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The mechanism of the inhibition of dehydrogenases by salicylate

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Salicylate inhibits rabbit muscle lactate dehydrogenase, horse liver alcohol dehydrogenase, pig heart malate dehydrogenase and pig heart isocitrate dehydrogenase *in vitro*. The inhibitions are reversible, involving competition with NAD, $NADH_2$ or NADP. The results are discussed with reference to some of the *in vivo* actions of the drug.

SALICYLATE causes an increased incorporation of [^{14}C] from labelled succinate into malate and citrate of mitochondrial preparations (Bryant, Smith & Hines, 1963) by inhibiting malate and isocitrate dehydrogenase activities. It was found that salicylate also inhibited several other dehydrogenase enzymes *in vitro* (Hines & Smith, 1964). It was suggested that the mechanism of inhibition of these enzymes involves a reversible competition with either NAD or NADP since the inhibitory effects of the drug were reduced by the further addition of the appropriate coenzyme to the reaction mixtures (Hines & Smith, 1964). The present paper describes kinetic studies, using purified lactate, alcohol, malate and isocitrate dehydrogenases, designed both to establish the validity of this mechanism of inhibition and to determine the inhibitor constants.

Experimental

MATERIALS

Rabbit muscle lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27), horse liver alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1), pig heart malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37), pig heart isocitrate dehydrogenase (L_S-isocitrate: NADP oxidoreductase EC 1.1.1.42), NAD, $NADH_2$, NADP, sodium pyruvate and tris were obtained from Boehringer Corporation (London) Ltd. L-Lactic acid (Grade 1), the monosodium salt of L-malic acid and the trisodium salt of DL-isocitric acid (Type 1) were obtained from the Sigma Chemical Co., St. Louis. Other chemicals were of analytical grade and deionized water was used throughout.

ENZYME ASSAYS

Lactate, alcohol, malate and isocitrate dehydrogenase activities were determined by measuring the formation of either oxidized or reduced coenzyme. Mixtures containing substrate, coenzyme, buffer and salicylate, when present, were allowed to come to thermal equilibrium in a 1 cm cell fitted into a constant-temperature cell housing. Full experimental details for each enzyme were as follows.

Lactate dehydrogenase. The reaction mixture contained L-lactate, 47·5 mM; NAD, 0·46 mM; sodium salicylate, 0–20 mM; in a total volume of 3 ml 0·0067M tris-acetate buffer, pH 8·0. The reaction was started by the addition of 10 μl of a solution containing approximately 1 μg of

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lactate dehydrogenase. The commercial lactate dehydrogenase preparation had previously been dialysed against the assay buffer, the dialysing medium being replaced three times over a period of 24 hr.

Alcohol dehydrogenase. The reaction mixture contained ethanol, 2·2 mM; NAD, 0·10 mM; sodium salicylate, 0–5 mM; in a total volume of 3 ml 0·067M glycine-sodium hydroxide buffer, pH 10·0. The reaction was started by the addition of 10 μl of a solution containing approximately 12 μg of alcohol dehydrogenase. The commercial alcohol dehydrogenase had previously been dialysed against 0·1 μ potassium phosphate buffer, pH 8·0, the dialysing medium being replaced three times over a period of 24 hr.

Malate dehydrogenase. The reaction mixture contained L-malate, 0·62 mM; NAD, 0·48 mM; sodium salicylate, 0–10 mM; in a total volume of 3 ml 0·067 M glycine-NaOH buffer, pH 10·0. The reaction was started by the addition of 1 μl of a solution containing approximately 0·5 μg malate dehydrogenase.

Isocitrate dehydrogenase. The reaction mixture contained DL-isocitrate, 0·028 mM (L$_S$-isocitrate, 0·014 mM); NADP, 0·024 mM; $MgCl_2$, 2 mM; and sodium salicylate, 0–20 mM; in a total volume of 9 ml 0·033M tris buffer, pH 7·3. The reaction was started by the addition of 2 μl of a solution containing approximately 20 μg isocitrate dehydrogenase. A portion of the mixture was poured into a 1 cm silica cell for measurement.

The reactions were followed by measuring the changes in extinction at 365 mμ in a Unicam SP800 recording spectrophotometer. Measurements of extinction were made at 365 mμ, not at 340 mμ, to avoid interference caused by the absorption of salicylate at the lower wavelength. The initial rates (v) were determined from the tracings obtained with an external recorder. The concentrations of substrates and coenzymes in solutions were determined enzymatically (Bergmeyer, 1963).

DIALYSIS EXPERIMENTS

In general the enzyme solutions, in either the absence or the presence of salicylate, were placed in cellulose tubing (inflatable diameter 6 mm, Visking Co.) and dialysed at 0° against the appropriate buffer, which was changed several times. The enzyme activities were measured before and after dialysis. Experimental details for each enzyme were as follows.

Lactate dehydrogenase. Enzyme solutions containing 10 μg enzyme protein and 1 mg serum albumin per ml of 0·1M tris-acetate buffer, pH 8·0, were mixed with equal volumes of either the same tris buffer or the buffer containing 60 mM sodium salicylate. Aliquots (1 ml) of the enzyme solutions, both before and after dialysis, were assayed in a 3 ml reaction mixture containing 47·5 mM L-lactate, 0·46 mM NAD and tris buffer at pH 8·0.

Alcohol dehydrogenase. Enzyme solutions containing 12 μg enzyme protein per ml of 0·1 μ phosphate buffer, pH 8·0, were mixed with equal volumes of either phosphate buffer or the same buffer containing 60 mM sodium salicylate. Aliquots (1 ml) of the variously treated enzyme

solutions were assayed in a 3 ml reaction mixture containing 2·2 mM ethanol, 0·1 mM NAD and glycine buffer, pH 10·0.

Malate dehydrogenase. Freshly diluted enzyme solutions containing 6 μg enzyme protein and 10 mg serum albumin per ml of 0·1M phosphate buffer, pH 7·5, were mixed with equal volumes of either the same buffer or the buffer containing 60 mM sodium salicylate. Aliquots (1 ml) of the enzyme solutions were assayed in a 3 ml reaction mixture containing 1·0 mM L-malate, 1·0 mM NAD and glycine buffer at pH 10·0.

Isocitrate dehydrogenase. Freshly prepared enzyme solutions containing 0·1 mg enzyme protein and 10 mg serum albumin per ml of 0·05M tris chloride buffer, pH 7·3, were mixed with equal volumes of either tris buffer or the buffer containing 120 mM sodium salicylate. Aliquots (1 ml) of the various enzyme solutions were assayed in a 3 ml reaction mixture containing 0·1 mM DL-isocitrate, 0·1 mM NADP, $MgCl_2$ and tris buffer, pH 7·3.

Analysis of kinetic data

Dalziel (1957, 1963) has derived a steady state initial rate equation for a compulsory order mechanism, with or without rate-limiting ternary complexes, in which the coenzyme forms a binary complex with the dehydrogenase enzyme but the substrate does not, and which may be written

$$\frac{E}{v} = \Phi_0 + \frac{\Phi_1}{[C]} + \frac{\Phi_2}{[S]} + \frac{\Phi_{12}}{[C][S]} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where E represents the total enzyme concentration, [C] and [S] are the initial concentrations of coenzyme and substrate respectively, and v is the initial rate. In the presence of an inhibitor (I), which competes with the coenzyme and forms an inactive complex (EI) with a dissociation constant of K_I, the initial rate equation becomes

$$\frac{E}{v} = \Phi_0 + \frac{\Phi_1\left(1 + \frac{I}{K_I}\right)}{[C]} + \frac{\Phi_2}{[S]} + \frac{\Phi_{12}\left(1 + \frac{I}{K_I}\right)}{[C]\,[S]} \quad .. \quad .. \quad .. \quad (2)$$

In the present work equations (3) and (4) were derived from equation 2 and primary plots, $\frac{[C]}{v}$ against [C] and $\frac{[S]}{v}$ against [S], were obtained by the Type B method described by Webb (1963) because this procedure allowed a greater significance to be attached to the results of the more accurate experiments using the higher substrate concentrations.

$$\frac{[S]E}{v} = \Phi_2 + \frac{\Phi_{12}\left(1 + \frac{I}{K_I}\right)}{[C]} + \left\{\Phi_0 + \frac{\Phi_1\left(1 + \frac{I}{K_I}\right)}{[C]}\right\}[S] \quad .. \quad (3)$$

$$\frac{[C]E}{v} = \Phi_1\left(1 + \frac{I}{K_I}\right) + \frac{\Phi_{12}\left(1 + \frac{I}{K_I}\right)}{[S]} + \left(\Phi_0 + \frac{\Phi_2}{[S]}\right)[C] \quad .. \quad (4)$$

To calculate the kinetic constants, secondary plots were made in which the slopes and intercepts from the primary plots were plotted against inhibitor concentrations. From equation 3 it can be shown that for a constant concentration of coenzyme [C_c] and for several concentrations of inhibitor, the slopes and intercepts on the $\frac{[C]}{v}$ axis of the primary plot can be represented by the following equations:

$$\text{Slopes of primary plot} = \Phi_0 + \frac{\Phi_1\left(1 + \frac{I}{K_I}\right)}{[C]} \quad .. \quad .. \quad (5)$$

$$\text{Intercepts of primary plot} = \Phi_2 + \frac{\Phi_{12}\left(1 + \frac{I}{K_I}\right)}{[C]} \quad .. \quad .. \quad (6)$$

Similarly for a constant concentration of substrate [S_c] the following equations can be derived:

$$\text{Slopes of primary plot} = \Phi_0 + \frac{\Phi_2}{[S]} \quad .. \quad .. \quad .. \quad (7)$$

$$\text{Intercepts of primary plot} = \Phi_1\left(1 + \frac{I}{K_I}\right) + \frac{\Phi_{12}\left(1 + \frac{I}{K_I}\right)}{[S]} \quad .. \quad (8)$$

It follows from equation 7 that the slopes of the primary plots must be constant, i.e. the plots are parallel lines, unless the inhibitor also competes with the substrate as well as the coenzyme. If the inhibitor competes with the substrate, the primary plots concerned in equation 5 should be parallel. The interpretation of the slopes and intercepts of the secondary plot are given in Table 1; from this Table the following kinetic parameters (see Dalziel, 1963) were calculated: K, Φ_0, Φ_1, Φ_2, Φ_{12}, $K_{mc}\left(=\frac{\Phi_1}{\Phi_0}\right)$, $K_{ms}\left(=\frac{\Phi_2}{\Phi_0}\right)$ and $K_c\left(=\frac{\Phi_{12}}{\Phi_2}\right)$.

TABLE 1. INTERPRETATION OF SECONDARY PLOTS FROM PRIMARY PLOTS. THE FOLLOWING EXPRESSIONS ARE DERIVED FROM EQUATIONS 5–8

		Slopes of secondary plots	Intercepts of secondary plots
[C_c]	Slopes of primary plots/[I] ..	$\frac{\Phi_1}{[C_c]\,K_I}$	$\Phi_0 + \frac{\Phi_1}{[C_c]}$
	Intercepts of primary plots/[I] ..	$\frac{\Phi_{12}}{[C_c]\,K_I}$	$\Phi_2 + \frac{\Phi_{12}}{[C_c]}$
[S_c]	Slopes of primary plots/[I] ..	0	$\Phi_0 + \frac{\Phi_2}{[S_c]}$
	Intercepts of primary plots/[I] ..	$\frac{\Phi_1}{K_I} + \frac{\Phi_{12}}{[S_c]\,K_I}$	$\Phi_1 + \frac{\Phi_{12}}{[S_c]}$

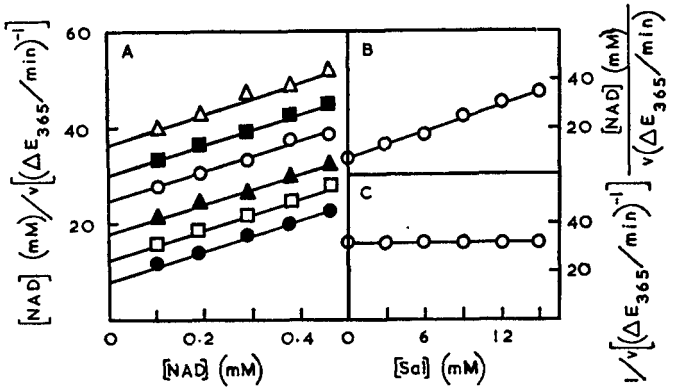

FIG. 1. Lactate dehydrogenase, kinetics with a constant concentration of lactate. The experimental conditions were as given in the text except that the coenzyme [NAD] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ●, Control; □, 3mM salicylate; ▲, 6mM salicylate; ○, 9mM salicylate; ■, 12mM salicylate; △, 15mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

Results

The results in Table 2 show that salicylate inhibits the activities of lactate, alcohol, malate and isocitrate dehydrogenases and that the degree of inhibition increases with salicylate concentration. The results of the dialysis experiments (Table 3) show that complete reactivation of the inhibited enzymes occurred after dialysis. The effects of varying the concentrations of NAD and salicylate in the presence of a constant concentration of lactate on the reaction rate of lactate dehydrogenase are shown in Fig. 1. Both the primary and secondary plots (see analysis of kinetic data) are presented in Fig. 1 and in subsequent figures. The results

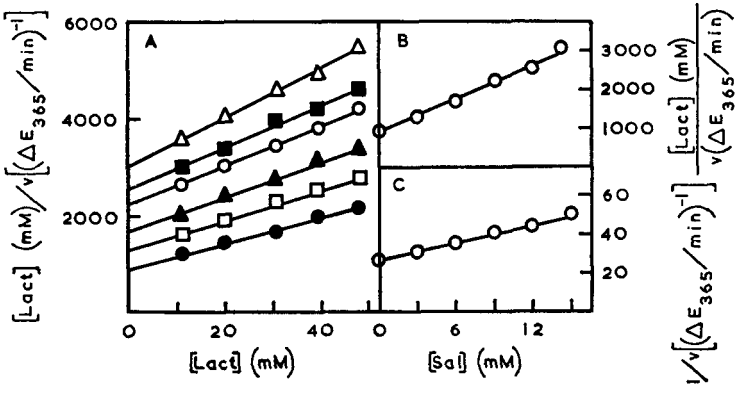

FIG. 2. Lactate dehydrogenase, kinetics with a constant concentration of NAD. The experimental conditions were as given in the text except that the L-lactate [Lact] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot ●, Control; □, 3mM salicylate; ▲, 6mM salicylate; ○, 9mM salicylate; ■, 12mM salicylate; △, 15mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

of similar experiments in which the concentration of lactate and salicylate were varied and that of NAD remained constant are shown in Fig. 2.

The lactate dehydrogenase reaction was also investigated in the reverse direction; Fig. 3 shows the results of experiments in which the concentrations of $NADH_2$ and salicylate were varied and pyruvate maintained constant and Fig. 4 those in which pyruvate and salicylate were varied in the presence of a constant concentration of $NADH_2$.

The results with alcohol dehydrogenase in which constant concentrations of either NAD or alcohol were maintained are given in Figs 5 and 6, with malate dehydrogenase using constant concentrations of either NAD or malate in Figs 7 and 8, and with isocitrate dehydrogenase and constant concentrations of either NADP or isocitrate in Figs 9 and 10.

The inhibitor constants and other kinetic parameters, calculated from the data contained in Figs 1 to 10, are given in Table 4.

Discussion

The Michaelis constants calculated from the present results (Table 4) are in good agreement with those reported by previous workers for lactate dehydrogenase (Anderson, Florini & Vestling, 1964), alcohol

TABLE 2. EFFECT OF SALICYLATE ON DEHYDROGENASE ENZYMES. Experimental details were as described in the Experimental section. The mean of three separate determinations was used to calculate each percentage inhibition. The initial velocities (ΔE_{365}/min) for the control reaction mixtures were as follows: lactate dehydrogenase, 0·057 ± 0·008; alcohol dehydrogenase, 0·036 ± 0·001; malate dehydrogenase, 0·021 ± 0·001; isocitrate dehydrogenase, 0·022 ± 0·001.

(a) Lactate dehydrogenase		(b) Alcohol dehydrogenase	
Salicylate (mM)	% Inhibition	Salicylate (mM)	% Inhibition
2·5	6	1·0	6
5·0	14	2·0	19
7·5	31	3·0	31
10·0	39	4·0	36
15·0	48	5·0	42
20·0	59		
(c) Malate dehydrogenase		**(d) Isocitrate dehydrogenase**	
Salicylate (mM)	% Inhibition	Salicylate (mM)	% Inhibition
2·5	8	5·0	6
5·0	16	10·0	20
7·5	36	15·0	26
10·0	45	20·0	34

TABLE 3. EFFECT OF DIALYSIS ON THE INHIBITION OF THE DEHYDROGENASES BY SALICYLATE. Experimental details were as described in the Experimental section. The results are expressed as ΔE_{365}/min and represent the mean ± standard deviations. The number of observations is given in parentheses

Dehydrogenase	Before dialysis		After dialysis	
	Control	Salicylate	Control	Salicylate
Alcohol	0·018 ± 0·001 (4)	0·010 ± 0·001 (4)	0·016 ± 0·001 (4)	0·015 ± 0·001 (4)
Lactate	0·063 ± 0·001 (5)	0·038 ± 0·002 (5)	0·032 ± 0·002 (5)	0·033 ± 0·002 (5)
Malate	0·135 ± 0·002 (6)	0·054 ± 0·002 (6)	0·134 ± 0·003 (6)	0·134 ± 0·003 (6)
Isocitrate	0·113 ± 0·003 (6)	0·076 ± 0·003 (6)	0·055 ± 0·001 (6)	0·057 ± 0·001 (6)

TABLE 4. KINETIC PARAMETERS OF LACTATE, ALCOHOL, MALATE AND ISOCITRATE DEHYDROGENASES. The values given here have been calculated from the constants defined under "Analysis of kinetic data." The three independent values of Φ_{12}, obtained during the calculations, have been included to indicate the internal consistency of the experimental data

Dehydrogenase	K_I mM	Φ_0 $\frac{1}{\Delta E_{365}/min}$	Φ_1 $\frac{mM}{\Delta E_{365}/min}$	Φ_2 $\frac{mM}{\Delta E_{365}/min}$	Φ_{12} $\frac{(mM)^2}{\Delta E_{365}/min}$	K_{mc} mM	K_{ms} mM	K_c mM
Lactate (lactate→ pyruvate) ..	3·79	21·0	2·66	522·5	211, 175, 216	0·127	24·9	0·39
Lactate (pyruvate→ lactate) ..	3·56	30·5	0·21	26·5	0·15, 0·17, 0·10	0·007	0·87	0·0053
Alcohol (ethanol→ acetaldehyde)	1·25	25·0	1·13	35·2	1·62, 2·04, 1·34	0·045	1·41	0·047
Malate (malate→ oxaloacetate) ..	1·33	33·4	2·21	2·88	1·45, 1·99, 1·12	0·066	0·086	0·53
Isocitrate (isocitrate→ oxoglutarate) ..	5·54	38·7	0·22	0·14	0·0013, 0·0016, 0·0026	0·0056	0·0035	0·013

dehydrogenase (Theorell, Nygaard & Bonnischen, 1955), malate dehydrogenase (Wolfe & Nielands, 1956; Cassman & England, 1966) and isocitrate dehydrogenase (Moyle, 1956).

The present work confirms that salicylate inhibits the four dehydrogenase activities *in vitro* (Table 2) and the results of the dialysis experiments (Table 3) show that the inhibitions are reversible. The plots of the kinetic data obtained in the present work demonstrate that salicylate competes with NAD and $NADH_2$ for lactate dehydrogenase (Figs 1C and 3C), with NAD for alcohol and malate dehydrogenases (Figs 5C and 7C) and with NADP for isocitrate dehydrogenase (Fig. 9C). The data exclude a mechanism involving competition with the respective substrates because the relevant plots (Figs 2C, 4C, 6C, 8C and 10C) do not have zero slopes. The pyridine nucleotide coenzymes used in the present experiments were not specially purified although it has been reported (Dalziel, 1963; Dalziel & Dickinson, 1965) that nucleotide impurities in commercial samples of NAD and NADP may act as competitive inhibitors of alcohol dehydrogenase and possibly other dehydrogenases. However, this type of interference only appeared to be of significance below pH 7 (Dalziel, 1963) and all reaction mixtures we used were at pH 7·3 or above.

The inhibitor constants (Table 4) show that salicylate inhibits the dehydrogenases to approximately the same degree as alanine aminotransferase but much more powerfully than aspartate aminotransferase (Gould, Dawkins, Smith & Lawrence, 1966). From Table 4 the ratios of K_c to K_I give an indication of the relative affinities of the coenzyme and of the salicylate for the enzyme. On this basis, malate dehydrogenase is the most sensitive and isocitrate dehydrogenase is the least sensitive to the inhibitory action of salicylate involving competition with the oxidized forms of the coenzymes. The lactate dehydrogenase $K_c : K_I$

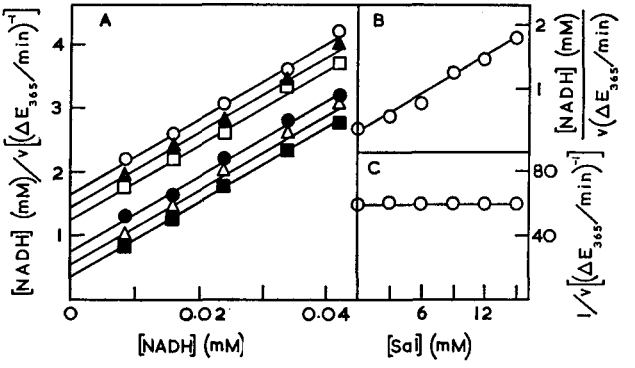

FIG. 3. Lactate dehydrogenase, kinetics with a constant concentration of pyruvate. Mixtures containing 0·93mM pyruvate and varying concentrations of coenzyme [NADH] and salicylate [Sal] as indicated, in a total volume of 3 ml of 0·0067 M tris-acetate buffer, pH 8·0, were allowed to come to thermal equilibrium at 25° in a 1 cm silica cell. The reaction was started by the addition of 10 μl of a solution containing approximately 1 μg of lactate dehydrogenase, which had been dialysed before use as described in Table 2. (A) Primary plot. ■, Control; △, 3mM salicylate; ●, 6mM salicylate; □, 9mM salicylate; ▲, 12mM salicylate; ○, 15mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

ratios show that salicylate inhibits the conversion of lactate to pyruvate much more strongly than the reverse reaction.

The present findings also suggest that salicylate is a general inhibitor of all pyridine nucleotide-linked dehydrogenase enzymes. The drug has been shown to interfere with the NADP-dependent glucose 6-phosphate and 6-phosphogluconate activities in preparations of human erythrocytes (Sturman & Smith, 1966) and with the $NADH_2$-cytochrome c reductase

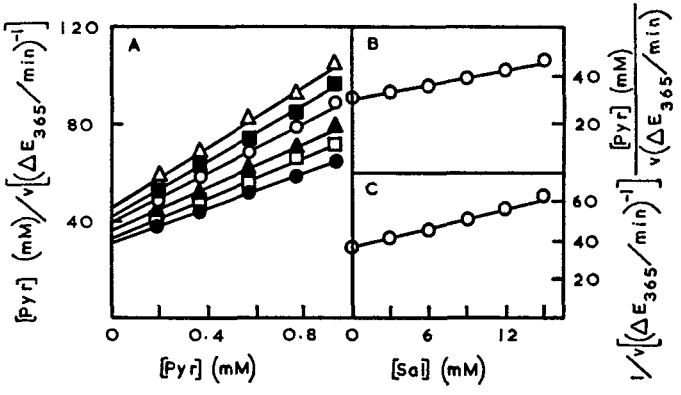

FIG. 4. Lactate dehydrogenase, kinetics with a constant concentration of $NADH_2$. Mixtures containing 0·042mM $NADH_2$ and varying concentrations of pyruvate [Pyr] and salicylate [Sal] as indicated, in a total volume of 3 ml of 0·0067 M tris-acetate buffer, pH 8·0, were allowed to come to thermal equilibrium at 25° in a 1 cm silica cell. The reaction was started by the addition of 10 μl of a solution containing approximately 1 μg of lactate dehydrogenase, which had been dialysed before use as described in Table 2. (A) Primary plot. ●, Control; □, 3mM salicylate; ▲, 6mM salicylate; ○, 9mM salicylate; ■, 12mM salicylate; △, 15mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

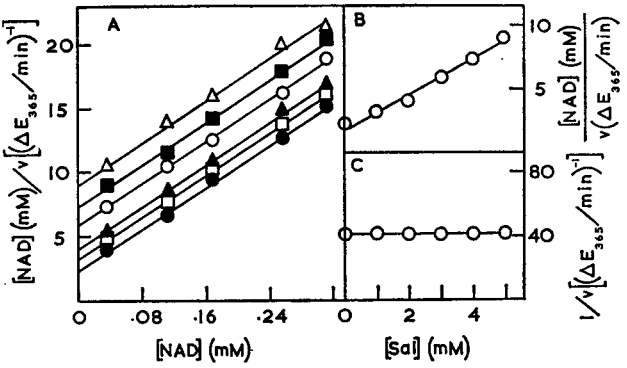

FIG. 5. Alcohol dehydrogenase, kinetics with a constant concentration of ethanol. The experimental conditions were as given in the text except that coenzyme [NAD] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ●, Control; □, 1mM salicylate; ▲, 2mM salicylate; ○, 3mM salicylate; ■, 4mM salicylate; △, 5mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

system in guinea-pig liver mitochondria (Hines, Bryant & Smith, 1963).

The *in vitro* results observed in the present work may have important implications in some of the *in vivo* actions of salicylate. Slater & Sawyer (1966) have reported that the injection of salicylate in the rat causes a rapid decrease in the content of NADP and $NADPH_2$ in the liver. They considered it unlikely that this effect would be due to salicylate displacing the nucleotides from binding sites into free solution, thereby making them more susceptible to enzymic breakdown. However, an increased accumulation of the nucleotides in free solution may enhance their leakage into the circulation especially as the uncoupling action of salicylate on oxidative phosphorylation reactions impairs normal

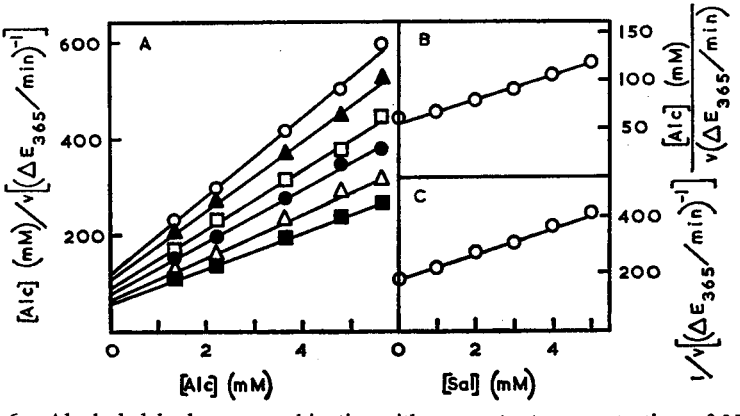

FIG. 6. Alcohol dehydrogenase, kinetics with a constant concentration of NAD. The experimental conditions were as given in the text except that the ethanol [Alc] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ■, Control; △, 1mM salicylate; ●, 2mM salicylate; □, 3mM salicylate; ▲, 4mM salicylate; ○, 5mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

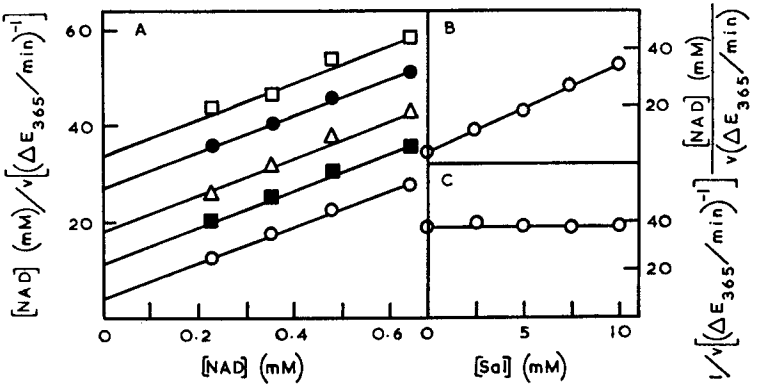

FIG. 7. Malate dehydrogenase, kinetics with a constant concentration of malate. The experimental conditions were as given in the text except that the coenzyme [NAD] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ○, Control; ■, 2·5mM salicylate; △, 5mM salicylate; ●, 7·5mM salicylate; ■, 10mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

membrane permeability (Mitidieri & Affonso, 1959). Such a diminution in intracellular nucleotide concentrations would be expected to reinforce an inhibitory action of the drug on NADP-dependent dehydrogenases.

It has also been shown that, in man and experimental animals, the administration of increasing amounts of salicylate causes a progressive rise in oxygen consumption. A similar effect occurs with isolated tissue preparations, except that the stimulation of oxygen uptake is succeeded by a depression of this function at higher salicylate concentrations. A typical result has been reported with isolated sacs of rat small intestine

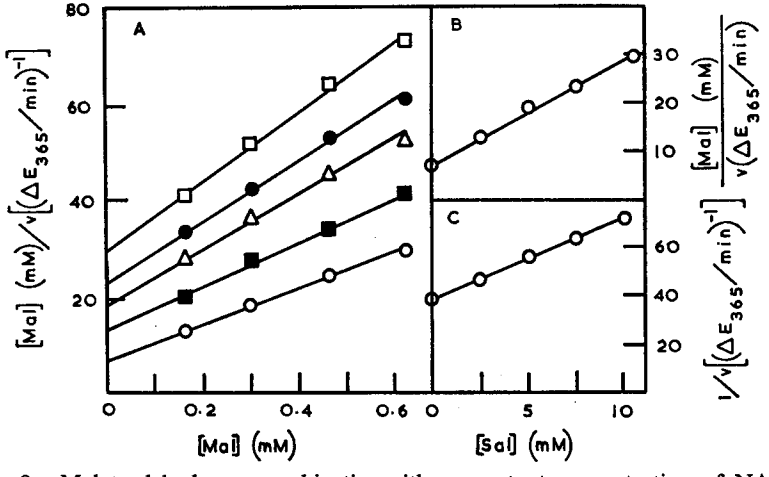

FIG. 8. Malate dehydrogenase, kinetics with a constant concentration of NAD. The experimental conditions were as given in the text except that the L-malate [Mal] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ○, Control; ■, 2·5mM salicylate; △, 5mM salicylate; ●, 7·5mM salicylate □, 10mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

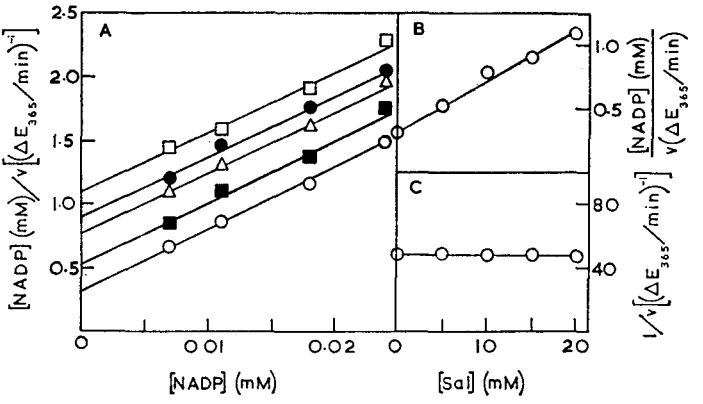

FIG. 9. Isocitrate dehydrogenase, kinetics with a constant concentration of isocitrate. The experimental conditions were as given in the text except that the coenzyme [NADP] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ○, Control; ■, 5mM salicylate; △, 10mM salicylate; ●, 15mM salicylate; □, 20mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

(Smith, 1958). 0 to 1 mM salicylate produced no effect, 1 to 5 mM caused an initial increase in the oxygen uptake and salicylate concentrations above 5 mM produced a marked depression in the oxygen consumption. Similar responses with mouse liver slices (Sproull, 1954) and rat cerebral cortex preparations (Fishgold, Field & Hall, 1951) have been observed. One possible explanation of these effects is that the initial stimulation of oxygen consumption results from the well-known uncoupling action of salicylate on oxidative phosphorylation processes, but that this effect is progressively antagonized and eventually overcome by the inhibitory action of increasing doses of the salicylate on the pyridine nucleotide-linked dehydrogenases.

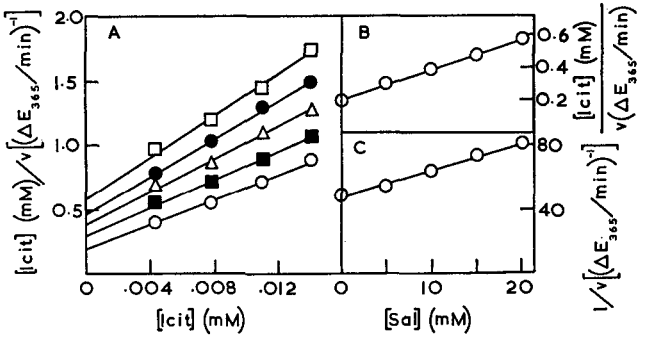

FIG. 10. Isocitrate dehydrogenase, kinetics with a constant concentration of NADP. The experimental conditions were as given in the text except that the isocitrate [Icit] and salicylate [Sal] concentrations were varied as shown. (A) Primary plot. ○, Control; ■, 5mM salicylate; △, 10mM salicylate; ●, 15mM salicylate; □, 20mM salicylate. (B) Secondary plot of intercepts of (A). (C) Secondary plot of slopes of (A).

A further interaction of the inhibitory effect of salicylate on dehydrogenase enzymes and body metabolism may be involved in the acid-base disturbances which occur in salicylate intoxication in man. Toxic amounts of the drug cause three basic actions which affect the acid-base equilibrium directly; an increased alveolar ventilation, an increased metabolic rate and an accumulation of organic anions in the plasma (Smith & Smith, 1966). The net result in an individual patient depends on the relative intensities of these actions and the human infant appears to be particularly vulnerable to the last action, which leads to a state of metabolic acidosis (Done, 1963). It has been shown that the plasma of salicylate-intoxicated infants and young children contains abnormally high concentrations of organic anions (Winters, 1959) although no detailed fractionation of the individual components has been accomplished. An inhibition of pyridine nucleotide-linked dehydrogenases by salicylate would be expected to cause decreased rates of metabolism of hydroxy acids in the tissues, which could lead to an accumulation of their anions in the plasma.

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