vico e α-chetoglutarico[2] con diminuzione dell'attività cocarbossilasica[3].

Ratti albini del peso medio di g 250 erano trattati con allossana per via endoperitoneale (mg 150/kg) e sacrificati circa 10 giorni dal trattamento, dopo aver controllato, a digiuno da 12 h, il contenuto ematico di glucosio[4], acido piruvico e α-chetoglutarico[5].

Negli organi rapidamente prelevati e messi in bicchiere contenente H_2O e ghiaccio, si dosava quindi la glutammico piruvico e la glutammico ossalacetico transaminasi (secondo il metodo descritto in una precedente nota[6], e seguendo il decorso da sinistra verso destra delle seguenti reazioni: glutammico + piruvico $\rightleftarrows$ α-chetoglutarico + alanina; aspartico + α-chetoglutarico $\rightleftarrows$ ossalacetico + glutammico).

I risultati si possono così riassumere:

a) I due chetoacidi transaminabili (piruvico e chetoglutarico) sono aumentati nel diabetico (ratto normale: ac. piruvico mg 1,6%; ac. α-chetoglutarico mg 0,64%; ratto diabetico: ac. piruvico mg 2,7%; ac. α-chetoglutarico mg 0,95%). Il loro aumento è proporzionale all'intensità dell'iperglicemia e al grado di diminuzione di cocarbossilasi.

b) Anche le transaminasi del fegato sono aumentate e il loro aumento è proporzionale a quello dei due chetoacidi sui quali si svolge la loro azione (ratto normale: glutammico piruvico transaminasi 28,5 μl di ac. piruvico scomparsi per mg peso secco; glutammico ossalacetico transaminasi 320 μl di ac. ossalacetico formati per mg peso secco. Ratto diabetico: glut. piruvico 47,2 μl di ac. piruvico scomparsi per mg tessuto secco; glut. ossalacetico 650 μl di ac. ossalacetico formati per mg tessuto secco).

Per controllare se l'aumento della transaminasi possa interpretarsi come un tentativo da parte dell'organismo di difendersi dalla minaccia dell'acidosi, ho trattato per 5 giorni un lotto di ratti diabetici con fruttoso (g 1 pro kg per via endoperitoneale): avevo precedentemente dimostrato[7] che il fruttoso normalizza nello scorbuto e migliora nel soggetto normale l'attività cocarbossilasica, migliorando pure la utilizzazione del glucoso, del piruvato, del chetoglutarato e del lattato; inoltre, come migliorava nello scorbuto l'utilizzazione dei chetoacidi attraverso la cocarbossilasi, ritornava alla norma anche l'aumentata attività degli enzimi transaminanti i chetoacidi stessi. D'altra parte anche nel diabete allossanico si ha normalizzazione dell'attività cocarbossilasica mediante somministrazione di fruttoso[8].

Ne è risultato che nel diabete allossanico, la somministrazione di fruttoso normalizza sia la concentrazione dei due chetoacidi nel sangue, sia la attività transaminasica epatica.

Questi risultati depongono a favore della ipotesi precedentemente da me formulata e cioè dell'intervento delle transaminasi nel tentativo di proteggere l'organismo dallo stato di α-chetoacidosi; essi inoltre fanno assumere a tali enzimi particolare importanza nella regolazione dell'equilibrio acido-base dell'organismo.

E. Barbieri

Istituto di Chimica Biologica dell'Università di Padova, il 21 dicembre 1956.

Summary

Pyruvic and α-ketoglutaric acid transaminase is increased in α-ketoacidosis caused in guinea pigs and rats by cocarboxylase deficiency (avitaminosis C, alloxan diabetes). Fructose administration not only normalizes the increased concentration of ketoacids and the decreased cocarboxylase activity, but also the increased amount of transaminase. It is suggested that in α-ketoacidosis from transaminable ketoacids the increased transaminating activity might be substitute for reduced cocarboxylase activity.

[2] C. E. Frohman, J. M. Orten e A. H. Smith, J. biol. Chem. *193*, 803 (1951). – A. Fidanza e F. Laviano, Boll. Soc. Ital. Biol. Sper. *27*, 1468 (1951).

[3] D. Siliprandi e N. Siliprandi, Acta Vitaminolog. *5*, 1 (1951).

[4] N. Nelson, J. biol. Chem. *153*, 375 (1944).

[5] J. W. Goodwin e C. R. Williams, Biochem. J. *51*, 708 (1952).

[6] E. Barbieri, La Ricerca Scientifica, Supplemento anno *25*, 319 (1955).

[7] E. Barbieri, Boll. Soc. Ital. Biol. Sper. *32*, 396, 398 (1956); Atti Congr. Biol. Sper., Pavia *1956* (in corso di stampa).

[8] C. R. Rossi, F. Rossi e C. S. Rossi, Exper. *12*, 389 (1956).

The Conversion of Ethanolamine into Taurine

The cleavage of S-aminoethylcysteine to cysteamine by the rat has been supported by the detection of cystamine in the urine of the rat injected with synthetic aminoethylcysteine[1]. Although cystine, when given in large amounts, produces an excretion of cystamine[2], cysteine injected in the same molar quantity as amino ethylcysteine did not produce any appreciable increase in non-cystine disulfide-sulfur[1]. This finding indicates that, in the case of aminoethylcysteine, the carbon skeleton of the recovered cystamine is likely to come from the aminoethyl moiety of the injected compound.

The biological cleavage of aminoethylcysteine to cysteamine cannot be taken as evidence for the occurrence of a transulfurative reaction from cysteine to ethanolamine unless aminoethylcysteine is found in biological material or the investigation with labelled compounds gives an unequivocal answer. The enzymatic cleavage of a number of thioethers of cysteine was in fact found to take place[3], although the reaction does not appear to have any biological significance.

In order to test the passage of the carbon of ethanolamine into that of cysteamine, a mouse was injected with C^{14} labelled ethanolamine and the radioactivity of the carbon of cystine and taurine extracted from the animal was analyzed. Cystamine was also isolated from the same animal, but the yield was very poor and the sample was not suitable for analysis. Nevertheless the comparative analysis of cystine and taurine extracted after the injection of ethanolamine gave the desired information. Both cystine and cysteamine are converted by the animal into taurine, and, in the case of the passage of ethanolamine into taurine through cysteamine, the isolated cystine should have given a lower specific activity than taurine.

C^{14} uniformly labelled ethanolamine was obtained according to the method of Pilgeram *et al.*[4], starting

[1] D. Cavallini, B. Mondovì, and C. De Marco, Biochim. biophys. Acta *18*, 122 (1955).

[2] D. Cavallini, C. De Marco, and B. Mondovì, Ric. Scient. *25*, 2901 (1955).

[3] F. Binley, J. biol. Chem. *186*, 287 (1950).

[4] L. O. Pilgeram, E. M. Gal, E. N. Sassenrath, and D. M. Greenberg, J. biol. Chem. *204*, 367 (1953).

from 0·5 mc uniformly labelled commercial ethylene oxide (Tracerlab). A total of 0·67 mM of C^{14} ethanolamine, with $5{\cdot}2 \times 10^7$ c/min/mM was injected in three portions at 24 h interval into a 20 g mouse. The animal was then killed, the skin removed, and homogenized in a Waring blendor with 70 ml water. The mixture was boiled for 30 min and filtered. The filtrate was used for the isolation of taurine and the insoluble coagulate was used for the extraction of cystine after hydrolysis in 3 vol. 6 *N* HCl. Taurine was isolated by a procedure similar to that described by VERLY *et al.*[5], using 50 mg of carrier. The final crystalline product was checked for purity by chromatography and it was recrystallized 6 times from water-ethanol to constant radioactivity. Cystine was obtained by the cuprous mercaptide precipitation and recrystallized 5 times at the isoelectric point to constant radioactivity. Purity was checked by chromatography and by the SULLIVAN method[6]. Radioactivity was measured after conversion of the compounds to $BaCO_3$ and correcting for self-absorption. The results are reported in the table.

Specific activity of injected ethanolamine and the extracted taurine and cystine

	c/min/mM
Ethanolamine	$3{\cdot}5 \times 10^7$
Cystine	$6{\cdot}6 \times 10^4$
Taurine	$1{\cdot}3 \times 10^4$

Inspection of the table shows that the mouse converted a small amount of ethanolamine into taurine and that cystine has a higher specific activity than taurine. This finding rules out the possibility that the taurine produced comes directly through: ethanolamine → cysteamine → taurine, in which case cystine, being excluded from the path, should not have been labelled. The results indicate that the route was probably: ethanolamine → glycine → serine → cystathionine → cysteine → taurine. All the steps of the last-mentioned route have in fact been found to be of biological occurrence[7].

As a result of the present work, it may be concluded that the transulfuration of ethanolamine is a process of secondary importance, if it exists at all. Thus the mechanism of the biosynthesis of cystamine represents a problem which still requires a solution.

D. CAVALLINI, F. POCCHIARI, and L. TENTORI

Institute of Biological Chemistry of the University, Departments of Biochemistry and Biology of the Istituto Superiore di Sanità, Rome, December 27, 1956.

Riassunto

L'etanolamina marcata con C^{14} è trasformata nel topo in taurina e cistina. La cistina ha una attività specifica più elevata della taurina. Questa constatazione indica che la transulfurazione della etanolamina nel topo è un processo biologico di scarsa importanza.

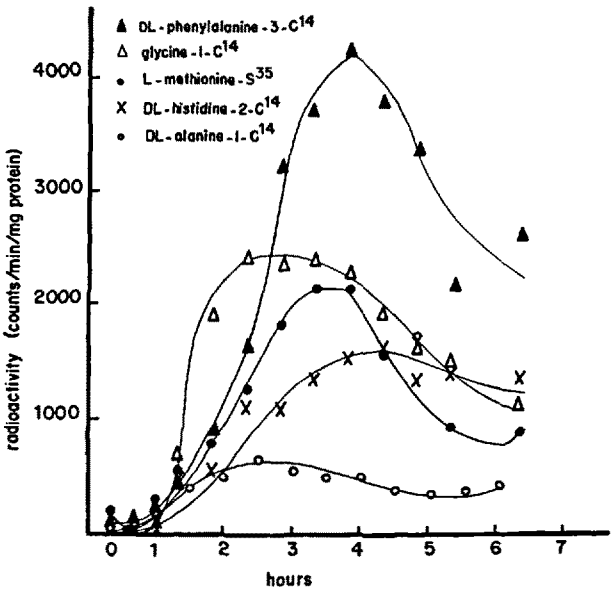
[5] W. G. VERLY and G. KOCH, Biochem. J. *58*, 663 (1954).

[6] M. X. SULLIVAN and W. C. HESS, J. biol. Chem. *116*, 221 (1936).

[7] A. WEISSBACH and D. B. S. SPRINSON, J. biol. Chem. *203*, 1031 (1953). – D. SHEMIN, J. biol. Chem. *162*, 297 (1946). – V. DU VIGNEAUD, *A Trail of Research* (Cornell University Press, 1953).

Radioactive Amino Acid Incorporation into the Rat Pancreatic Juice Proteins*

In a previous paper[1] results were presented studying the rate of incorporation of glycine-C^{14} into the rat pancreatic juice proteins. The data obtained suggest that these proteins are built up from blood amino acids and not from preexisting plasma proteins. As our results are based on observations performed only with glycine-C^{14}, it is desirable to extend them to other amino acids. In this paper we present the results obtained utilizing radioactive phenylalanine, alanine, histidine and methionine besides glycine.



Incorporation of radioactive amino acids into the rat pancreatic proteins. Abscissa: Time after injection of radioactive amino acid. Each point is the average of three animals.

Material and methods. Adult male Wistar rats weighing from 300 to 400 g were used throughout. In the glycine experiment the weight of the animals was 240 g. The anesthesia, stimulation of pancreatic secretion and collection of pancreatic juice was performed as already described[2]. Endovenous injection of 3·3 μC of the following amino acids was performed 5 min after the secretin-carbamoylcholine stimulus: DL-phenylalanine-3-C^{14}, DL-alanine-1-C^{14}, DL-histidine-2-C^{14}, glycine-1-C^{14} and L-methionine-S^{35}. Three rats were injected with each of the amino acids. 5 μl samples of pancreatic juice were collected every 20 min during the first 2 h and every half hour for the next 4 h. These samples were spread on stainless steel planchets and their radioactivity measured with a thin mica window Geiger-Müller counter (TGC-2 of Tracerlab). Under these conditions no selfabsorption is observed. The protein content of each planchet was determined by the method described by LOWRY *et al.*[3].

* Aided partially by grants from the 'Conselho Nacional de Pesquisas' and 'Deutsche Forschungsgemeinschaft'.

[1] L. C. U. JUNQUEIRA, G. C. HIRSCH, and H. A. ROTHSCHILD, Biochem. J. *61*, 275 (1955).

[2] L. C. U. JUNQUEIRA, G. C. HIRSCH, and H. A. ROTHSCHILD, Biochem. J. *61*, 275 (1955). – G. C. HIRSCH, L. C. U. JUNQUEIRA, H. A. ROTHSCHILD, and S. R. DOHI, Pflügers Arch. ges. Physiol. (1956) (in press).

[3] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).