exclude the presence of protected *N*-terminal groups. Moreover, since thyroglobulin is a glycoprotein, containing also sialic acid[18], steric effects of the carbohydrate moieties may influence the reaction with phenylisothiocyanate. Gottschalk[19] has observed such effects in applying the Edman method to sequence determinations in glycopeptides, while Johansen, Marshall and Neuberger[20] found that a glycopeptide from egg albumin required special conditions for reaction with fluorodinitrobenzene. When the sialic acid component of a DEAE cellulose purified preparation of thyroglobulin was removed by treating the protein for 18 h at 37° C with neuraminidase[21], we found the values of *N*-terminal amino-acids to be the same as those of an incubated control (sialic acid recovered from diffusate equivalent to 0·85 mg/100 mg protein). However, the residual carbohydrate structures could be responsible for any inhibitory effects.

In none of the quantitative assays reported here nor in a series of qualitative tests could we confirm the finding[2,3] of iodinated amino-acids as *N*-terminal groups. This negative result was supported when hog thyroglobulin, labelled *in vivo* with iodine-131 and isolated by ammonium sulphate fractionation, followed by chromatography on DEAE cellulose, was submitted to the Edman procedure; no radioactive phenylthiohydantoins could be detected.

We thank Dr. Pehr Edman, St. Vincent's School of Medical Research, for advice on his assay method, and Miss P. Jablonski, who prepared several of the thyroglobulin samples. The National Health and Medical Research Council (Commonwealth of Australia) provided a personal grant to one of us (T. A. A. D.) and contributed towards the cost of this investigation.

T. A. A. Dopheide
V. M. Trikojus

Russell Grimwade School of Biochemistry,
University of Melbourne,
Australia.

[1] Derrien, Y., Michel, R., and Roche, J., *Biochim. Biophys. Acta*, **2**, 454 (1948).
[2] Roche, J., Michel, R., Nunez, J., and Lacombe, G., *Bull. Soc. Chim. Biol.*, **37**, 219 (1955).
[3] Roche, J., Michel, R., and Nunez, J., *Bull. Soc. Chim. Biol.*, **37**, 229 (1955).
[4] Edman, P., *Acta Chem. Scand.*, **4**, 277, 283 (1950); **7**, 700 (1953).
[5] Sanger, F., *Biochem. J.*, **39**, 507 (1945).
[6] Ingbar, S. H., Askonas, B. A., and Work, T. S., *Endocrinol.*, **64**, 110 (1959).
[7] Talanti, S., and Hopsu, V. K., *Acta Endocrinol.*, **35**, 481 (1960).
[8] McQuillan, M. T., Mathews, J. D., and Trikojus, V. M., *Nature*, **192**, 333 (1961).
[9] Ui, N., and Tarutani, O., *J. Biochem.*, **50**, 508 (1961).
[10] Edman, P. (private communication).
[11] Niall, H., and Edman, P., *J. Gen. Physiol.*, **45**, Supp., 185 (1962).
[12] Edman, P., *Proc. Roy. Austral. Chem. Inst.*, **24**, 434 (1957).
[13] Ilse, D., and Edman, P., *Austral. J. Chem.*, **16**, 411 (1963).
[14] Sjöquist, J., *Acta Chem. Scand.*, **7**, 448 (1953).
[15] Edman, P., and Sjöquist, J., *Acta Chem. Scand.*, **10**, 1507 (1956).
[16] McQuillan, M. T., and Trikojus, V. M., *Austral. J. Biol. Sci.*, **6**, 617 (1953).
[17] Menzies, C. A., McQuillan, M. T., and Trikojus, V. M. (to be published: see also ref. 9).
[18] Gottschalk, A., and Ada, G. L., *Biochem. J.*, **62**, 681 (1956). Gottschalk, A., *Yale J. Biol. Med.*, **28**, 525 (1956). Spiro, R. G., and Spiro, M. J., *Fed. Proc.*, **22**, Abs., 588 (1963).
[19] Gottschalk, A. (private communication).
[20] Johansen, P. G., Marshall, R. D., and Neuberger, A., *Biochem. J.*, **78**, 518 (1961).
[21] Ada, G. L., *Biochim. Biophys. Acta*, **73**, 276 (1963). Dr. Ada kindly provided a sample of neuraminidase.

Effect of β-Methylaspartate on Ornithine Cycle Reactions

β-METHYLASPARTATE (β-MA) was identified[1] as a product of glutamate metabolism in cell-free extracts of *Clostridium tetanomorphum*. β-MA is a strong antimetabolite toward aspartate: it is an inhibitor of pyrimidine biosynthesis from aspartate[2] and of urea synthesis from citrulline + aspartate[3]. In addition, β-MA seems to have certain interesting metabolic activities: it participates in transamination reactions in rat and rabbit liver and brain[4] and in *Escherichia coli*[5], and it may also be a precursor in the *de novo* synthesis of thimine[6].

The present communication deals with the *in vitro* effects of Dl-threo-β-MA (Sigma) on urea synthesis from carbamyl-phosphate (CP), aspartate and ornithine as well as from citrulline and aspartate. Evidence is also presented on the inhibitory effect on the same reactions of β-MA injected *in vivo* in rats.

The results are reported in Table 1.

Table 1. UREA SYNTHESIS IN RAT LIVER AFTER ADDITION OF β-MA TO HOMOGENATES OR INJECTION OF IT *in vivo*

β-Methyl-aspartate addition	Extra-formed urea from CP + ornithine + aspartate (μmoles/200 mg tissue)	Inhibition (%)	Extra-formed urea from citrulline + aspartate (μmoles/200 mg tissue)	Inhibition (%)
—	4·75	—	3·6	—
In vitro (40 μmoles/200 mg tissue)*	1·6	66·5	1·5	58·3
In vitro (80 μmoles/200 mg tissue)*	1·1	76·8	1·0	72·2
In vivo (3·4 mmoles/kg)†	0·43	90·9	1·4	61·1
In vivo (2·4 mmoles/kg)†	0·93	80·4	2·6	27·7

The incubation mixture was the following: rat liver homogenate (40 per cent in 0·1 M phosphate buffer *p*H 7·4): 0·5 ml.; $MgCl_2$: 10 μmoles; ATP, fumarate and pyruvate: 5 μmoles each; L-ornithine, L-aspartate, L-citrulline, dilithium carbamyl-phosphate (Calbiochem): 10 μmoles each when added. Final volume: 2 ml., *p*H 7·4; incubation at 37° C for 1 h in Warburg vessels in O_2—CO_2 (95 : 5 per cent) atmosphere. The reaction was stopped by cooling the vessels at 0° C; urea was then determined by urease method (Sigma, type V) following Krebs and Henseleit (ref. 7).
Non-enzymatic urea, as well as urea formed without substrates, was subtracted.
* β-MA was added to the incubation mixtures in the amounts given above.
† β-MA was injected intraperitoneally; 2 h later rats were killed and livers were removed and homogenized.

They show that β-MA added to liver homogenate inhibits urea synthesis from CP + ornithine + aspartate as well as from citrulline + aspartate. The rate of inhibition is of approximately the same order for both reactions.

In vivo injection of β-MA produces inhibition of urea synthesis in liver removed 2 h after the injection. The degree of inhibition appears greater with urea from CP + ornithine + aspartate than from aspartate + citrulline.

Severina[3] claimed that β-MA had a seven-fold stronger inhibition on urea synthesis *in vitro* than α-methylaspartate (α-MA).

On the contrary, the *in vivo* results (Table 1), if compared with those with α-MA obtained in previous work[8], show that α-MA is a stronger inhibitor of urea synthesis in rat liver than β-MA when the compounds are injected *in vivo*. In fact, the injection into rats of 3·4 mmoles/kg of α-MA effects 100 per cent inhibition of urea synthesis from both citrulline + aspartate and CP + ornithine + aspartate; the same dose of β-MA inhibits urea synthesis from CP + ornithine + aspartate by 90 per cent and from citrulline + aspartate by 61·1 per cent.

Experiments are under way to find out the reasons for these differences between the action of α-MA and β-MA.

This work was carried out under the auspices of the Impresa Enzimologia of the Italian National Research Council.

F. De Lorenzo
G. Illiano
G. Molea
G. Della Pietra

Institute of Biological Chemistry,
Medical School,
University of Naples.

[1] Barker, H. A., Weissbach, H., and Smyth, R. D., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1093 (1958).
[2] Woolley, D. W., *J. Biol. Chem.*, **235**, 3238 (1960).
[3] Severina, I. S., *Biokhimiya*, **27**, 943 (1962).
[4] Mardashew, S. R., *Clin. Chim. Acta*, **6**, 157 (1961).
[5] Galegov, G. A., and Mardashew, S. R., *Doklady Akad. Nauk S.S.S.R.*, **129**, 209 (1959).
[6] Isenberg, H. D., Seifter, E., and Berkman, J., *Biochim. Biophys. Acta*, **39**, 187 (1960).
[7] Krebs, H. A., and Henseleit, K., *Z. physiol. Chem.*, **210**, 33 (1932).
[8] Cedrangolo, F., Della Pietra, G., Cittadini, D., Papa, S., and De Lorenzo, F., *Nature*, **195**, 708 (1962); *Enzymologia*, **25**, 308 (1963).