Table 2. *Identification of the structure of some naturally occurring mono- and di-unsaturated acids*

Source	Nature of acid	Fragments identified	Structure
Cod-liver oil	C ₁₆ mono-unsaturated	<i>n</i> -Heptanoic, azelaic	Hexadec-9-enoic
Human sebum	C ₁₄ mono-unsaturated	Glutaric	Tetradec-5-enoic
Human sebum	C ₁₆ mono-unsaturated	<i>n</i> -Decanoic	Hexadec-6-enoic
Cardiolipin	C ₁₆ mono-unsaturated	<i>n</i> -Heptanoic, azelaic	Hexadec-9-enoic
Cardiolipin	C ₁₈ di-unsaturated	<i>n</i> -Hexanoic, azelaic	Octadeca-9:12-dienoic
Faecal fatty acids	C ₁₈ mono-unsaturated	<i>n</i> -Nonanoic, azelaic	Octadec-9-enoic

This unsaturated acid when subjected to oxidative degradation gave three acids: *n*-hexanoic, malonic and glutaric acids. It was thus identified as arachidonic acid (Mowry, Brode & Brown, 1942).

SUMMARY

1. The separation of the methyl esters of mono- and di-carboxylic acids on the gas-liquid chromatogram is described.

2. Oxidation with permanganate in acetic acid followed by identification of the split products on the gas-liquid chromatogram is used to establish the structures of some naturally occurring mono- and di-unsaturated acids.

3. The presence of vaccenic acid in human plasma is confirmed by this technique and cow's milk is shown to contain a variety of mono-unsaturated octadecenoic acids.

4. The isolation of an unsaturated acid present as a major component in human red cells and plasma is described, and is shown by reduction and oxidation to be arachidonic acid.

We thank Dr M. G. MacFarlane for the fatty acids from a purified sample of cardiolipin. Thanks are also due to Mr H. Hadaway for much experimental assistance.

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Pyridoxamine Phosphate Transaminase

By R. B. BEECHEY AND FRANK C. HAPPOLD

Department of Biochemistry, The University of Leeds

(Received 8 October 1956)

The starting material for the preparation of tryptophanase in these laboratories has been a 0.8M-potassium chloride extract of cells of *Escherichia coli*. Happold & Struyvenberg (1954) showed that when this was resolved into apoenzyme and coenzyme (pyridoxal phosphate) by dialysis against water, ammonium, potassium or rubidium ions were also required for reactivation, but that when the holoenzyme was dialysed against 0.25M-potassium chloride the dissociation of apoenzyme and coenzyme occurred only very slowly, over a period of days.

In later studies on this enzyme we observed that aqueous extracts were devoid of tryptophanase activity but did contain apotryptophanase, and that this was remarkably stable; it naturally requires pyridoxal phosphate and potassium, ammonium or rubidium ions for activation. The further observa-

tion that pyridoxamine phosphate also caused an increase in tryptophanase activity was, however, an unexpected discovery; our previous failure, and that of Wood, Gunsalus & Umbreit (1947), to observe this fact may have been due to the use of ammonium sulphate in the partial purification of the enzyme or, in our experiments, to the use of potassium chloride solutions as the enzyme solvent. We now suggest that, whilst pyridoxal phosphate is the coenzyme of tryptophanase, as of so many other enzymes associated with the intermediary metabolism of amino acids, a specific transaminase exists which catalyses the reversible interconversion of pyridoxamine phosphate and pyridoxal phosphate. We have called this enzyme pyridoxamine phosphate transaminase (Beechey & Happold, 1955). The importance of this reaction in the physiology of the cell needs no emphasis.

EXPERIMENTAL

Measurement of activity

The enzyme preparations used were obtained from acetone-ether-dried cells of *Escherichia coli* which had been grown for 18 hr. at 37° on casein-digest medium solidified with agar (Dawes, Dawson & Happold, 1947). The cells were extracted for 1 hr. with water (20 mg. of cells/ml. of water) at 2–3° and then centrifuged at 12 000 *g* for 30 min. at 3°. In later experiments the aqueous cell extract was freeze-dried in 15 ml. batches and stored in stoppered ampoules at –18°. Pyridoxamine phosphate transaminase activity was retained for at least 6 weeks. The aqueous extract was concentrated for use by dissolving the dried powder, from 15 ml. of aqueous extract, in 2 × 2.5 ml. of water or 0.05 *M*-NaHCO₃-Na₂CO₃ buffer, pH 10.1. Insoluble matter was removed by centrifuging at 1500 rev./min. for 10 min.

Tryptophanase activity (initial activity) is expressed as μ g. of indole formed in 5 or 10 min. at 37° and was determined by measuring the initial rate of indole formation in a total volume of 2.5 ml. The 0.5 ml. of aqueous cell extract, 0.5 ml. of 0.2 *M*-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 8.3, and 1 ml. of additions were incubated for 10 min. at 37°, and 0.5 ml. of 1.96×10^{-2} *M*-L-tryptophan was then added. The reaction was stopped after 5 or 10 min. by the addition of 0.5 ml. of 10% (w/v) trichloroacetic acid.

Indole was determined by a modification of the method of Happold & Hoyle (1934). The rose-indole was run off from the light petroleum (b.p. 40–60°) into centrifuge tubes and centrifuged at 1500 rev./min. for 5 min. to remove any suspended protein fragments. Its absorption was then measured at 560 *m* μ in a Unicam diffraction-grating spectrophotometer, model SP. 400, and compared with a standard curve.

Protein was estimated by a quantitative biuret procedure.

Optimum conditions for enzyme assay

Principle of the method. The absorption of light at 390 *m* μ by pyridoxal phosphate is maximal at pH 14, whereas pyridoxamine phosphate does not absorb light at this wavelength and pH (Williams & Niellands, 1954). If then the transaminase action were stopped by adding NaOH to the reaction mixture (both pyridoxal phosphate and pyridoxamine phosphate are stable under alkaline conditions) and the changes in light absorption at 390 *m* μ were measured, the rate of formation, or conversion, of pyridoxal phosphate could be obtained.

Experimental method. Measurements were made in a Unicam spectrophotometer, model SP. 500. A volume (1 ml.) of 0.05 *M*-NaHCO₃-Na₂CO₃ buffer, pH 10.1 (Delory & King, 1945) and 1 ml. of 0.05 *M*-MgSO₄ or water, 1 ml. of substrate (100 μ g./ml.) and 1 ml. of water or additions were added to both the experimental reaction tubes and the control reaction tubes. After temperature equilibration at 37°, 1 ml. of the concentrated aqueous extract, also at 37°, was added at zero time to the experimental reaction tubes. After a 60 min. reaction period, 1 ml. of 2.5 *N*-NaOH was added to both the experimental tubes and the control tubes. A volume (1 ml.) of the concentrated enzyme, which had also been maintained at 37°, was then added to the control tubes. Where Mg²⁺ ions had been added, the precipitated magnesium hydroxide was removed by centrifuging at 1500 rev./min. for 10 min. Transaminase activity is expressed as μ g. of pyridoxal phosphate produced from, or converted into, pyridoxamine phosphate.

(a) *Conversion of pyridoxamine phosphate into pyridoxal phosphate.* Magnesium ions were always added to the reaction tubes. To determine the optical-density changes at 390 *m* μ due to the formation of pyridoxal phosphate, the optical density of the contents of the experimental reaction tubes was measured, the contents of the corresponding control reaction tubes being used as a blank.

(b) *Conversion of pyridoxal phosphate into pyridoxamine phosphate.* Magnesium ions were not added to the reaction tubes. To determine the optical-density changes at 390 *m* μ due to the removal of pyridoxal phosphate, the optical density of the contents of the control tubes was measured, the contents of the corresponding experimental tubes being used as a blank.

The optical densities so obtained were referred to a standard graph which had been prepared by incubating 1 ml. of solutions containing known amounts of synthetic pyridoxal phosphate with 2 ml. of 0.05 *M*-NaHCO₃-Na₂CO₃ buffer, pH 10.1, and 2 ml. of water for 60 min., and then adding 1 ml. of 2.5 *N*-NaOH. The optical densities of these tubes were measured with a blank in which the pyridoxal phosphate solution was replaced by water. There is a linear relationship between optical density and pyridoxal phosphate concentration up to at least 40 μ g./ml.

The pyridoxal phosphate and pyridoxamine phosphate (both from Roche Products Ltd.) were shown to be free of contaminating members of the vitamin B₆ group by filter-paper electrophoresis (Siliprandi, Siliprandi & Lis, 1954).

RESULTS

Apparent activation of apotryptophanase by pyridoxamine phosphate

When 100 μ g. of pyridoxal phosphate or pyridoxamine phosphate is incubated separately for varying periods of time with standard preparations of cell extract, buffer and tryptophan and the initial rate of indole production is determined, activation occurs with both the vitamin B₆ derivatives. The results are recorded in Table 1 and show that whereas 10 min. incubation time is sufficient to obtain the maximum degree of activation with pyridoxal phosphate, 30 min. or more is required with pyridoxamine phosphate, and the latter attains only one-half the activity which occurs with the co-enzyme pyridoxal phosphate itself. Since pyridoxamine phosphate will not activate apotryptophanase we assumed that the aqueous extract was causing the conversion of pyridoxamine phosphate into pyridoxal phosphate.

Effect of bivalent metal ions upon the apparent activation of apotryptophanase by pyridoxamine phosphate

The effect of Mg²⁺ ions upon the tryptophanase activity of the aqueous extracts of *Escherichia coli* cells in the presence of pyridoxal phosphate or pyridoxamine phosphate, and in their absence, is

Table 1. Variation of the tryptophanase activity of aqueous cell extracts on incubation with pyridoxal phosphate (Pal P) and pyridoxamine phosphate (Pamine P)

Aqueous cell extract (0.5 ml.), 0.5 ml. of 0.2M-tris buffer (pH 8.3), 0.5 ml. of water and 0.5 ml. of vitamin B₆ derivative (100 µg./ml.) or water were incubated at 37° for the stated times. A volume (0.5 ml.) of 1.96×10^{-2} M-L-tryptophan was then added. The reaction was stopped 5 or 10 min. later and the indole production was determined.

Time of incubation (min.)	Indole produced (µg./5 min.)			Indole produced (µg./10 min.)		
	No additions	With Pal P added	Pal P effect	No additions	With Pamine P added	Pamine P effect
0	5.8	13.1	7.3	9.4	10.7	1.3
10	0.7	13.2	12.5	0.9	3.0	2.1
20	0.0	12.6	12.6	0.7	4.7	4.0
30	0.0	12.5	12.5	0.6	5.8	5.2
40	0.0	12.4	12.4	0.0	5.6	5.6
50	0.0	12.6	12.6	0.1	6.0	5.9
60	0.0	12.5	12.5	0.0	5.5	5.5
70	0.0	12.0	12.0	0.0	5.0	5.0
80	0.0	12.4	12.4	0.0	5.3	5.3

Fig. 1. Acceleration of the conversion of pyridoxamine phosphate into pyridoxal phosphate by Mg^{2+} ions. Aqueous cell extract (0.5 ml.), and 0.5 ml. of 0.2M-tris buffer (pH 8.3), 0.5 ml. of $MgSO_4$ solution (water in control) and 0.5 ml. of addition (as indicated below) were incubated for 40 min. at 37°. A volume (0.5 ml.) of 1.96×10^{-2} M-L-tryptophan was then added. The reaction was stopped 5 or 10 min. later and indole determined. Additions: Δ , 0.5 ml. of pyridoxal phosphate (100 µg./ml.), 5 min. reaction with tryptophan; $\bullet$, 0.5 ml. of pyridoxamine phosphate (100 µg./ml.), 10 min. reaction with tryptophan; $\square$, 0.5 ml. of water, 10 min. reaction with tryptophan.

shown in Fig. 1. In the presence of pyridoxal phosphate there is no increase in tryptophanase activity on the addition of Mg^{2+} ions. However, with pyridoxamine phosphate, tryptophanase activity is enhanced by their addition over the range 2×10^{-3} to 2.5×10^{-5} M. Above 2×10^{-3} M, Mg^{2+} ions inhibit the tryptophanase activity of the extracts. Since Mg^{2+} ions do not activate tryptophanase, the apparent activation of this enzyme in

the aqueous extract by pyridoxamine phosphate can only be accounted for by assuming that Mg^{2+} ions activate a pyridoxamine phosphate transaminase present in the extract, and that pyridoxal phosphate produced by this enzyme then activates apotryptophanase.

Cobalt, Cu^{2+} , Mn^{2+} and Fe^{2+} ions are without effect upon the initial rate of indole production by these extracts in the presence of pyridoxamine phosphate or pyridoxal phosphate, except where the concentration is sufficiently high to cause inhibition.

Spectrophotometric evidence for the enzymic inter-conversion of pyridoxal phosphate and pyridoxamine phosphate

At pH 8.3 pyridoxal phosphate shows an absorption maximum at 390 m μ and absorbs comparatively weakly at 320 m μ , whereas pyridoxamine phosphate absorbs strongly at 320 m μ and does not absorb light at 390 m μ (Williams & Nielsens, 1954). Systems in which pyridoxal phosphate or pyridoxamine phosphate were separately incubated at 37° with aqueous cell extract buffered at pH 8.3, were set up both with and without Mg^{2+} ions. Controls, to which no pyridoxal phosphate or pyridoxamine phosphate had been added, were incubated at 37° and used as blanks, the optical-density changes at 390 m μ being measured. The results and details of the experiment are recorded in Fig. 2.

When aqueous extract is incubated with pyridoxamine phosphate the optical density at 320 m μ decreases and there is a simultaneous increase in that at 390 m μ . This indicates the conversion of pyridoxamine phosphate into pyridoxal phosphate. In the presence of 2.5×10^{-3} M- Mg^{2+} ions these

changes are accentuated. On incubating the extract with pyridoxal phosphate, there is a decrease in optical density at 390 $m\mu$ and an increase at 320 $m\mu$, corresponding to the conversion of pyridoxal phosphate into pyridoxamine phosphate. These latter changes are not significantly affected by the presence of $2.5 \times 10^{-3} M$ - Mg^{2+} ions. In the absence of the extract there are no changes in absorption at either wavelength. No gaseous exchange occurred when the buffered enzyme-substrate systems were allowed to react in Warburg manometers. We can therefore assume that an enzyme system is present in the aqueous extract which catalyses the interconversion of pyridoxal phosphate and pyridoxamine phosphate.

Fig. 2. Interconversion of pyridoxamine phosphate and pyridoxal phosphate. A volume (10 ml.) of 0.2M-tris buffer (pH 8.3), and 10 ml. of substrate (100 $\mu g./ml.$), 10 ml. of water or $10^{-3} M$ - $MgSO_4$ solution and 10 ml. of aqueous cell extract were incubated at 37°. At indicated times samples were taken and the optical densities at 320 $m\mu$ and 390 $m\mu$ were read against a blank in which water replaced the substrate. Substrates: (a), pyridoxal phosphate; (b), pyridoxamine phosphate. ●, Optical density at 320 $m\mu$; ○, optical density at 390 $m\mu$.

Effect of pH on enzyme activity

In these experiments the enzyme preparation contained 10–12 mg. of protein/ml. Buffers, 0.05M- $NaHCO_3$ - Na_2CO_3 , pH range 8.9–10.6, and 0.2M-tris, pH range 7.2–8.2, were added to the reaction tubes, which also contained enzyme, substrate and water or a solution of $MgSO_4$. After 90 min. incubation NaOH was added and, where present, the precipitated magnesium hydroxide was removed by centrifuging at 1500 rev./min. for 10 min. The changes in optical density at 390 $m\mu$ were then measured. The results are illustrated in Fig. 3, and show that the conversion of pyridoxamine phosphate into pyridoxal phosphate is sensitive to changes in pH, with a sharp optimum at pH 10.1. The conversion of pyridoxal phosphate into pyridoxamine phosphate is somewhat less sensitive to changes in pH, the optimum lying between pH 9.8 and 10.

Variation of enzyme activity with varying substrate concentration

In these and following experiments the concentrated enzyme was dissolved in 0.05M- $NaHCO_3$ - Na_2CO_3 buffer, pH 10.1. The results obtained when the substrates in different concentrations were incubated with the usual systems for 90 min. are illustrated in Fig. 4. The rate of reaction is linear up to a substrate concentration of 20 $\mu g./ml.$, both for the conversion of pyridoxamine phosphate into pyridoxal phosphate and the reverse reaction.

Fig. 3. Effect of pH on enzyme activity. Reaction period, 90 min. Substrates: pyridoxamine phosphate, Mg^{2+} ions present, in 0.05M- $NaHCO_3$ - Na_2CO_3 buffer, ○, or in 0.2M-tris buffer, ●; pyridoxal phosphate, no Mg^{2+} ions present, 0.05M- $NaHCO_3$ - Na_2CO_3 buffer, △.

Hence in subsequent experiments a substrate concentration of $20 \mu\text{g./ml.}$ was used.

The Michaelis constants for these reactions were calculated by the method of Lineweaver & Burk (1934), as shown in Fig. 5. For the conversion of pyridoxal phosphate into pyridoxamine phosphate

Fig. 4. Effect on enzyme activity of variation of substrate concentration: 2 ml. of $0.05\text{M-NaHCO}_3\text{-Na}_2\text{CO}_3$ buffer, pH 10.1, 1 ml. of concentrated enzyme solution, 1 ml. of 10^{-2}M-MgSO_4 solution or water and 1 ml. of substrate solution were incubated at 37° for 90 min. A volume (1 ml.) of 2.5N-NaOH was then added. Substrates: pyridoxamine phosphate, magnesium present, O; pyridoxal phosphate, no magnesium present, Δ .

Fig. 5. Evaluation of Michaelis constants. O, Conversion of pyridoxamine phosphate into pyridoxal phosphate, $K_m = 4.1 \times 10^{-4}\text{M}$; Δ , conversion of pyridoxal phosphate into pyridoxamine phosphate, $K_m = 6.1 \times 10^{-4}\text{M}$. These values are only approximations (see text).

the value of the constant is $6.1 \times 10^{-4}\text{M}$, and for the conversion of pyridoxamine phosphate into pyridoxal phosphate is $4.1 \times 10^{-4}\text{M}$. These values can only be regarded as approximations, since in these experiments a large fraction of the substrate reacted during the incubation period and therefore the measured velocities are averages over ranges of substrate concentrations.

Effect of time on reaction velocity

The rates of enzymic action with either pyridoxal phosphate or pyridoxamine phosphate as substrate are illustrated in Fig. 6; both show that the rate is

Fig. 6. Effect of time on reaction velocity. A volume (2 ml.) of $0.05\text{M-NaHCO}_3\text{-Na}_2\text{CO}_3$ buffer, pH 10.1, and 1 ml. of concentrated enzyme solution, 1 ml. of 10^{-2}M-MgSO_4 and 1 ml. of substrate ($100 \mu\text{g./ml.}$) were incubated for the indicated times at 37° . A volume (1 ml.) of 2.5N-NaOH was then added. Substrates: pyridoxamine phosphate, magnesium present, O; pyridoxal phosphate, no magnesium present, Δ .

Fig. 7. Variation of enzyme concentration. The substrate was pyridoxal phosphate (final concn. $20 \mu\text{g./ml.}$); the pH was 10.1 and time of incubation 1 hr.

linear for a reaction period of 60 min. This was the reaction time used in subsequent experiments. Again, a large fraction of the substrate reacted during the incubation period and the measured velocities are averages over ranges of substrate concentrations.

Variation of enzyme concentration

The concentrated enzyme preparation usually contained 10–12 mg. of protein/ml. The concentrated enzyme was diluted with buffer and the diluted enzyme solutions were added to reaction tubes, which contained pyridoxal phosphate buffered at pH 10.1. After 60 min. incubation NaOH was added and the changes in optical density at 390 m μ were measured. Fig. 7 shows that there is a linear relationship between enzyme activity and protein concentration up to a final protein concentration of 1.6 mg./ml. In later experiments therefore the freeze-dried powder was dissolved in one-half the original volume of aqueous extract of buffer.

Effect of other compounds on the transaminase action

The effect upon the present system of certain compounds which are normally associated with transamination reactions was next studied. Oxaloacetate, α -oxoglutarate, pyruvate, phenylpyruvate, L-aspartate, L-glutamate, DL-alanine, L-glutamine and DL-asparagine were added to the reaction mixture at concentrations based on 1.0, 0.1 and 0.01 mg. of the L-isomer/ml. Inhibition of enzymic action was noted at the two higher concentrations by these substances and none of them increased the enzymic activity.

Effect of dialysis

Concentrated enzyme (25 ml.), dissolved in 0.05 M-NaHCO₃-Na₂CO₃ buffer, pH 10.1, was di-

alysed in a rotating sac against 1.5 l. of the same buffer at 2–3°. This treatment gave 50 % inactivation after 5 hr. Addition of sodium L-glutamate and L-aspartic acid (1 mg./ml.) neither activated nor inhibited the dialysed enzyme, whereas non-dialysed enzyme was inhibited by similar concentrations of these substances.

Effect of hydroxylamine on the conversion of pyridoxamine phosphate into pyridoxal phosphate

Whilst studying the conversion of pyridoxamine phosphate into pyridoxal phosphate we observed that neutralized hydroxylamine over a range 10⁻²–10⁻⁷ M inhibited the reaction in solutions which also contained enzyme, substrate, buffer and Mg²⁺ ions (see Table 2). Since hydroxylamine is known to combine with pyridoxal phosphate Davison (1956), 30 μ g. of pyridoxal phosphate (the amount which would be expected to be produced by the enzymic conversion of the pyridoxamine phosphate) was also incubated for the reaction period with buffer, Mg²⁺ ions and hydroxylamine, and used as a control; NaOH was subsequently added. The percentage inhibition of enzyme activity produced by the added hydroxylamine was always greater than could be accounted for by the inactivation of pyridoxal phosphate by hydroxylamine. If pyridoxal phosphate were itself the coenzyme for this transformation this could account for the inhibition.

Pyridoxal kinase, which catalyses the phosphorylation of pyridoxal by adenosine triphosphate, has been shown to be present in brain (Roberts & Frankel, 1951), in *Escherichia coli* (Binkley & Christensen, 1951) and *Streptococcus faecalis* R (Gunsalus, Bellamy & Umbreit, 1944). No pyridoxal kinase was present in any of our extracts.

Table 2. *Effect of hydroxylamine on the conversion of pyridoxamine phosphate into pyridoxal phosphate (Pal P)*

'Experimental' tubes contained 1 ml. of 0.05 M-NaHCO₃-Na₂CO₃ buffer (pH 10.1), 1 ml. of concentrated enzyme, 1 ml. of pyridoxamine phosphate (100 μ g./ml.), 1 ml. of 10⁻² M-MgSO₄ and 1 ml. of hydroxylamine solution. 'Control' tubes contained 2 ml. of 0.05 M-NaHCO₃-Na₂CO₃ buffer (pH 10.1), 1 ml. of pyridoxal phosphate (30 μ g./ml.), 1 ml. of 10⁻² M-MgSO₄ and 1 ml. of hydroxylamine solution. These tubes were incubated at 37° for 60 min. together with appropriate blanks; 1 ml. of 2.5 N-NaOH was then added and the changes in optical density at 390 m μ were measured.

Hydroxylamine concn. (M)	Control		Experimental	
	Apparent concn. of Pal P (μ g./ml.)	Anticipated inhibition (%)	Apparent Pal P production (μ g./ml.)	Inhibition (%)
0	30	0	16.5	—
0	—	—	15.5	—
10 ⁻²	11.5	61	4.0	75
10 ⁻³	—	—	3.0	81
10 ⁻⁴	—	—	4.0	75
10 ⁻⁵	—	—	16.5	0
10 ⁻⁶	—	—	15.0	6
10 ⁻⁷	28.5	5	17.0	0

DISCUSSION

Umbreit, O'Kane & Gunsalus (1948) observed that pyridoxal phosphate, but not pyridoxamine phosphate, could reactivate purified pig-heart glutamate-aspartate apotransaminase, a finding which was not compatible with the original hypothesis of transamination, whereby the interconversion of the amine and the aldehyde forms of the vitamin B₆ derivatives was involved in the mechanism. Meister (1955) in his review on amino acid metabolism discusses this; Meister, Sober & Peterson (1954) found that pyridoxal phosphate and pyridoxamine phosphate were equivalent in their activation of the purified apotransaminase, though a longer period of pre-incubation was required for the activation of the apotransaminase. Moreover, conversion of pyridoxamine phosphate into pyridoxal phosphate before enzyme-coenzyme combination could not be demonstrated either by enzymic assay or by ultraviolet-absorption studies, results which Meister (1955) states are compatible with the original concept that both the B₆ derivatives are coenzymes of the transamination reactions, and that their interconversion occurs after coenzyme-enzyme combination has occurred.

It may be that the apotransaminase preparation of Umbreit *et al.* (1948) was free from the enzyme which catalyses the interconversion of pyridoxamine phosphate into pyridoxal phosphate, whereas the preparation of Meister *et al.* (1954) was not.

In the present study we have presented evidence that the interconversion of pyridoxamine phosphate and pyridoxal phosphate is catalysed by an enzyme present in aqueous extracts of *Escherichia coli* which also contain apotryptophanase, an enzyme which is activated by pyridoxal phosphate but not directly by pyridoxamine phosphate. Thus apotryptophanase can be indirectly activated by pyridoxamine phosphate when the pyridoxamine phosphate transaminase is present. We have not yet determined the nature of the amino group acceptor in this transamination, but it does not appear to be oxaloacetate, α -oxoglutarate, pyruvate or phenylpyruvate. Magnesium ions activate the conversion of pyridoxamine phosphate into pyridoxal phosphate only. The results illustrated in Fig. 2 suggest that either Mg²⁺ ions affect the equilibrium point of the reaction or that the enzyme is less stable in the absence of Mg²⁺ ions. Whereas the possibility of two separate enzymes cannot be excluded at this stage of the investigation, their presence would not clarify this point.

These findings have obvious bearing upon the mechanism of transamination reactions. Pyridoxal phosphate is the coenzyme of many enzymes associated with protein metabolism, and if this is true for whole cells, and since pyridoxamine phos-

phate is the predominating member of the B₆ group within the cell (Rabinowitz & Snell, 1947), the control of this one enzyme, pyridoxamine phosphate transaminase, could affect the whole pattern of the amino acid metabolism of the cell. That the cell has some form of control of the enzymes involved in vitamin B₆ metabolism can be deduced from the fact that pyridoxal kinase also phosphorylates pyridoxine. The product pyridoxine phosphate has not been shown to be present in cells; what is more, it is a potent inhibitor of pyridoxal phosphate-linked enzymes.

In continuation of this work Happold & Turner (unpublished results) have found a similar system in preparations of glutamic transaminase, prepared from sheep heart by the method of Cammarata & Cohen (1951).

SUMMARY

1. Evidence for the enzymic interconversion of pyridoxal phosphate and pyridoxamine phosphate has been presented. Magnesium ions activate the conversion of pyridoxamine phosphate into pyridoxal phosphate.
2. The enzyme was present in aqueous extracts of *Escherichia coli* but not in the 0.8M-potassium chloride extracts used in the preparation of tryptophanase.
3. Optimum conditions for enzymic assay have been established.

We are indebted to the Medical Research Council for support of these studies. One of us (R. B. B.) is also grateful for a Medical Research Council training grant. Pyridoxal phosphate and pyridoxamine phosphate were gifts from Roche Products Ltd.

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A Comparison of Sodium Salicylate and 2:4-Dinitrophenol as Metabolic Stimulants *in vitro*

By D. H. SPROULL

From the Clinical Chemotherapeutic Research Unit of the Medical Research Council, Western Infirmary, Glasgow

(Received 19 November 1956)

Sodium salicylate as a peripheral metabolic stimulant exhibits the outstanding pharmacological feature of 2:4-dinitrophenol (DNP) and related compounds (Sproull, 1954*b*). DNP inhibits oxidative phosphorylation (Loomis & Lipmann, 1948), which is considered to be the source of its metabolic stimulant property (see Simon, 1953, for review). Brody (1956) has shown that salicylate also inhibits oxidative phosphorylation. The similarity of salicylate and DNP, although contrary to therapeutic and toxicological experience of these agents in man, is of great interest from the viewpoint of therapeutics, and suggests that the actions of the two compounds, as metabolic stimulants, may be identical. Some comparisons were made in the present investigation of the effects of salicylate and DNP on the respiration of tissue slices, in the hope of obtaining evidence which would permit a definite conclusion regarding difference or identity of the drugs' primary actions.

Peiss & Field (1952) found that DNP increased the metabolic rate of rat-brain slices with glucose or pyruvate as substrate, but not when succinate or α -oxoglutarate was employed. The first part of the present investigation was to determine whether this property of DNP is possessed by salicylate.

The second part of this investigation was to compare the concentration-response curves of the two compounds on mouse-liver slices, and to study the combined action of the compounds on this preparation.

METHODS

Brain slices. The rate of O_2 consumption of brain slices from fed male Wistar albino rats was determined by the direct method in Warburg respirometers at 37°. The slices, nominally 0.3 mm. thick, were prepared in a mechanical chopper and were separated and selected individually before

being placed in the Warburg flasks; the medium was Krebs-Ringer phosphate, pH 7.4 (Krebs & Henseleit, 1932). The results were expressed as μ l. of O_2 /hr./mg. final dry wt. of tissue (Q_{O_2}). For each rat the Q_{O_2} values of the control and treated tissues were simultaneously determined in runs of 30 min. duration. Sodium pyruvate was prepared by neutralization of an iced ethanolic solution of redistilled pyruvic acid, and was recrystallized twice; α -oxoglutaric acid, m.p. 112°, was obtained commercially and was neutralized in aqueous solution immediately before use; substrate concentration was generally 0.01 M.

Liver slices. These were prepared from 3- to 8-month-old fed CBA mice, an improved chopper (Sproull, 1956) being used. The details of the manometric technique were as described above, with the exception that the mouse liver was transferred directly from the chopper to the flask without preliminary washing and separation of the slices. Glucose (0.011 M) was incorporated into the medium. The rate of O_2 uptake of the liver slices was, as usual, referred to the dry weight of the tissue after the run.

RESULTS

Rat brain

These experiments were to determine whether or not some concentration of sodium salicylate alters the metabolic rate of the tissue slices under certain experimental conditions. For a given concentration of sodium salicylate and a given substrate the hypothesis that the mean difference between the values of Q_{O_2} for the control and treated tissue (ΔQ_{O_2}) was zero (H_0) was tested sequentially against the alternative hypothesis that it differed from zero (H_1), with the sequential test of Student's hypothesis proposed by Wald (1947) and the tables of Arnold (1951). In each case, where the mean ΔQ_{O_2} was μ and the variance σ^2 , it was decided whether $|\mu| < 2\sigma$ (i.e. H_0) or whether $|\mu| \geq 2\sigma$ (i.e. H_1). The maximum probability assigned to a decision in favour of