INTENSE CONCENTRATION OF \(\alpha,\gamma\)-DIAMINOBUTYRIC ACID BY CELLS*

BY HALVOR N. CHRISTENSEN, THOMAS R. RIGGS, HERBERT FISCHER,†
AND IRENE M. PALATINE

(From the Department of Biochemistry and Nutrition, Tufts College Medical School, Boston, Massachusetts)

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During an exploration of the relation between amino acid structure and concentrative uptake by the Ehrlich mouse ascites carcinoma cell, the case of L-\(\alpha,\gamma\)-diaminobutyrate was encountered. This basic amino acid was concentrated so strongly as to displace most of the potassium from the cells and to cause large shifts of other electrolytes. Our observations upon this amino acid are recorded here.

EXPERIMENTAL

The \(\alpha,\gamma\)-diaminobutyric acid and L-\(\alpha,\beta\)-diaminopropionic acid were synthesized for us by Miss Lillian Alonso, from L-glutamic acid (1) and L-asparagine (2). The diamino acids were included as their hydrochlorides in Krebs' Ringer-bicarbonate medium, and this was added to the ascitic fluid suspension or to the separated cells or tissues at levels of 15 to 40 mM per liter. The methods of incubation, separation, and analysis of the cells for electrolytes (3) and diamino acids (4) have been recorded. Magnesium was determined by the method of Garner (5) applied to nitric or trichloroacetic acid extracts of the cells. Water uptake by the cells was measured by their increase of weight; shifts of water were taken into account in calculating shifts of ions from or into the cells.

In the experiments in mice bearing the ascites tumor, 0.3 M solutions of diaminobutyric acid monohydrochloride were fed or injected under the loose skin in the scapular region by passing the needle under the skin for a cm. or more to obviate leakage from the site. After an hour the mouse was decapitated and the ascitic fluid removed and centrifuged, and the liver, kidney, and musculature of the hind legs excised quickly and weighed on a direct reading torsion balance. For electrolyte determinations (3)

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† Visiting investigator from the University of Frankfurt. Exchange Fellow, United States State Department; Charlton Fellow.
nitric acid extracts were made; for the amino acid analysis (4) the tissues were ground with five portions of 7 per cent trichloroacetic acid. The phosphotungstate of the diamino acid was then precipitated from this extract.

Results

The approximate electrolyte pattern typical of the cells of the Ehrlich mouse ascites tumor is shown in Fig. 1, A. The sodium values are slightly depressed and the chloride values slightly increased by the small but variable contamination of the neoplastic cells with erythrocytes which we encountered. The electrolyte patterns attained upon incubation with α,γ-diaminobutyrate are shown in Figs. 1, B, 2, and 3, A. Increases of the volume of cellular water are shown by the thickening of the bars; this varied from 28 to over 100 per cent. The extracellular fluids corresponding to these diagrams were abnormal only in containing (after incubation) K at levels from 15 to 30 m.eq. per liter and the amino acid cation at 6 or 8 mM per liter. The bars of Fig. 1, C and 4, B illustrate the balance among the ions transferred to and from the cells, expressed in milliequivalents per liter of original cell water. The balance obtained was ordinarily better than that of Fig. 1, C. There were no indications that the exchange of the organic cation for inorganic cations was other than 1:1. Fig. 3 shows the inhibition of the entrance of diaminobutyrate produced when the initial extracellular level of K was 30 m.eq. per liter. The diaminobutyrate appeared a stronger competitor for concentration by the cell than K; when both initial levels were 30 mM, K reached a distribution (cellular)/(extracellular) of 79/43 = 1.8, and the diamino acid of 65/11.5 = 5.7. When the extracellular K level was low to begin with, the corresponding ratios were (for K) 60/20 = 3 and (for the amino acid) 96/5.5 = 17.4.

At an initial extracellular level of 30 mM, the transfer of the amino acid into the cell continued for at least 5 hours. The loss of K, however, was almost complete in the 1st hour. After that, the readjustment was mainly by transfer of chloride and water. There was a consequent further dilution of the remaining cell potassium, and a gradual increase in the concentration of the organic cation. Even after swelling to double the normal water content during 2 hours, the cells upon reinoculation produced the tumor, although with a lag period compared with normal cells. Breakdown of the osmotic barrier with the disappearance of the amino acid gradient occurred in one case after 2 hours at an initial extracellular level of 60 mM of diamino acid. Observation of the cells by phase contrast microscopy during uptake of diaminobutyrate showed intense swelling of the cytoplasm of all the neoplastic cells (not of the contaminating erythro-
cytes), but swelling of the nucleus could not be detected. The optical
density of the cytoplasm decreased, Brownian movements of the cyto-
plasmic granules became prominent, and eventually many granules ap-
peared in the extracellular fluid. Disruption of the cytoplasm was not
observed.

Clearly the cells were restrained in the uptake of the diamino acid by
the necessary concomitant transfers of K, Na, Cl, and water. It was
reasoned that the entrance of the diamino acid hydrochloride could occur from a hypertonic solution without the necessity of water transfer, and that more would be taken up as the chloride and less by replacement with K. Fig. 4, A shows the results when the extracellular fluid was hypertonic by 40 mM of the amino acid hydrochloride plus 40 mM of NaCl. Still larger uptake of the organic cation and of Cl was observed, but the K displacement was as large as ever. The displacement of Na against a gradient is noteworthy (final distribution 24 m.eq. per kilo of cell water, 183 m.eq. per liter in the extracellular fluid). As in every other case ob-

![Fig. 3](http://www.jbc.org/)  
**Fig. 3.** Inhibition of diaminobutyrate uptake by high extracellular K levels. Symbols and scales as in Fig. 1.

![Fig. 4](http://www.jbc.org/)  
**Fig. 4.** Uptake of diaminobutyrate from hypertonic medium. Symbols and scales as in Fig. 1.

served, Mg failed to be displaced, suggesting that it was mainly in a bound state. Higher tonicities prevented the swelling, but the amino acid gradients were smaller.

L-α,β-Diaminopropionic acid produced similar but somewhat smaller shifts of K; e.g., loss of 44 m.eq. per liter of original cell water, compared with 64 m.eq. with diaminobutyrate. Swelling and uptake of the amino acid and of Cl were also observed, but the distribution of this amino acid was not accurately measured.

**Uptake by Other Cells and Tissues**—Hemidiaphragms of rats showed no uptake of diaminobutyrate after 2 hours of incubation in Krebs' Ringer-bicarbonate solution containing sodium pyruvate (6) and diaminobutyrate at 20 mM levels. Human and canine erythrocytes likewise failed to ad-
mit the amino acid upon incubation in vitro in Raker's medium (7) plus plasma.

Table I shows the distribution of diaminobutyrate upon feeding to or injection into mice bearing the ascites tumor. Upon feeding by stomach tube, a voluminous diarrhea was produced, the gut became distended, and absorption manifestly was poor. In spite of their powerful uptake of the diamino acid in vitro, the carcinoma cells captured only a very small portion in competition with the liver until the liver had exchanged about half of its K for diaminobutyrate. Skeletal muscle and kidney also showed an appreciable activity for concentrating the amino acid. Very little of the diamino acid remained in the extracellular fluid, the ascitic plasma serving as a sample of that fluid. Essentially all of the administered acid was recovered in the whole mouse after an hour. High levels were attained in the carcinoma cells only when the dose was large enough to exceed the ability of the liver to take up the diamino acid.

**Table I**

*Distribution of Diaminobutyrate Fed (Experiment 1), or Injected Subcutaneously, into Mice with Ascites Tumor*

Normal diamino acid levels in millimoles per kilo, carcinoma cell 4, liver 3.8, muscle 2.7. The animals were sacrificed 2 hours after feeding, 1 hour after injection.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Dose</th>
<th>Diamino acid, mm per kilo</th>
<th>Changes in ions of liver, m.eq. per kilo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ascitic plasma</td>
<td>Carcinoma cell</td>
</tr>
<tr>
<td>1</td>
<td>750</td>
<td>3.8</td>
<td>11.3</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>1.2</td>
<td>14.1</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>1.1</td>
<td>11.6</td>
</tr>
<tr>
<td>4</td>
<td>225</td>
<td>2.0</td>
<td>61</td>
</tr>
</tbody>
</table>

DISCUSSION

The behavior of the neoplastic cells toward diaminobutyrate appears to establish that the amino acid is in the free state within cells, or at least has its full osmotic activity and electric charge. Similarly the results provide good evidence for the validity of the widely held view that K⁺ is in the free state within the cell and that water moves freely into and out of the cell. In contrast, Mg showed no detectable displacement by diaminobutyrate.

With reference to intensity of the concentrative uptake of the natural food amino acids, glycine and alanine, the mouse tissues fall in the order,
carcinoma cells > liver > muscle (8), whereas for diaminobutyrate the order was liver > carcinoma cells > muscle. To begin with, the neoplastic cells concentrate this amino acid far more powerfully than any naturally occurring amino acid yet encountered; the intensity of the uptake by the liver can be gaged only from the apparent failure of the tumor cells to share in an administered dose until the liver reached nearly maximal levels.

Observations of the natural occurrence of \(\alpha,\gamma\)-diaminobutyric acid and \(\alpha,\beta\)-diaminopropionic acid seem to be limited so far to antibiotics (9, 10). Amino acid and peptide structures related to these amino acids are under exploration.

**SUMMARY**

\(\alpha,\gamma\)-Diaminobutyric acid was observed to be so strongly concentrated by the Ehrlich mouse ascites carcinoma cell as to displace most of the cellular potassium. Chloride and water were taken up until the cell water was more than doubled. Sodium was also displaced from the cells, but not magnesium. The response to \(\alpha,\beta\)-diaminopropionic acid was similar but weaker. Erythrocytes and rat diaphragm failed to admit diaminobutyrate into the cells in vitro.

Almost all of a moderate dose of diaminobutyrate injected subcutaneously into a mouse was captured by the liver, and only after the liver appeared to reach a maximal level (with displacement of about half its potassium) was the amino acid captured by the carcinoma cells. Skeletal muscle and kidney concentrated the amino acid to a lesser but appreciable extent under these conditions.

**BIBLIOGRAPHY**

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