 | 4.1 |
| Valine | 2.0 | 2.3 | 2.5 | 10.3 | 3.3 | 6.3 |

* B.I., before incubation; A.I., after incubation.

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after the first resuspension. *Supernatant (S).* The supernatant fluid from a 1:1 liver homogenate in buffer, centrifuged for 30 minutes at 4,000 r.p.m. (2700 x g) was employed for this fraction. *Preparation of filtrates for microbiological assay.* These were prepared according to Schurr *et al.*(3) by adding 2.5 ml of distilled water per ml of protein preparation in each tube and heating at 100°C for 5 minutes. The heated solutions were homogenized, and freshly prepared 10% sodium tungstate and 0.6 N $H_2SO_4$ (5:7) was added in the amount of 1.5 ml per ml of original protein preparation. To remove all precipitated material, the mixtures were centrifuged twice at 2000 r.p.m. (700 x g) for 10 minutes, once at 10000 r.p.m. (6590 x g) for 20 minutes and then filtered. Aliquots of the filtrates were employed for microbiological assay. The final dilution of the mitochondria and supernatant fractions was 10 times the original volumes in fresh tissue.

*Microbiological assays.* All preparations were adjusted to pH 7.0 and the free amino acids determined microbiologically according to the method of Henderson, Brickson, and Snell(4). A Cannon dispenser and titrator was employed to dispense the samples and media and to titrate the lactic acid formed. The samples were dispensed in volumes from 0.05 to 0.25 ml, the total volume per assay tube being made up to 0.50 ml.

The following modifications in procedure were made for assay of certain individual amino acids: For determination of alanine the samples were irradiated and $B_6$ emitted from the basal medium. Glutamic acid was determined after glutamine had been decomposed by autoclaving samples at 18 pound steam pressure for 20 minutes. A basal medium described by Dunn and coworkers(5) was purchased† for the determination of proline and aspartic acids. Good agreement was found for the different assay levels over the range of the standard curves of the amino acids tested.

*Discussion.* The data for the free amino acid concentrations expressed as γ/mg protein N, are given in Table I. The change in amino acid concentrations (determined as γ per ml, of each preparation suspended in an equal volume of buffer) during incubation are plotted in Fig. 1. The deviations from the means are represented by the vertical lines. Besides the 13 amino acids shown in the figure, determinations were also made of methionine, but

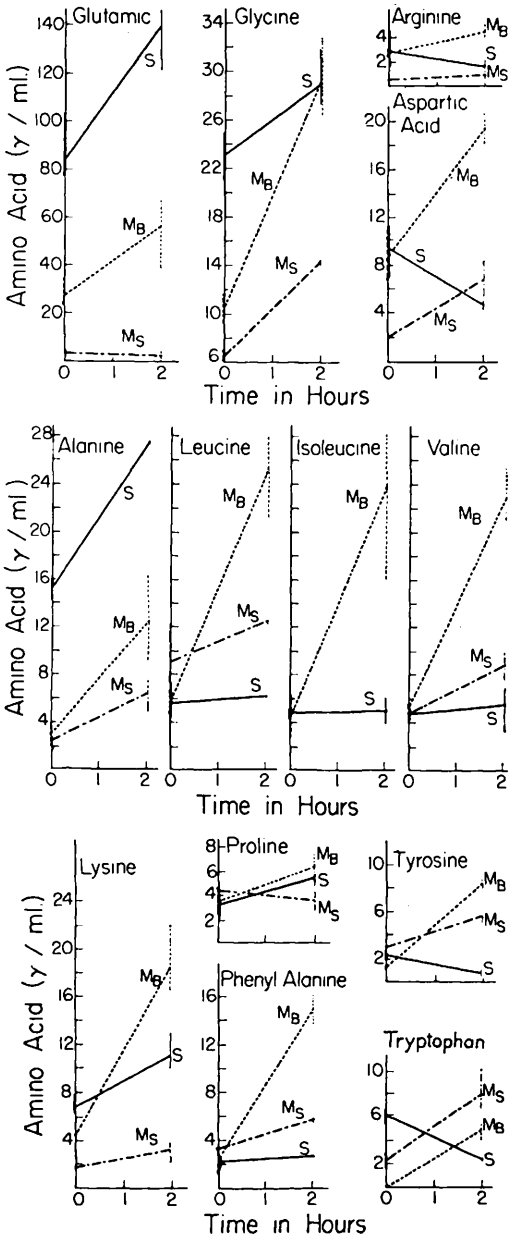


FIG. 1. Free amino acids of cytoplasmic fractions of liver before and after incubation at 37°C. S, supernatant fluid; $M_B$, buffer-prepared mitochondria; $M_s$, 0.25 M sucrose-prepared mitochondria.

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the values were too low ($<1$ γ/ml) to be reliable and therefore are omitted. Analysis of sucrose-prepared mitochondria for isoleucine was not complete due to a variation in the properties of the organism which developed at that point.

It is clear that the concentrations of amino acids should be lower than the true values *in situ* because of the losses due to the washing procedures employed in the isolation. The sucrose preparations have a one third less nitrogen content and, in general, show a lower concentration of the free amino acids, although there is no 3:2 proportionality, as would be expected from the nitrogen analysis. Table I shows that for certain of the amino acids the concentration is the same prior to incubation for the buffer or sucrose-prepared mitochondria. In other instances the buffer-prepared mitochondria and the supernatant fluid have closely agreeing concentrations. The free amino acid concentrations in the mitochondrial preparations were calculated to range between 25 to 75% of that determined in our laboratory for whole liver homogenate.

Those amino acids which are relatively stable metabolically and are also essential amino acids (Fig. 1), or are derived from essential amino acids, increase in both mitochondrial fractions upon incubation, but remain approximately unchanged in the microsome-containing supernatant. This may indicate an extensive hydrolysis of protein within the mitochondria and but little in the supernatant and microsomal fractions. In the group of essential amino acids, a significantly greater increase occurs in the buffer-prepared than in the sucrose-prepared mitochondria with the exception of phenylalanine.

The data show that there are appreciable concentrations in the mitochondria of the 6 essential amino acids determined, as well as of the non-essential amino acids and that, with two apparent exceptions, the concentrations of the free amino acids increase upon incubation. Therefore, all the required amino acids appear to be available as substrates for the biosynthesis of proteins.

*Summary.* The concentrations of 13 free amino acids were determined in mitochondrial preparations of liver and in the supernatant fluid. Appreciable concentrations of the amino acids were found and, in general, these increased during a 2-hour period of incubation.

1. Peterson, E. A., and Greenberg, D. M., *J. Biol. Chem.*, 1952, v194, 359.

2. Schneider, W. C., and Hogeboom, G. W., *J. Biol. Chem.*, 1950, v183, 123.

3. Schurr, P. E., Thompson, H. T., Henderson, L. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v182, 29.

4. Henderson, L. M., Brickson, W. L., and Snell, E. E., *J. Biol. Chem.*, 1948, v172, 15, 31.

5. Dunn, M. S., Camien, M. N., Malin, P. R., Murphy, E. A., and Riever, P. J., *Univ. of California Pub. Physiol.*, 1949, v8, 293.

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## Effects of Total-Body X-Irradiation on the Tissue Mast Cell. (20068)

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Hemorrhage is a very striking characteristic of the syndrome which follows total-body irradiation. Strong indications that hyperheparinemia is the primary cause of post-irradiation bleeding have been found in the X-irradiated dog where 1) clotting time is greatly prolonged, 2) toluidine blue or protamine administration effects normal clotting time and controls hemorrhage, 3) material having the properties of heparin appears in the blood, and 4) toluidine blue or protamine is effective in controlling hemorrhage in the presence of marked thrombocytopenia(1,2). The importance of hyperheparinemia has been questioned, however, by workers who have failed to establish prolonged clotting time as an essential component of the radiation syndrome(3-8), and the recent finding that