THE MECHANISM OF THE INHIBITION OF DECARBOXYLASES BY *ISO*NICOTINYL HYDRAZIDE

by

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The anti-tubercular drug *iso*nicotinyl hydrazide (isoniazid) has been shown to react with a number of enzyme systems[1]. Since some of the enzymes inhibited are known to require pyridoxal phosphate as co-enzyme, it has been suggested that inhibition is due to competition between pyridoxal and *iso*nicotinyl hydrazide, for there is a structural resemblance between these two substances[2,3,4,5]. This hypothesis is supported by experimental results obtained by inhibition of bacterial arginine decarboxylase and tryptophanase activity by *iso*nicotinyl hydrazide and reversal of this inhibition by pyridoxine[2,3]; on *in vivo* experiments with rats deficient in pyridoxine[4] and on the competitive antagonism for the vitamin B_6 group as measured by the growth of *Lactobacilli* or *Saccharomyces*[5].

However, this view is contested by VILTER, BIEHL, MUELLER AND FRIEDMAN[6] who suggest that inhibition of vitamin B_6-requiring enzymes is due to the formation of a pyridoxal-*iso*nicotinyl hydrazone. No evidence, to the knowledge of the author, has been presented so far to support this hypothesis.

Rat liver L-cysteine sulphinic acid decarboxylase[7,8] has been chosen as a suitable model vitamin B_6-requiring enzyme for studying the mechanism of inhibition by *iso*nicotinyl hydrazide. Experiments reported in this paper support the view that this inhibition is due to the formation of pyridoxal phosphate *iso*nicotinyl hydrazone. Rat brain cysteine sulphinic and glutamic acid decarboxylases, and liver cysteine sulphinic acid and DOPA decarboxylases are also similarly inhibited. The inhibition reaction is slow, not readily reversed, and in these respects differs markedly from the inhibition by hydroxylamine which is rapid and reversible[9]. It is therefore suggested that the rate of inhibition of enzyme activity by *iso*nicotinyl hydrazide may be used as a means for demonstrating the presence of pyridoxal phosphate as the prosthetic group of an enzyme.

MATERIALS

Adult white male rats have been used throughout this work to provide brain and liver enzyme preparations. The animals were stunned, the tissues dissected out, cooled in ice and a 25% w/v liver suspension prepared in 0.067 *M* Sørensen's phosphate buffer pH 6.8 using a Potter-Elvehjem all glass homogenizer.

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Pyridoxal, pyridoxine and pyridoxamine phosphates were kindly supplied by Dr. A. L. MORRISON, Roche Products, Welwyn Garden City, England. *Iso*nicotinyl hydrazide was obtained from Organon, Oss, Holland. 1-*iso*nicotinyl-2-*iso*propyl hydrazine (Marsilid) and nicotinic acid hydrazide were gratefully received from Dr. S. BLAU, Produits Roche, Paris. L-cysteine sulphinic acid was prepared in this laboratory by Dr. B. BERGERET to whom my thanks are also due.

METHODS

The decarboxylation of amino acid substrates is followed manometrically in the Warburg apparatus at 35° C. Readings are taken every five minutes and providing that the output of carbon dioxide is sensibily linear, the output of gas is calculated by the method of least squares[10]. The kinetics of enzyme inhibition are treated in a similar way to those for the inhibition of cholinesterase by organophosphates[11].

One ml of liver suspension is pipetted into the centre well of a two-armed Warburg flask, phosphate buffer pH 6.8 is added to give a final volume of 3.2 ml. Substrate (sodium salt of final concentration $1 \cdot 10^{-2}$ *M*) is placed in one side arm and inhibitor solution in the other. After gassing in the bath with nitrogen for 6 min, inhibitor is tipped in at stated time intervals from the side arm, substrate is then added at the end of the incubations and enzyme activity determined. Activity is calculated as a percentage of the uninhibited enzyme activity. Output of carbon dioxide is uncorrected for gas retention. The apparent energy of activation is obtained by determining the rate of inhibition on the same liver suspension and with the same inhibitor solution at 19°, 30°, 35° and 40° C and calculating the energy involved from the Arrhenius expression.

Beckmann spectrophotometric studies

In these studies the U.V. absorption spectrum of pyridoxal ($5.6 \cdot 10^{-5}$ *M*), and *iso*nicotinyl hydrazide ($3 \cdot 10^{-4}$ *M*) in Sørensen's phosphate buffer (0.067 *M*, pH 6.8) are determined separately at room temperature (Fig. 1). Steadily increasing absorption occured in a band between 290 mμ and 350 mμ on mixing the two solutions. This reaction could be followed to completion, and under these conditions where the *iso*nicotinyl hydrazide is in excess it is possible to apply the first order expression and calculate the rate of the reaction. By determining the rate of reaction at 19°, 30°, 35° and 40° C an approximate value for the energy of activation is derived. Hydroxylamine reacts rapidly with pyridoxal phosphate and the rate of increase of absorption is too fast to measure, however $3 \cdot 10^{-4}$ *M* 1-*iso*nicotinyl-2-*iso*propyl hydrazine (Marsilid) does not appear to react with pyridoxal phosphate.

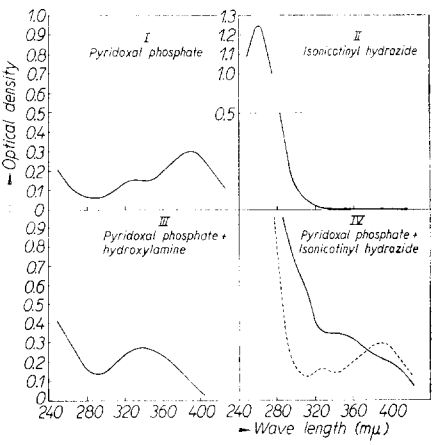

Fig. 1. U.V. spectrograms of pyridoxal phosphate, *iso*nicotinyl hydrazide and pyridoxal phosphate plus hydroxylamine or *iso*nicotinyl hydrazide. Pyridoxal phosphate concentration was $5.6 \cdot 10^{-5}$ *M* in 0.067 *M* phosphate buffer pH 6.8. Hydroxylamine and *iso*nicotinyl hydrazide concentrations were $1 \cdot 10^{-4}$ *M* and $3 \cdot 10^{-4}$ *M* respectively. The solutions after mixing were kept for 90 min before making the measurements. The sum of the absorptions of pyridoxal phosphate plus *iso*nicotinyl hydrazide alone (same concentration) is shown by the broken line (IV).

RESULTS

Initial experiments in this laboratory showed that *iso*nicotinyl hydrazide inhibited rat brain cysteine sulphinic acid decarboxylase. The inhibition was greater if the *iso*nicotinyl hydrazide was incubated with the brain suspension before addition of

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substrate*. In the following experiments liver suspensions have been used as a source of the enzyme, since the activity is higher than that of the brain and the co-enzyme is firmly bound to the apoenzyme[12].

The kinetics of inhibition of cysteine sulphinic acid decarboxylase

It seemed interesting to compare the mechanism of inhibition by hydroxylamine and *iso*nicotinyl hydrazide, since the former is a well known example of an inhibitor of pyridoxal phosphate-requiring enzymes[9,13,14]. Suspensions of rat liver in 0.067 *M* Sørensen's phosphate buffer pH 6.8 are incubated for 30 min at 35° C with varying concentrations of either hydroxylamine or *iso*nicotinyl hydrazide, and the remaining activity determined by the addition of neutralised cysteine sulphinic acid. The concentration producing 50% inhibition is calculated by plotting percentage inhibition against the negative log of the molar concentration and reading the concentration producing 50% inhibition directly from the curves (Fig. 2 and Table I).

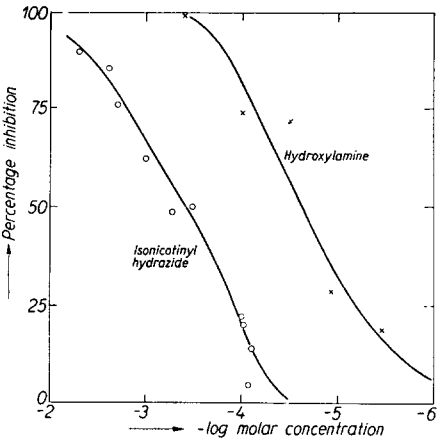

Fig. 2. The inhibition of liver cysteine sulphinic acid decarboxylase by *iso*nicotinyl hydrazide and hydroxylamine. Inhibitor and rat liver suspension were incubated for 30 min at 35° C before the enzyme activity was determined.

TABLE I

CONSTANTS FOR THE INHIBITION OF CYSTEINE SULPHINIC ACID DECARBOXYLASE

These results were obtained by methods described in the text, using 250 mg (wet weight) of liver per flask.

Constant	*Substrate*	*Inhibitor*	
	Cysteine sulphinic acid	*Isonicotinyl hydrazide*	*Hydroxylamine*
Inhibitor concentration producing 50% inhibition after 30 min incubation at 35° C	—	$3 \cdot 10^{-4}$ *M*	$2.5 \cdot 10^{-5}$ *M*
Dissociation constants (k_s and k_1) obtained by the method of LINEWEAVER AND BURK (1934)[15]	$1.3 \cdot 10^{-3}$	$4 \cdot 10^{-2}$	$1.3 \cdot 10^{-5}$
First order inhibition constant for the reaction between *iso*nicotinyl hydrazide and the enzyme at pH 6.8 and 35° C	—	92 $min^{-1} \cdot l \cdot mol^{-1}$	—

In the presence of substrate, different results are obtained. 3.5, 5 and $10 \cdot 10^{-5}$ *M* hydroxylamine or 2, 4 and $8 \cdot 10^{-2}$ *M* *iso*nicotinyl hydrazide have been added at 35° C to the liver decarboxylase preparation, together with varying concentrations of cysteine sulphinic acid. The reciprocals of the rate of output of carbon dioxide (in the first 8 min) have been plotted against the reciprocals of the substrate concentration according to the method of LINEWEAVER AND BURK[15]. The results demonstrate that inhibition by both substances is competitive with the substrate: the Michaelis

* Private communication from Dr. BERGERET of this laboratory.

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constants and thus the affinities are different (Table I). Furthermore, these results (a difference of inhibitor concentration of 2,000 times) show that in comparison with hydroxylamine, *iso*nicotinyl hydrazide is a much weaker inhibitor of the decarboxylase activity than is suggested by the ten-fold difference in values for 50% inhibition obtained by incubation of inhibitor and enzyme for 30 min before adding substrate. If *iso*nicotinyl hydrazide or hydroxylamine is incubated with liver suspension at 35° C for different times, before addition of substrate, a striking difference between their action is seen. In Fig. 3 the log percentage decarboxylase activity of a control without inhibitor is plotted against the time of incubation with inhibitor. In the case of hydroxylamine, inhibition is almost instantaneous and maximum inhibition is reached in 10 min at a concentration of $3 \cdot 10^{-5}$ *M*. Under these special conditions, this form of the inhibition curve is characteristic of a reversible inhibitor. Inhibition by *iso*nicotinyl hydrazide is slow and exponential. The rate of inhibition obeys the first order expression for a *pseudo*-monomolecular-reaction, when one component (*iso*nicotinyl hydrazide) is in excess and the amount of enzyme is constant. The rate constant can best be calculated by plotting the log percentage activity against concentration × time[11] (Table I).

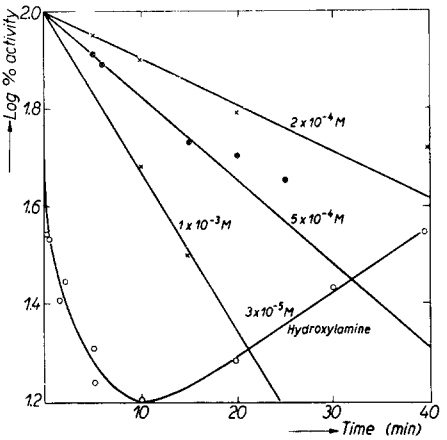

Fig. 3. The kinetics of inhibition of rat liver cysteine sulphinic acid decarboxylase by *iso*nicotinyl hydrazide and hydroxylamine. *Iso*nicotinyl hydrazide (×) or hydroxylamine (○) have been incubated with rat liver suspensions for varying times before determining the enzyme activity.

Inhibition by *iso*nicotinyl hydrazide is not easily reversed, while that of hydroxylamine is easily reversible. This is clearly shown in Table II, where the dilution method of NACHMANSOHN, ROTHENBERG AND FELD[16] modified by ALDRIDGE[17] has been employed. Since the affinity of *iso*nicotinyl hydrazide for the enzyme is less than that of the substrate, it is possible to stop the progressive inhibition by addition of the substrate and on diluting either with substrate alone or substrate plus inhibitor to demonstrate reversibility.

The apparent energy of activation for the inhibition of cysteine sulphinic acid decarboxylase has been found by plotting reciprocal of the absolute temperature against the log rate constant for different temperatures. Approximate values of 16,000 cals/mole for the inhibition process and 7,000 cals/mole for substrate decarboxylation have been obtained. These values suggest that the process of inhibition is a chemical reaction and probably not enzyme mediated.

The reaction of pyridoxal phosphate with isonicotinyl hydrazide

Brain cysteine sulphinic acid decarboxylase requires the addition of pyridoxal phosphate for maximum activity[12]. However, pyridoxal phosphate is more firmly bound to the liver decarboxylase and can only be separated by feeding animals on a diet deficient in vitamin B_6[8] or by the following method. The supernatant obtained by centrifuging a 10% w/v rat liver suspension in 0.25 *M* sucrose for 10 min at

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TABLE II

THE REVERSIBILITY OF THE INHIBITION BY *iso*NICOTINYL HYDRAZIDE AND HYDROXYLAMINE

Male rat liver (50% w/v) was freshly suspended in cold phosphate buffer, pH 6.8. 0.5 ml of rat liver suspension with inhibitor or without inhibitor was placed in the side arm of a Warburg flask, the centre well contained either buffer and substrate (final concentration 0.01 *M*) or buffer plus inhibitor and substrate. After 20 min shaking and incubation at 35° C, the contents of the side arm were tipped into the centre and the evolution of carbon dioxide determined. The final volume in each flask was 3.9 ml.

Side arm contents. 0.5 ml of the following mixtures	*Centre well contents. 3.2 ml buffer + 0.01 M cysteine sulphinic acid*	*µl CO_2/min*	*% activity*
0.4 ml of buffer + 1.6 ml of liver suspension	without inhibitor	3.13	100
0.3 ml of *iso*nicotinyl hydrazide (final concentration $2 \cdot 10^{-3} M$) + 1.2 ml of suspension	with *iso*nicotinyl hydrazide ($2 \cdot 10^{-3} M$)	0.7	23
0.3 ml of *iso*nicotinyl hydrazide (final concentration $2 \cdot 10^{-3} M$) + 1.2 ml of suspension	without inhibitor	0.85	27
0.4 ml of buffer + 1.6 ml of liver suspension	with *iso*nicotinyl hydrazide ($2 \cdot 10^{-3} M$)	3.12	100
0.3 ml of hydroxylamine (final concentration of $6 \cdot 10^{-5} M$) + 1.2 ml of suspension	with hydroxylamine ($6 \cdot 10^{-5} M$)	1.39	45
0.3 ml of hydroxylamine (final concentration of $6 \cdot 10^{-5} M$) + 1.2 ml of suspension	without inhibitor	2.84	91
0.4 ml of buffer + 1.6 ml of liver suspension	with hydroxylamine ($6 \cdot 10^{-5} M$)	1.44	46

8,000 *g* was 50% saturated with ammonium sulphate. The precipitate obtained by centrifuging at 8,000 *g* for 10 min was then dialysed for 3 days and stored for a week at —15° C. The dialysed enzyme preparation had a low decarboxylase activity (5.7 µl CO_2/25 min/200 mg dry weight), but could be activated by about 10 times by addition of $2.3 \cdot 10^{-4} M$ pyridoxal phosphate (calcium or sodium salt) and addition of $1 \cdot 10^{-4} M$ ferrous sulphate further improved this activity. Pyridoxal phosphate could not be replaced by pyridoxine phosphate and only by high concentrations of pyridoxamine phosphate (10 × pyridoxal phosphate concentration).

Since other enzymes requiring vitamin B_6 are, in general, inhibited by keto reagents, it seemed possible that *iso*nicotinyl hydrazide combines with the formyl group of pyridoxal phosphate to form *iso*nicotinyl pyridoxal phosphate hydrazone. Thus, in 1954 SAH[18] demonstrated a chemical reaction between pyridoxal and *iso*nicotinyl hydrazide. The rate of reaction between pyridoxal phosphate and *iso*nicotinyl hydrazide was determined at different temperatures in the Beckman spectrophotometer by measuring the increased absorption at 300 mµ or 330 mµ. An approximate value of 14,000 cals/mole for the energy of activation was obtained. This value is of the same order of that obtained for the enzyme inhibition reaction and is therefore in accord with the hypothesis that the inhibition of the enzyme is simply due to inactivation of the formyl group of pyridoxal phosphate.

Another experiment was designed to show that the apoenzyme alone was not inactivated by *iso*nicotinyl hydrazide, whereas the holoenzyme was. Table III shows the effect of incubating the apoenzyme of liver for an hour with a concentration of *iso*nicotinyl hydrazide which produces 70% inhibition of the holoenzyme. The slight inhibition of the apoenzyme is due (a) to the presence of unseparated holoenzyme and (b) to a lag in the reactivation of the enzyme activity after addition of pyridoxal phosphate and substrate. The experiment illustrated in Table IV shows that the rate

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of inhibition of decarboxylase activity is the same if pyridoxal phosphate and *iso*-nicotinyl hydrazide are incubated for 30 min in the side arm of a Warburg flask, before adding to the apoenzyme, or if the incubation is effected in the presence of the enzyme.

TABLE III

THE EFFECT OF *iso*NICOTINYL HYDRAZIDE ON THE INHIBITION OF HOLOENZYME AND APOENZYME DECARBOXYLASE ACTIVITY

Each flask contained 2 ml of a dialysed enzyme preparation (100 mg dry weight/ml), 0.8 ml of 0.25 *M* phosphate buffer, pH 6.8 and ferrous sulphate $1 \cdot 10^{-4}$ *M*. The final volume was 3.2 ml. Pyridoxal phosphate ($1.25 \cdot 10^{-4}$ *M*) *iso*nicotinyl hydrazide ($3 \cdot 10^{-4}$ *M*) and cysteine sulphinic acid ($1 \cdot 10^{-2}$ *M*) were added as indicated. The temperature was 35° C. Two-armed Warburg flasks were used, one arm contained substrate and the other pyridoxal or inhibitor.

Flask contents Apoenzyme plus	*Side arm contents (Added after 1 h followed by addition of the substrate)*	*Enzyme activity Maximum rate of CO_2 evolution µl CO_2 10 min*
—	pyridoxal	16
*iso*nicotinyl hydrazide	pyridoxal	11.5
pyridoxal	*iso*nicotinyl hydrazide	14
pyridoxal + *iso*nicotinyl hydrazide	—	4.5

TABLE IV

THE EFFECT OF INCUBATING PYRIDOXAL PHOSPHATE AND *iso*NICOTINYL HYDRAZIDE TOGETHER, WITH AND WITHOUT THE ENZYME

Each flask contained 2 ml of dialysed enzyme preparation (100 mg dry weight/ml), 0.8 ml of 0.25 *M* phosphate buffer, pH 6.8 and ferrous sulphate ($1 \cdot 10^{-4}$ *M*). The final volume was 3.2 ml. Final concentrations were pyridoxal phosphate ($1.25 \cdot 10^{-4}$ *M*) and *iso*nicotinyl hydrazide ($3.3 \cdot 10^{-4}$ *M*). After 30 min, at 35° C, the contents of the side arm were tipped into the centre and substrate added.

Flask contents	*Maximum rate of CO_2 evolution µl CO_2/25 min*	*% inhibition*
Apoenzyme only	5.7	—
Apoenzyme + pyridoxal	39	—
0.05 ml of a mixture of *iso*nicotinyl hydrazide ($2 \cdot 10^{-2}$ *M*) and pyridoxal phosphate ($7.5 \cdot 10^{-3}$ *M*) are added		
(1) to the apoenzyme in the centre well	12.6	79
(2) into the side arm	14.5	74
0.5 ml of a mixture of *iso*nicotinyl hydrazide ($2 \cdot 10^{-3}$ *M*) and pyridoxal phosphate ($7.5 \cdot 10^{-4}$ *M*) are added		
(1) to the apoenzyme in the centre well	12.2	80
(2) into the side arm	13.8	75

The affinity of pyridoxal phosphate *iso*nicotinyl hydrazone for the enzyme appears to be less than the affinity of pyridoxal phosphate for the enzyme, for addition of excess pyridoxal phosphate to the holoenzyme prevents inhibition by *iso*nicotinyl hydrazide and furthermore addition of pyridoxal phosphate to the inhibited holoenzyme results in a slow reversal of inhibition. It has also been found that the presence of 2:3 dimercaptopropanol (BAL) interferes with inhibition, and indeed addition of BAL reverses the inhibition.

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Inhibition by other inhibitors in vitro and in vivo

Table V shows that other hydrazides are equally potent inhibitors of the liver decarboxylase, except for Marsilid. However, when injected into the rat Marsilid inhibits decarboxylase activity in a similar way to *iso*nicotinyl hydrazide.

TABLE V

CONSTANTS FOR *in vitro* INHIBITION OF LIVER DECARBOXYLASE ACTIVITY

The concentration of inhibitor producing 50% inhibition of rat liver decarboxylase activity has been determined as previously described.

Inhibitor	*50% inhibition concentration (M)*
*iso*Nicotinyl hydrazide	$3 \cdot 10^{-4}$
Nicotinic acid hydrazide	$4.5 \cdot 10^{-4}$
1-*iso*nicotinyl-2-*iso*propylhydrazine (Marsilid)	$2 \cdot 10^{-1}$ *
Hippuric hydrazide	$3 \cdot 10^{-4}$

* This concentration inhibits by 35%.

TABLE VI

INHIBITION *in vivo* OF LIVER DECARBOXYLASE ACTIVITY

Male rats of about 250 g were injected intraperitoneally with the dose of hydrazide indicated. After two or six hours the animals were killed and the liver suspended (25% w/v) in phosphate buffer. Cysteine sulphinic decarboxylase activity was determined as previously described. The mean enzyme activity of nine control rats is recorded together with the standard error of the observations.

Substance injected and dose	*Enzyme activity* $\mu l CO_2/250$ *mg wet weight/min*	
	After 2 h	*After 6 h*
Control rats (9)	7.78 ± 0.6	—
*iso*Nicotinyl hydrazide (250 mg/kg)	2.0 3.65	2.25
Marsilid (500 mg/kg)	5.05 3.57 3.24	2.41 1.32 3.29
Nicotinic acid hydrazide (250 mg/kg)	3.22	—

The effect of isonicotinyl hydrazide on other pyridoxal-requiring enzymes

The rate of inhibition of brain glutamic acid and liver DOPA decarboxylase by *iso*nicotinyl hydrazide has been compared with the rate of inhibition of cysteine sulphinic acid decarboxylase in the same tissue. It will be seen that there is good agreement between the inhibition curves of each pair of enzymes in brain and liver (Figs. 4 and 5).

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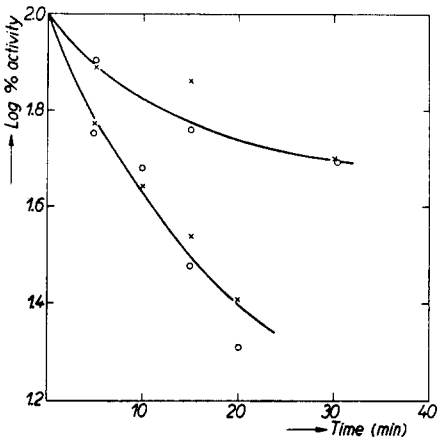

Fig. 4. Comparison of the rate of inhibition of rat brain glutamic and cysteine sulphinic acid decarboxylases by *iso*nicotinyl hydrazide. In experiment (1) brain concentration was 300 mg wet weight in 3 ml of phosphate buffer pH 6.8 with *iso*nicotinyl hydrazide $2 \cdot 10^{-3}$ *M* and pyridoxal phosphate 16.6 μg/ml. In experiment (2) brain concentration was 500 mg wet weight in 3 ml of phosphate buffer pH 6.8 with *iso*-nicotinyl hydrazide $4 \cdot 10^{-3}$ *M* and pyridoxal phosphate 33.3 μg/ml, the temperature was 35° C. Glutamate (×) and cysteine sulphinic (○) acid (0.01 *M*) were added to determine the residual enzyme activity, after incubating *iso*-nicotinyl hydrazide and brain suspension together for varying times.

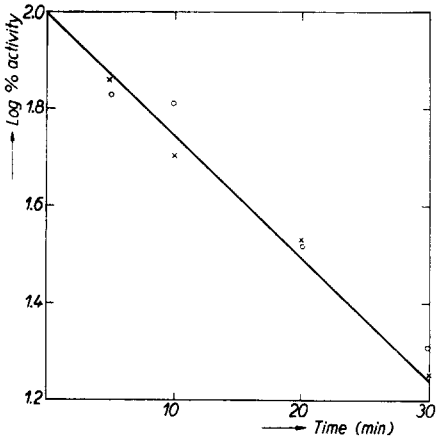

Fig. 5. Comparison of the rate of inhibition of rat liver cysteine sulphinic acid and 3:4 dihydroxyphenylalanine. 2 ml of 50% w/v rat liver suspended in phosphate buffer pH 6.8 were added to each flask, the final volume being 3.2 ml. *Iso*nicotinyl hydrazide ($1 \cdot 10^{-3}$ *M*) was added to the enzyme, and after incubating for varying times either 0.01 *M* cysteine sulphinic acid or 0.05 *M* 3:4 dihydroxyphenylalanine (DOPA) was added. Readings were taken every 2.5 min and the rates calculated from the plot of carbon dioxide evolution in the first ten minutes (DOPA) and 15 min for cysteine sulphinic acid decarboxylase. The experiment was otherwise as previously described.

DISCUSSION

In this paper a study has been made of the mechanism of inhibition by *iso*nicotinyl hydrazide of a pyridoxal phosphate requiring enzyme, namely rat liver cysteine sulphinic acid decarboxylase. Although decarboxylase inhibition by a typical carbonyl reagent (hydroxylamine) was found to be rapid and readily reversible, inhibition by *iso*nicotinyl hydrazide was relatively slow and not easily reversed. Inhibition by both substances was competitive with the substrate and thus possibly occurs at the active group of the enzymic system. This active group is probably the formyl group of pyridoxal phosphate[19] for pyridoxal cannot be replaced by pyridoxine or pyridoxamine phosphate.

This possibility has now been investigated in some detail, for the progressive, irreversible inhibition of decarboxylase activity proved readily amenable to kinetic study. Rates of inhibition at different temperatures were calculated; the high value for the apparent energy of activation obtained from these values suggested that a chemical reaction had occured. The reaction between pyridoxal phosphate and *iso*-nicotinyl hydrazide alone could be followed in the spectrophotometer. The energy of activation for this reaction was found to be of the same order as that for the enzyme inhibition. This suggests that the reaction is not catalysed by the enzyme. This conclusion was confirmed in experiments showing that inhibition of holoenzyme

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by *iso*nicotinyl hydrazide, or inhibition by adding to the apoenzyme pyridoxal phosphate plus *iso*nicotinyl hydrazide, incubated separately for the same time, resulted in the same amount of inhibition (Table IV). Using a dialysed enzyme preparation it was found that incubation of apoenzyme and *iso*nicotinyl hydrazide with addition of pyridoxal at the end of the experiment, resulted in slight inhibition. However, if pyridoxal phosphate, apoenzyme and *iso*nicotinyl hydrazide are incubated together marked inhibition is obtained. Experiments with other inhibitors also support the hypothesis that inhibition is due to the formation of the *iso*nicotinyl hydrazone. Thus, hydrazides of hippuric and nicotinic acids are about as equally potent inhibitors *in vitro*. Marsilid, with a substituted terminal amino group, is not an efficient inhibitor *in vitro*, neither does it react with pyridoxal phosphate chemically.

It is interesting to note that if large single doses of the different anti-tubercular drugs are given *in vivo*, decarboxylase activity is inhibited by Marsilid as well as by the nicotinic acid hydrazides. This suggests that Marsilid undergoes a conversion in the body.

Therefore it might be that the action of these drugs against the tubercle bacillus corresponds in part to a similar mechanism although of course the doses given are much larger than clinical doses. Recently pyridoxine together with *iso*nicotinyl hydrazide has been given *in vitro* to tubercle cultures and *in vivo* to infected guinea pigs[20,21]. On the basis of these results it is suggested that there is no relationship between *iso*nicotinyl hydrazide action and vitamin B_6. However, it is possible in these experiments that since pyridoxine itself will not react with *iso*nicotinyl hydrazide, sufficient of this latter compound penetrates the tubercle bacillus before any pyridoxal formed has a chance to act.

It is suggested that since *iso*nicotinyl hydrazide reacts with pyridoxal phosphate and the apoenzyme does not appear to activate this reaction, that the rate of inhibition should be the same for all pyridoxal requiring enzymes. Such inhibition curves might then be used to detect cases where pyridoxal phosphate is tightly bound to the enzyme, and it is difficult to show the co-enzyme's presence by desoxy-pyridoxin phosphate or even by vitamin B_6 deficiency experiments. In support of this hypothesis it has been shown that there is a parallel rate of inhibition between brain cysteine sulphinic acid and glutamic acid decarboxylase, and between liver cysteine sulphinic acid and DOPA decarboxylase. It is also interesting to note that *iso*nicotinyl hydrazide has been shown to inhibit transaminases[22]. However, it should be stressed that the method should be used with caution, inasmuch as that certain tissues such as brain and kidney have been shown to destroy *iso*nicotinyl hydrazide[23]. Furthermore, the presence of substrates (such as glutamic acid in brain) would interfere with inhibition. Nevertheless study of inhibition rates of two enzymes in the same preparation, one known to be a pyridoxal phosphate requiring enzyme and the other not, or inhibition of a purified enzyme seems to be a technique with promise.

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SUMMARY

1. The kinetics of inhibition of liver cysteine sulphinic acid decarboxylase by *iso*nicotinyl hydrazide and hydroxylamine have been compared.

2. Evidence is presented to suggest that decarboxylase inhibition is the result of interaction between the formyl group of pyridoxal phosphate and the inhibitor.

3. It is suggested that the rate of inhibition of enzymes by *iso*nicotinyl hydrazide may be used to demonstrate the presence of pyridoxal phosphate as a prosthetic group.

RÉSUMÉ

1. Les cinétiques de l'inhibition de la cystéine sulfinique décarboxylase du foie par l'*iso*nicotinyl hydrazide et par l'hydroxylamine ont été comparées.

2. Les résultats décrits suggèrent que l'inhibition de la décarboxylase est le résultat d'une interaction entre le groupe formyl du phosphate de pyridoxal et l'inhibiteur.

3. L'auteur propose d'utiliser la vitesse d'inhibition des enzymes par l'*iso*nicotinyl hydrazide pour démontrer la présence du phosphate de pyridoxal comme groupement prosthétique.

ZUSAMMENFASSUNG

1. Die Kinetik der Hemmung von Cysteinsulfinsäuredekarboxylase der Leber durch *Iso*nikotinylhydrazid und Hydroxylamin wurde verglichen.

2. Auf Grund der Ergebnisse darf angenommen werden, dass sich die Dekarboxylasehemmung aus einer Reaktion zwischen der Formylgruppe von Pyridoxalphosphat und dem Hemmungsfaktor ergibt.

3. Es wird vorgeschlagen, die Hemmungsgeschwindigkeit von Enzymen durch *Iso*nikotinylhydrazid zum Beweis der Gegenwart von Pyridoxalphosphat als prosthetische Gruppe zu verwenden.

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