J. Enzyme Inhibition, 1995, Vol. 10, pp. 65–72
Reprints available directly from the publisher
Photocopying permitted by license only

DIFFERENT EXTENT OF INHIBITION OF PYRUVATE DEHYDROGENASE AND 2-OXOGLUTARATE DEHYDROGENASE BOTH CONTAINING ENDOGENOUS THIAMINE PYROPHOSPHATE, BY SOME ANTICOENZYME ANALOGUES

SLAWOMIR STRUMILO,* MARIA CZYGIER and JOLANTA MARKIEWICZ

Department of Biochemistry, Institute of Biology, University of Warsaw, Branch in Bialystok, Swierkowa 20B, 15–950 Bialystok, Poland

(Received 13 March 1995; in final form 26 June 1995)

Inhibitory effects of 4′-oxythiamine pyrophosphate (OTPP) and tetrahydrothiamine pyrophosphate (ThTPP) on the purified bison heart pyruvate dehydrogenase complex (PDC) semisaturated with endogenous thiamine pyrophosphate (TPP) and 2-oxoglutarate dehydrogenase complex (OGDC) saturated about 85% with endogenous TPP, were studied. It has been established that the thiamine derivatives strongly inhibit not only the PDC apoenzyme moiety, but also the PDC holoenzyme moiety. The apparent I_{50} values for the holoenzyme were 0.006 μM and 0.046 μM for OTPP and ThTPP, respectively. The inhibition of the PDC is reversible. After removal of the anticoenzyme analogues by gel filtration the endogenous TPP within the PDC is retained as it is evidenced by complete recovery of the enzyme activity without added TPP. In contrast with the PDC, OGDC holoenzyme form is weakly inhibited by the anticoenzyme analogues.

KEY WORDS: Pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, inhibition, thiamine pyrophosphate analogues

INTRODUCTION

Pyruvate dehydrogenase (EC 1.2.4.1) and 2-oxoglutarate dehydrogenase (EC 1.2.4.2) are the first components (E_1) of the multienzyme complexes, which catalyse thiamine pyrophosphate (TPP)-dependent decarboxylation and dehydrogenation of pyruvic and 2-oxoglutaric acids, respectively. Overall the pyruvate dehydrogenase complex (PDC)- and 2-oxoglutarate dehydrogenase complex (OGDC)-reactions are expressed by the following equation:

$$\text{R-CO-COOH} + \text{HS-CoA} + \text{NAD}^+ \rightarrow \text{R-CO-S-CoA} + \text{NADH} + \text{CO}_2 + \text{H}^+,$$

where: R = CH_3-, for pyruvate; R = HOOC-CH_2-CH_2-, for 2-oxoglutarate.

*Correspondence.

The PDC in mitochondria responds to a number of regulatory signals by means of reversible phosphorylation mechanisms.[1,2] The OGDC is a limiting unit of the citric acid cycle[3] and has allosteric mechanisms of regulation of its activity.[4–7]

With respect to coenzyme-anticoenzyme regulation, interactions between the multienzyme complexes, TPP and its derivatives may also be of interest.

In an earlier work it was shown that 4′-oxythiamine, tetrahydrothiamine and thiamine-thiazolone pyrophosphates were potent competitive inhibitors of PDC versus TPP.[8–10] However, in those experiments, TPP was almost absent in the PDC preparation used.

The enzymes in the present study were the bison heart PDC and OGDC which after purification contain bound endogenous TPP in an amount providing more than 50% and 85% of the maximum PDC[11] and OGDC activity, respectively. The aim of this research was to elucidate whether well known anticoenzyme inhibitors, 4′-oxythiamine pyrophosphate and tetrahydrothiamine pyrophosphate, can inhibit not only the apoenzyme moiety of PDC and OGDC, but also the holoenzyme moiety of E_1 in the multienzyme complexes constitution.

MATERIALS AND METHODS

The PDC and OGDC were isolated and purified from the hearts of 7 European bisons (*Bison bonasus*) by the method suggested for the same complexes from bovine heart.[12] The purified PDC and OGDC had a specific activity of about 11 IU/mg protein and showed on SDS-polyacrylamide gel electrophoresis a set of subunits[11] typical for the same complexes from other mammalian tissues.[4,13–15]

The velocity of the PDC- and OGDC-reactions was recorded by measuring NADH formation at 340 nm with spectrophotometers DU-640 (Beckman, Fullerton, USA) or Specord UV/VIS (Carl Zeiss, Jena, Germany) using a thermostated cell (30°C). The reaction medium (1 ml) contained 50 mM potassium phosphate buffer (pH 7.8), 1 mM dithiothreitol, 1 mM $MgCl_2$, 0.1 mM CoA, 2 mM NAD^+, 2 mM pyruvate-K^+ (in the case of PDC) or 10 mM 2-oxoglutarate-Na^+ (in the case of OGDC), various TPP concentrations and various inhibitor concentrations, as it is shown in the legends for Figures 1, 3, 4 and 6. TPP (0.2 mM) was used as a saturating concentration. The reaction was started by the addition of 2–5 μg of the enzyme preparations. The assays were carried out in duplicate. When a lag phase was observed in the kinetic curve, the steady-state rate after the lag phase was measured. For preliminary kinetic analysis Lineweaver-Burk plots were used.[16]

4′-Oxythiamine was prepared according to the method of Rydon[17] and tetrahydrothiamine according to Bonvicino and Hennessy[18] with subsequent phosphorylation and purification[19] of these thiamine analogues by Dr S. Zabrodskaya and Dr D. Oparin (Institute of Biochemistry, Academy of Sciences, Grodno, Belarus). TPP was obtained from Sigma (Milwaukee, USA). The concentrations of thiamine pyrophosphate analogues were determined spectrophotometrically using the molar absorption coefficients of 8550 $M^{-1}cm^{-1}$ (267 nm), 8900 $M^{-1}cm^{-1}$ (266 nm) and 6080 $M^{-1}cm^{-1}$ (269 nm) for thiamine, 4′-oxythiamine and tetrahydrothiamine,[19] respectively.

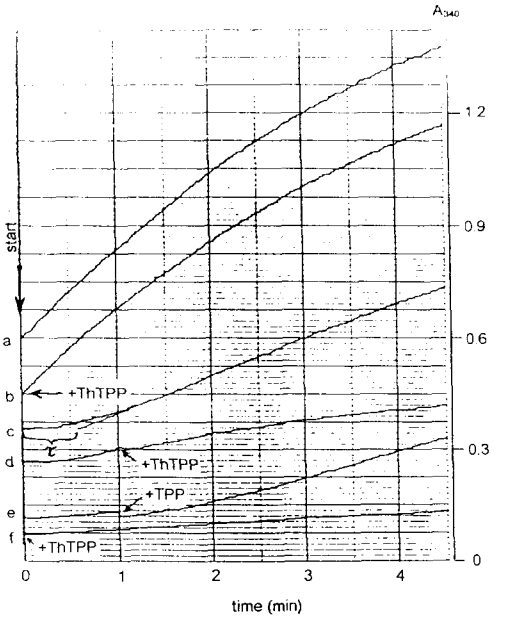
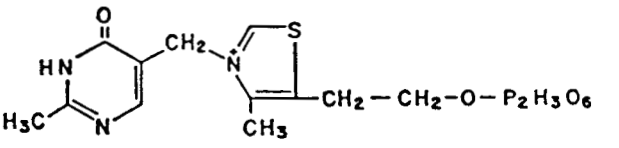


FIGURE 1 Original kinetic curves of the PDC-catalysed reaction at different conditions: (a) in the presence of 0.2 mM thiamine pyrophosphate (TPP), (b) in the presence of 0.2 mM TPP and 0.5 μM tetrahydrothiamine pyrophosphate (ThTPP), (c) without exogenous TPP, (d) without TPP, +0.5 μM ThTPP 1 min after start, (e) without TPP, +0.5 μM ThTPP + 0.2 mM TPP 1 min after start, (f) without TPP, +0.5 μM ThTPP.

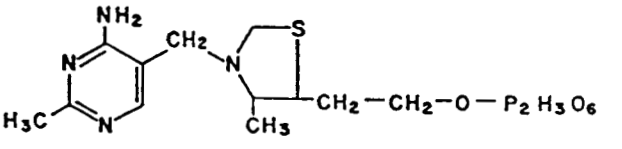
RESULTS AND DISCUSSION

The rate of the overall bison heart PDC reaction observed in the absence and presence of exogenous thiamine pyrophosphate (Figure 1, curves (a) and (c)) suggested that the purified PDC was apparently semisaturated with endogenous TPP. Earlier it was shown that the PDC from *Escherichia coli*,[20] pig heart,[21] bovine adrenal glands[22] and human heart[23] also did not completely lose TPP during purification but bison heart PDC contained the greatest amount of endogenous TPP. When the PDC acted with residual TPP in the absence of exogenous coenzyme, a lag phase was observed after the contact with substrate (Figure 1, curve (c)). Such kinetic phenomenon was explained as a result of partial, limited dissociation of the endogenous bound TPP in the absence of pyruvate and its complete reassociation in the region of the pyruvate dehydrogenase active sites in the presence of the substrate.[24]

Using the PDC preparation semisaturated with endogenous TPP we studied the possibility of its inhibition by anticoenzyme thiamine derivatives, 4′-oxythiamine pyrophosphate and tetrahydrothiamine pyrophosphate (Figure 2).



4'- Oxythiamine Pyrophosphate



Tetrahydrothiamine Pyrophosphate

FIGURE 2 Formulae of the thiamine anticoenzyme analogues used.

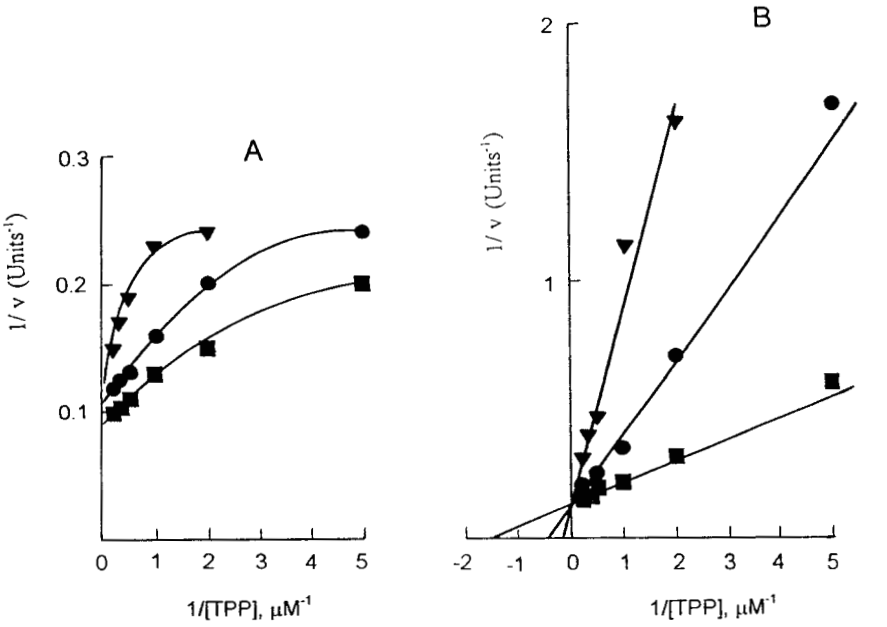


FIGURE 3 Lineweaver-Burk plots of the dependence of the PDC-catalysed reaction rate on the concentration of exogenous thiamine pyrophosphate (TPP): (A) disregarding of endogenous TPP; (B) after subtraction of the reaction rate provided by endogenous TPP: ■ – in the absence of inhibitor; ● – in the presence of 0.01 μM 4′-oxythiamine pyrophosphate; ▼ – in the presence of 0.1 μM tetrahydrothiamine pyrophosphate.

First, the initial PDC-reaction rate (ν) was measured at different concentrations of added exogenous TPP in the absence and presence of the inhibitors. Lineweaver-Burk plots of the dependence of ν on concentrations of exogenous TPP were evidently nonlinear (Figure 3A), because endogenous TPP also contributed to the reaction rate. On the other hand, after subtraction from the overall PDC-reaction rate of the PDC activity exerted with endogenous TPP we obtained data which indicated the apparent competitive inhibition of the PDC by 4′-oxythiamine and tetrahydrothiamine pyrophosphates versus added TPP (Figure 3B). However, such subtraction was not exact, because it disregarded the degree of inhibition of PDC by the anticoenzymes versus endogenous TPP. Therefore, from Figure 3B only two clear conclusions can be drawn: (1) the apparent K_m value for exogenous TPP amounts to 0.6 μM, (2) 4′-oxythiamine pyrophosphate is about 3-fold more potent as an inhibitor than tetrahydrothiamine pyrophosphate, because a 10-fold lower concentration of OTPP caused only a 3-fold lower inhibition.

Interestingly, TPP-dependent yeast pyruvate decarboxylase[19] and bovine heart PDC[9] were inhibited by tetrahydrothiamine pyrophosphate more strongly than by 4′-oxythiamine pyrophosphate. Instead, the PDC from bovine adrenals had almost equal K_i values (0.07 and 0.10 μM) for both inhibitors under similar conditions.[22] Thus, PDC of different origin showed distinctly different sensitivity to the anticoenzymes.

In the next step of the investigation the activity of the PDC was measured in the presence of different concentrations of the anticoenzyme derivatives without addition of exogenous TPP. It was found that 4′-oxythiamine pyrophosphate and tetrahydrothiamine pyrophosphate at appropriate concentrations completely inhibited the PDC (Figure 4). This means that the anticoenzymes can inhibit not only the PDC apoenzyme moiety competitively with respect to TPP,[22] but also the PDC holoenzyme moiety. Concentrations of the inhibitors which caused 50% inhibition (I_{50}) of the PDC activity were 0.006 μM and 0.046 μM for 4′-oxythiamine pyrophosphate and tetrahydrothiamine pyrophosphate, respectively. Probably, the inhibitors can compete with both added and endogenous TPP. This may be possible due to limited dissociation of the endogenous TPP, as represented in Figure 5.

Returning to Figure 1 (curve (d)), it should be noted that inhibition of the PDC occurs in the absence of exogenous TPP, by added tetrahydrothiamine pyrophosphate 1 min after the start of the PDC reaction. Apparently, the inhibitor having high affinity for the pyruvate dehydrogenase can partially push out endogenous TPP during the PDC reaction. On the other hand, TPP at a high concentration can push off the added anticoenzyme analogue from the active sites of the pyruvate dehydrogenase (Figure 1 curve (e)). Similar results were obtained using 4′-oxythiamine pyrophosphate.

After anticoenzyme treatment the bond between endogenous TPP and the PDC was maintained; Sephadex G-25 gel filtration removed 4′-oxythiamine pyrophosphate and tetrahydrothiamine pyrophosphate and recovered the initial PDC activity, detected without addition of exogenous TPP (Table 1). Thus, the profound inhibitory effects of the anticoenzyme analogues were completely reversible.

The second multienzyme complex, OGDC, after purification contained bound endogenous TPP in an amount providing for 85% of the maximum activity (Figure 6).

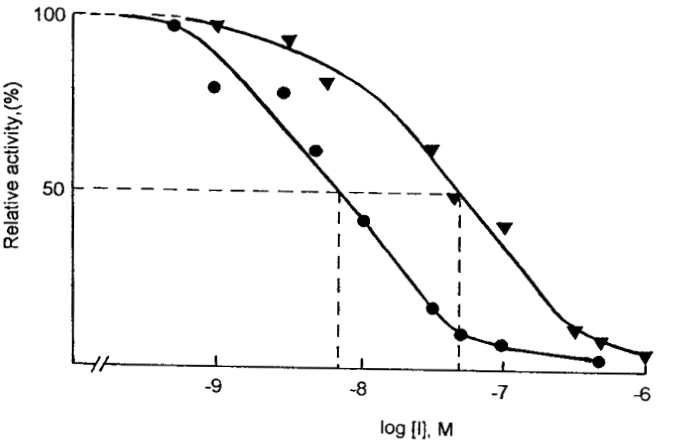


FIGURE 4 Relative activity of the PDC with endogenous thiamine pyrophosphate (TPP) in the presence of varied 4′-oxythiamine pyrophosphate (●) and tetrahydrothiamine pyrophosphate (▼) concentrations.

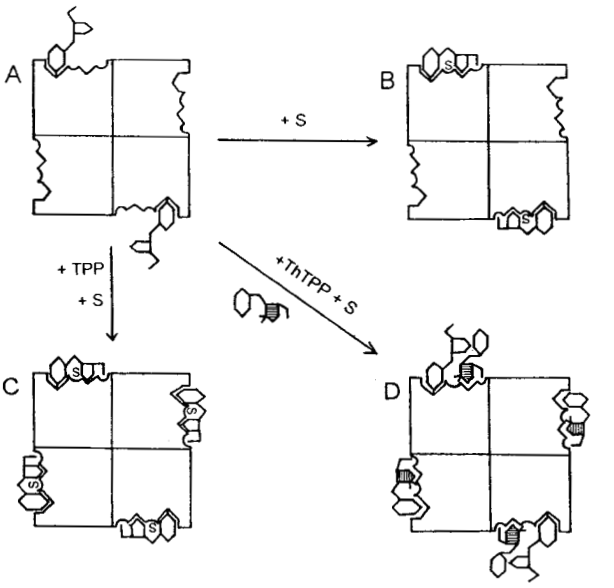


FIGURE 5 Scheme illustrating the possible mechanism of functioning of the PDC semisaturated with endogenous bound TPP: (A) Partial dissociation of the endogenous TPP in the absence of pyruvate (S); (B) Reassociation of the endogenous TPP in the presence of the substrate (S); (C) Status of PDC in the presence of exogenous TPP and the substrate; (D) Inhibition of apoenzyme and holoenzyme moieties of PDC by the anticoenzyme analogue (ThTPP).

TABLE 1
PDC activity with endogenous TPP (IU/mg protein) after anticoenzyme treatment followed by Sephadex G-25 gel filtration.

Initial activity	+0.5 μM oxythiamine pyrophosphate	+2.0 μM tetrahydrothiamine pyrophosphate	activity after gel filtration
5.46	0.30	—	5.41
5.67	—	0.43	5.59

Such a high degree of saturation with endogenous TPP is typical for the OGDC isolated from different animal sources.[4,12,15] Contrary to PDC, the OGDC does not exert a lag phase whilst functioning with endogenous TPP in the absence of added TPP (data not presented). All this may be interpreted in terms of a firmer TPP-OGDC bond.

Addition to the reaction medium without exogenous TPP of 4′-oxythiamine pyrophosphate and tetrahydrothiamine pyrophosphate over a broad range of concentrations showed low inhibitory effect of the anticoenzyme analogues with respect to the OGDC (Figure 6). The inhibition of OGDC arose only at relatively high concentrations (above 5 μM) of the thiamine analogues. 4′-Oxythiamine pyrophosphate inhibited the OGDC stronger ($I_{50} \approx 24$ μM) than tetrahydrothiamine

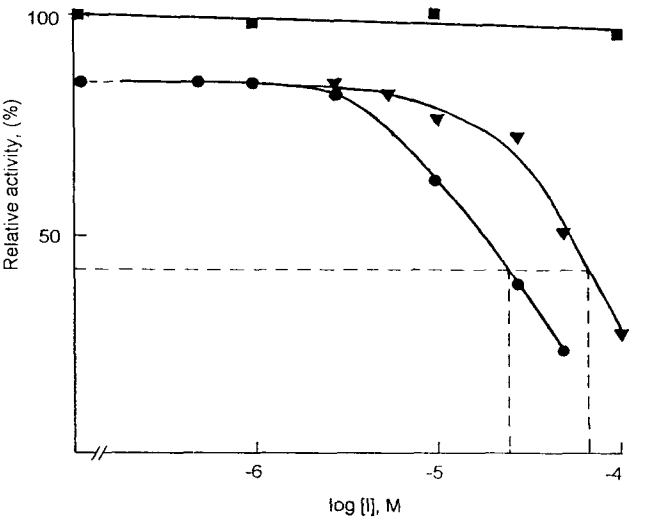


FIGURE 6 Relative activity of the OGDC with 0.2 mM TPP (■) and without exogenous TPP (• ▼): • – in the presence of varied concentrations of 4′-oxythiamine pyrophosphate; ▼ ■ – in the presence of varied concentrations of tetrahydrothiamine pyrophosphate.

pyrophosphate ($I_{50} \approx 67\ \mu M$). These approximate I_{50} values for the OGDC were about 3 orders higher than those for the pyruvate dehydrogenase complex (Figure 4). It may be explained on the basis of firmer screening of the 2-oxoglutarate dehydrogenase active sites by endogenous TPP. However, the weak inhibition of the OGDC by the thiamine analogues is probably an anticoenzyme effect because a high concentration of added exogenous TPP protects OGDC against inhibition (Figure 6).

The data obtained are in agreement with results of experiments performed *in vivo*[25,26] which indicated that the OGDC activity in rat tissues was more resistant than pyruvate dehydrogenase activity to injections of 4′-oxythiamine as an anticoenzyme precursor.[27]

Acknowledgements

We would like to thank very much Dr S.V. Zabrodskaya and Dr D.A. Oparin from the Institute of Biochemistry, Academy of Science of Belarus in Grodno for providing us with the TPP-analogues.

References

1. Patel, M.S. and Roche, T.E. (1990) *FASEB J.*, **4**, 3224.
2. Yeaman, S.J. (1989) *Biochem. J.*, **257**, 625.
3. Cooney, C.J., Taegtmeyer, H. and Newsholme, E.A. (1981) *Biochem. J.*, **200**, 701.
4. McCormack, J.C. and Denton, R.M. (1979) *Biochem. J.*, **180**, 533.
5. Strumilo, S.A, Vinogradov, V.V. and Senkevich, S.B. (1980) *Ukrainian Biochem. J.*, **52**, 321.
6. Lawlis, V.B. and Roche, T.E. (1980) *Mol. Cell. Biochem.*, **32**, 147.
7. Strumilo, S.A., Taranda, N.I. and Vinogradov, V.V. (1984) *Biomed. Biochim. Acta*, **43**, 237.
8. Severin, S.E., Khailova, L.S. and Bernhardt, R. (1976) *Ukrainian Biochem. J.*, **48**, 503.
9. Strumilo, S.A., Kiselevsky, Y.V., Taranda, N.I., Zabrodskaya, S.V. and Oparin, D.A. (1989) *Voprosy medicinskoj chimii*, (Moskow), **35**, 102.
10. Yakovleva, G.M., Strumilo, S.A., Gorenshtein, B.I. and Ostrovsky, Y.M. (1986) *Biokhimia*, (Moskow), **51**, 59.
11. Czygier, M. and Strumilo, S.A. (1994) *Acta Biochim. Polonica*, **41**, 453.
12. Stanley, C.J. and Perham, R.W. (1980) *Biochem. J.*, **191**, 147.
13. Roche, T.E. and Cate, R.L. (1977) *Arch. Biochem. Biophys.*, **183**, 664.
14. Strumilo, S.A., Senkevich, S.B. and Vinogradov, V.V. (1980) *Biokhimia*, (Moskow), **45**, 883.
15. Strumilo, S.A., Taranda, N.I., Senkevich, S.B. and Vinogradov, V.V. (1981) *Acta Biolog. Med. Germanica*, **40**, 257.
16. Cornish-Bowden, A. (1976) *Principles of Enzyme Kinetics*. London: Butterworths.
17. Rydon, H.N. (1951) *Biochem. J.*, **48**, 383.
18. Bonvicino, G.E. and Hennessy, D.J. (1957) *J. Amer. Chem. Soc.*, **79**, 6325.
19. Wittorf, J.H. and Gubler, C.J. (1971) *Eur. J. Biochem.*, **22**, 544.
20. Bisswanger, H. (1974) *Eur. J. Biochem.*, **48**, 377.
21. Walsh, D.A., Cooper, R.H., Denton, R.M., Bridges, B.J. and Randle, P.J. (1976) *Biochem. J.*, **157**, 41.
22. Strumilo, S.A., Senkevich, S.B., Zabrodskaya, S.V. and Vinogradov, V.V. (1981) *Ukrainian Biochem. J.*, **53**, 65.
23. Kiselevsky, Y.V., Ostrovtsova, S.A. and Strumilo, S.A. (1990) *Biomed. Biochim. Acta*, **49**, 285.
24. Strumilo, S.A., Senkevich, S.B. and Vinogradov, V.V. (1981) *Biokhimia*, (Moskow), **46**, 1938.
25. Ostrovsky, Y.M. (1975) *Active centres and grouping in thiamine molecule*. Minsk: Nauka i Tekhnika.
26. Strumilo, S.A., Senkevich, S.B. and Vinogradov, V.V. (1984) *Biomed. Biochim. Acta*, **43**, 159.
27. Rindi, G., de Giuseppe, L. and Ventura, U. (1963) *J. Nutrition*, **81**, 147.