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THE INHIBITION OF TERMINAL OXIDATION BY PORPHYRINOGENIC DRUGS*

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Abstract—A total of 30 drugs representing a wide variety of molecular species were investigated for their ability: (1) to inhibit reduced nicotinamide adenine dinucleotide oxidase purified from beef heart mitochondria, and (2) to induce increased glycolysis, as measured by lactate production, in cultured mouse fibroblast cells. Most of the drugs tested were chosen because they have been associated with the precipitation of symptoms in clinical cases of porphyria, or have been shown to induce porphyria in an experimental system, or both. The constant relationship between these two properties, namely, the inhibition of electron transport and the exacerbation or induction of porphyria, supports the thesis of a direct metabolic link between impaired terminal oxidation and porphyrinogenesis.

BARBITURATES have frequently been associated with the precipitation of symptoms in individuals with the genetic disorder AIP,† a disease characterized clinically by abdominal pain and neurological signs, and chemically by the urinary excretion of excessive porphyrin precursors, PBG and ALA, and usually uroporphyrin and coproporphyrin. Many barbiturates and related compounds such as the amides and carbamides also induce a chemically similar experimental porphyria in animals,[1] in chick embryos,[2] and in cells in culture,[3] such compounds being referred to as porphyrinogenic drugs. A second distinctive property of the barbiturates and related compounds is their ability to inhibit a specific segment of the terminal oxidation system.[4] Lipid solubility and the possession of the —CONH— grouping appeared to be important requirements for such inhibition.

More recently it has become evident that barbiturates are not unique in inducing porphyrinogenesis and inhibiting NADH oxidase activity. Actually a wide variety of molecular species, many of which find frequent use in clinical medicine, produce both biochemical effects. Our attention was directed to several of these drugs because of our own clinical experiences as well as those of others in observing patients with various disorders of porphyrin metabolism. Such findings prompted us to study further the structure-function relationships of these porphyrinogenic compounds as an

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† Abbreviations used: acute intermittent porphyria, AIP; porphobilinogen, PBG; δ-aminolevulinic acid, ALA; reduced nicotinamide adenine dinucleotide, NADH; allylisopropylacetamide, AIA; diethyl-1,4-dihydro-2,4,6-trimethyl pyridine, DDC.

aid to the elucidation of their mode of action. The effects of this large group of drugs on NADH oxidase activity and on oxidative metabolism of L-929 cells as expressed in lactate excretion have been studied. The results are considered in relation to a hypothesis that inhibition of NADH oxidation and accelerated porphyrin biosynthesis are associated metabolic changes. A preliminary report has appeared.[5]

EXPERIMENTAL

The methodology employed has been described previously.[4, 6] Briefly, NADH oxidase, a particulate enzyme purified from beef heart mitochondria, catalyzed the oxidation of added NADH by molecular oxygen. Its activity was measured spectrophotometrically by observing the rate of disappearance of NADH at 340 mμ.[7] Since the enzyme preparations had different specific activities, comparisons could better be made by expressing all results as a per cent of the control value for a given experiment. A typical enzyme preparation catalyzed the oxidation of 3·4 μmole NADH/mg protein/min. Beef heart was chosen as the enzyme source because it provides a standard preparation that has been used widely in terminal oxidation studies.

Tissue culture experiments were carried out by introducing drugs into replicate cultures of glass-grown Earle's strain L-929 cells in a modified Eagle's medium containing calf serum. The per cent increase of lactate production above that of control cells (2·5 mg/100 ml medium/24 hr) was taken as an index of inhibited terminal oxidation.[6, 8] Each experiment was performed with individual drug concentrations tested in triplicate flasks, the contents of which were then pooled for the lactate determinations by the method of Barker.[9] The lactate values represent the average of two to four separate experiments. Lactate excretion is reported as a gross change in the flask, which does not take into account the frequent reduction in cell count in the drug-containing flasks; such a correction would quantitatively increase the apparent effect on lactate, but would not alter the conclusions drawn from the data.

Because of its limited solubility, data on griseofulvin were obtained by making multiple cell counts and, as an exception, were expressed as the increase in lactate production per cell rather than per flask. In this way changes in lactate metabolism were observable at a lower drug concentration. However, such a procedure did not allow a comparison to be made with other drugs.

Since many of the drugs had very limited water solubility, several solvents were employed including dimethylsulfoxide, dimethylformamide, and ethyl alcohol, the solvent then being added to the controls. Solubility characteristics of some drugs such as hexachlorobenzene precluded their being studied adequately in either biological system. Significant drug effects in tissue culture cells occasionally required 5- to 10-fold higher concentrations over those used for the enzyme assays, possibly reflecting the fact that the results were not expressed on a per cell basis; for this reason, a few drugs could be examined only with the NADH oxidase system.

Meprobamate and isopropyl meprobamate were donated by Wallace Laboratories, Cranbury, N.J.; methsuximide, phensuximide, diphenylhydantoin, and ethosuximide by Parke Davis & Co., Detroit, Mich.; griseofulvin by Schering Corp., Bloomfield, N.J.; and AIA and chlordiazepoxide by Hoffman-La Roche, Inc., Nutley, N.J. The additional carbamates were synthesized and donated by Dr. Ralph J. Fessenden, Department of Chemistry, San Jose State College, San Jose, Calif. Phenobarbital

and propoxyphene HCl were obtained from E. R. Squibb & Sons, New Brunswick, N.J.; secobarbital from Eli Lilly & Co., Indianapolis, Ind.; meperidine HCl from Winthrop Laboratories, New York, N.Y.; and glutethimide from Ciba Pharmaceutical Co., Summit, N.J. Diethylstilbestrol and ethinyl estradiol were purchased from Nutritional Biochemical Corp., Cleveland, Ohio; β-estradiol and estrone from Sigma Chemical Co., St. Louis, Mo.; antipyrine from Aldrich Chemical Company, Milwaukee, Wis.; chlorobutanol and DDC from Eastman Organic Chemicals, Rochester, N.Y. Analogues of the latter compound were generously supplied by Dr. G. S. Marks, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada.

RESULTS AND DISCUSSION

The data obtained from several barbiturates and related compounds are arranged in Table 1 in decreasing order of their inhibition of NADH oxidase, which approximates that for lactate excretion by cells in tissue culture. For comparison, two previously

TABLE 1. EFFECTS OF BARBITURATES AND RELATED DRUGS IN RESPIRATORY ENZYME AND TISSUE CULTURE SYSTEMS

Compound	Concn. (10^{-4} M)	Inhibition of NADH oxidase activity (%)	Increase in lactate excretion by L-929 cells (%)
Diphenylhydantoin	1	39 (2)†	15
(Dilantin)*	2	61 (2)	
	3	70 (2)	
	5		124
Secobarbital	5	64 (4)	122
(Seconal)	10	90 (3)	
	15		159
Glutethimide	10	64 (4)	235
(Doriden)	20	94 (3)	
Methsuximide	10	25 (2)	28
(Celontin)	20	52 (2)	
	30	73 (2)	
	50		142
Phensuximide	10	15 (2)	26
(Milonin)	20	39 (2)	
	30	54 (2)	
	50		273
Phenobarbital	10	14 (4)	
	20	30 (5)	
	30	55 (5)	
	40		20
	50		135
Allylisopropyl-	50	27 (2)	37
acetamide (AIA)	100	59 (2)	118
Ethosuximide	100	20 (3)	33
(Zarontin)	200	38 (1)	
	400	62 (1)	

* Names in parentheses are abbreviations or trade names.
† Numbers in parentheses indicated separate determinations averaged.

tested compounds are included: secobarbital, a frequently used inhibitor for electron transport studies, and AIA, used most often for the experimental induction of porphyria. The drugs include also several anticonvulsants (diphenylhydantoin, methsuximide, phensuximide, phenobarbital, and ethosuximide). A patient diagnosed as having hereditary coproporphyria experienced a precipitation of AIP-like symptoms

by several of these anticonvulsants, which were administered for seizure control.[10] Diphenylhydantoin and ethotoin administration have also been associated with the onset of porphyria in patients with primary seizure disorders.[11, 12]

Relatively high concentrations (10^{-2} M) of AIA were required to achieve > 50 per cent inhibition of NADH oxidase activity, possibly because AIA is more water soluble than most of the other drugs investigated. Liver cells in primary culture become porphyrinogenic in response to much smaller concentrations of AIA (2×10^{-5} M).[3] These discrepancies in response to drug level might be attributable to a concentrating of the drugs by cells or cell organelles.

Ethosuximide is more hydrophilic than AIA, and perhaps on this basis acted only poorly in either system. Clinically ethosuximide did not appear to influence the course of a patient with hereditary coproporphyria, whereas the more effective oxidation inhibitor, methsuximide, was directly responsible for increased PBG excretion.[10]

Glutethimide, a nonbarbiturate hypnotic, caused 64 per cent inhibition of NADH oxidase at 10^{-3} M; concomitantly it increased lactate excretion by 235 per cent. This drug and several of the anticonvulsants also inhibited NADH oxidation by beef heart mitochondria as well as ox brain NAD(P)H dehydrogenase.[13] Increased porphyrin production in chick embryo liver cultures has been induced by glutethimide and several anticonvulsants.[3]

Attention was initially directed to the carbamates and dicarbamates (Table 2) because of an aggravation of clinical symptoms in a porphyric patient being treated with meprobamate. In the present studies meprobamate was at least as inhibitory of NADH oxidase as phenobarbital, a drug frequently incriminated in inducing acute

TABLE 2. EFFECTS OF CARBAMATES AND DICARBAMATES IN RESPIRATORY ENZYME AND TISSUE CULTURE SYSTEMS

	Compound	Concn. (10^{-4} M)	Inhibition of NADH oxidase activity (%)	Increase in lactate excretion by L-929 cells (%)
A.	2,2-Dimethyl-1-hexane-ol carbamate	1	25 (6)*	23
		2·5	54 (7)	122
		5	76 (5)	173
		10		661
B.	2-Methyl-2-butyl-1,3-propanediol dicarbamate	2·5	19 (2)	
		5	52 (6)	
		10	68 (6)	39
		25		158
C.	2,2-Dimethyl-1-pentane-ol carbamate	5	22 (3)	33
		10	56 (7)	47
		15	87 (2)	68
D.	N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate (isopropyl meprobamate)†	5		39
		10	55 (2)	106
		20	90 (2)	
E.	2,2-Dimethyl-1,5-pentanediol dicarbamate	5	14 (5)	
		10	40 (5)	28
		15	52 (5)	
		25		68
F.	2-Methyl-2-propyl-1,3-propanediol dicarbamate (meprobamate)	10	31 (4)	18
		20	50 (6)	
		30	53 (4)	
		50		292

* Numbers in parentheses indicate separate determinations averaged.

† Names in parentheses are generic names.

attacks of porphyria. Meprobamate was porphyrinogenic in chick embryo liver cells.[3] Isopropyl meprobamate, another commonly used clinical drug, inhibited NADH oxidase to about twice the degree of meprobamate at a given concentration.

As indicated by the results in Table 2, several additional carbamates were inhibitors of NADH oxidase and inducers of glycolysis in tissue culture cells. Lengthening an alkyl side chain of a carbamate by one carbon increased by about 3-fold the inhibitory capacity (compounds A and C). Lengthening the side chain of a dicarbamate by one carbon atom was associated with more than a doubling in inhibition (compounds B and F). A single cabamate (compound C) was about 1·5 times more inhibitory than its corresponding dicarbamate (compound E). Although this series is small, the results demonstrate the effectiveness of the alkyl substituted amide structure as an inhibitor of terminal oxidation.

Several reports have described the unusual effectiveness of DDC for the induction of porphyria in animals.[14, 15] At 2×10^{-4} M, DDC inhibited NADH oxidase and stimulated lactate formation to the same degree as 5×10^{-4} M secobarbital (Tables 1 and 3). The three analogues of DDC were slightly more effective oxidation inhibitors

TABLE 3. EFFECTS OF PYRIDINE DERIVATIVES IN RESPIRATORY ENZYME AND TISSUE CULTURE SYSTEMS

Compound	Concn. (10^{-4} M)	Inhibition of NADH oxidase activity (%)	Increase in lactate excretion by L-929 cells (%)
Diethyl-1,4-dihydro-2,6-dimethyl-4-ethyl pyridine-3,5-dicarboxylate	0·25	15 (3)*	
	0·50	44 (4)	69
	1		127
	2		165
Diethyl-1,4-dihydro-2,6-dimethyl-4-propyl pyridine-3,5-dicarboxylate	0·50	56 (7)	18
	1		51
	2		184
Diethyl-1,4-dihydro-2,6-diethyl-4-methyl pyridine-3,5-dicarboxylate	0·25	17 (5)	
	0·50	54 (5)	30
	1	71 (3)	74
	2		209
Diethyl-1,4-dihydro-2,4,6-trimethyl pyridine-3,5-dicarboxylate (DDC)	1	21 (3)	8
	2	61 (3)	152
	3	77 (3)	

* Numbers in parentheses indicate separate determinations averaged.

than the parent compound; similarly they were more porphyrinogenic in guinea pigs.[16]

Endocrine factors are known to influence the course of AIP.[1] Likewise, several steroid hormones induced an increased porphyrin production in chick embryo liver cell cultures.[3] As inhibitors of NADH oxidase activity, both estrogenic and progestational compounds were effective (Table 4). The two components of Enovid, the 3-methyl-ether of ethinyl estradiol plus a progestational compound, were the most inhibitory steroid compounds tested, inhibiting NADH oxidase > 50 per cent at concentrations of $5·0 \times 10^{-7}$ M. The synthetic estrogen, diethylstilbestrol caused 45 per cent inhibition of NADH oxidase at $5·0 \times 10^{-6}$ M, whereas progesterone caused 42 per cent inhibition at 10^{-4} M; the latter oxidation inhibitor has been studied in greater detail by others.[17, 18] Two characteristics of AIP—(1) that it is rarely

TABLE 4. EFFECTS OF STEROID HORMONES AND RELATED STRUCTURES IN RESPIRATORY ENZYME AND TISSUE CULTURE SYSTEMS

Compound	Concn. (M)	Inhibition of NADH oxidase activity (%)	Increase in lactate excretion by L-929 cells (%)
Diethylstilbestrol	5×10^{-6}	45 (2)*	
	1×10^{-5}	67 (4)	36
	2×10^{-5}	84 (2)	
	5×10^{-5}		120
β-Estradiol	5×10^{-5}	14 (3)	24
	1×10^{-4}	42 (3)	53
	2×10^{-4}		303
Estrone	1×10^{-4}	66 (3)	9
	2×10^{-4}		150
Ethinyl estradiol	5×10^{-6}	54 (3)	
	1×10^{-5}	60 (3)	
	5×10^{-5}	95 (1)	32
	1×10^{-4}		103
	2×10^{-4}		228
Progesterone	1×10^{-4}	42 (2)	
	$2{\cdot}5 \times 10^{-4}$	67 (2)	190
Enovid			
Norethynodrel	$2{\cdot}5 \times 10^{-7}$	32 (5)	
	5×10^{-7}	54 (4)	
	1×10^{-6}	86 (3)	
	5×10^{-5}		38
	1×10^{-4}		194
Mestranol	5×10^{-7}	62 (5)	
	1×10^{-6}	86 (3)	
	5×10^{-5}		101
	1×10^{-4}		288

* Numbers in parentheses indicate separate determinations averaged.

manifested clinically before puberty, and (2) that some women experience an exacerbation at specific times during the menstrual cycle[1]—may be at least partially explained in terms of the inhibitory effect of the steroid hormones on terminal oxidation.

The role of hormones in porphyria has produced many conflicting reports. Several of these hormones have in our experience, as well as that of others, been associated with increased porphyrin and porphyrin precursor production in porphyric patients, and in some cases have induced clinical symptoms in an otherwise latent condition.[19–22] Wetterberg[23] described a patient whose clinical condition probably worsened, and Redeker[20] observed a marked increase in urinary pyrrole excretion of a patient after the administration of an oral contraceptive. An increased urinary excretion of ALA in a number of healthy women taking oral contraceptives have been observed.[24] On the other hand, oral contraceptives reportedly alleviated the discomfort of porphyric patients experiencing menstrual exacerbation of pain;[25, 26] there appeared to be no clinical improvement from oral contraceptives in other patients. Perhaps in patients experiencing menstrual exacerbation of pain, administered steroids altered a delicate hormonal balance such that the overall net effect was actually a decreased level of inhibitory hormones.

In Table 5 are summarized the results obtained on a group of miscellaneous drugs. Chlordiazepoxide has, in two instances in our experience, been associated with the precipitation of clinical symptoms or induced excretion of PBG in patients, one having AIP and the other hereditary coproporphyria. This drug inhibited NADH oxidase

TABLE 5. EFFECTS OF MISCELLANEOUS DRUGS IN RESPIRATORY ENZYME AND TISSUE CULTURE SYSTEMS

Compound	Concn. (M)	Inhibition of NADH oxidase activity (%)	Increase in lactate excretion by L-929 cells (%)
Chlordiazepoxide hydrochloride (Librium)*	1×10^{-4}		37
	2×10^{-4}	24 (3)†	
	3×10^{-4}	29 (6)	
	5×10^{-4}	53 (6)	74
	1×10^{-3}		61
	5×10^{-3}		178
1,1,1-Trichloro-2-methyl-2-propanol (Chlorobutanol)	5×10^{-4}		178
	1×10^{-3}	14 (2)	234
	2×10^{-3}	44 (2)	381
	3×10^{-3}	78 (2)	
2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one (Antipyrine)	2×10^{-3}	35 (2)	
	5×10^{-3}	46 (2)	
	1×10^{-2}	58 (3)	65
Hexachlorobenzene	4×10^{-5}‡	0 (1)	0
Griseofulvin	2×10^{-4}‡	0 (2)	0

* Names in parentheses are trade names.
† Numbers in parentheses indicate separate determinations averaged.
‡ Highest concentration that could be tested.

53 per cent at 5×10^{-4} M. Chlorobutanol and antipyrine, although quite hydrophilic compounds, inhibited NADH oxidase 44 per cent at 2×10^{-3} M and 46 per cent at 5×10^{-3} M, respectively. Both compounds were porphyrinogenic in cultured liver cells.[27] The chlorobutanol inhibition of electron transport has been previously reported.[13] Unfortunately, both hexachlorobenzene and griseofulvin were too lipophilic to test in the enzyme system, although it was possible to demonstrate increased glycolysis in tissue culture by griseofulvin when the increase in lactate production was calculated on a per viable cell basis (Table 6), since the effect was always amplified by this method of expressing the results. Both compounds are porphyrinogenic in human beings and in animals.[28–31]

TABLE 6. EFFECTS OF GRISEOFULVIN ON LACTATE PRODUCTION BY L-929 CELLS

Concn. (10^{-4} M)	Increase in lactate excretion per cell (%)	Increase in lactate excretion per viable cell (%)
0·10	21*	15
0·20	30	24
0·50	29	34
1·0	129	170
2·0	126	292

* All values except the highest concentration represent the average of two separate experiments.

Three additional drugs, chlorpromazine, propoxyphene hydrochloride, and meperidine hydrochloride, were of particular interest because they are frequently used for the symptomatic treatment of AIP. All three drugs inhibited purified NADH oxidase. Chlorpromazine inhibited 56 per cent at a concentration of 3×10^{-5} M. However, added to intact tissue culture cells, this drug increased oxygen uptake suggesting that

its primary effect may be that of an uncoupler of oxidative phosphorylation rather than an inhibitor of electron transport. In long-term growth in tissue culture, other toxic effects of chlorpromazine became prominent before inhibition of electron transport so that increased lactate excretion could not be demonstrated. Mice given chlorpromazine (36 mg/kg) showed a 4-fold increase in liver ALA synthetase activity. Chlorpromazine reportedly has a variety of effects in intact animal cells including inhibition of respiratory and glycolytic enzymes, uncoupling of oxidative phosphorylation, inhibition of cholinesterase, and altered cell permeability and DNA synthesis. The drug thus is very complex in its action, producing various effects in intact cells at concentrations too low to inhibit NADH oxidase. Dinitrophenol, a classical uncoupler of oxidative phosphorylation, had no effect on liver ALA synthetase activity in mice. Unfortunately, toxicity of the compound restricted its administration to 15 mg/kg.

Propoxyphene hydrochloride inhibited NADH oxidase activity by 51 per cent at 1×10^{-3} M. This drug also exerted toxic effects on intact cells at concentrations too low to inhibit terminal oxidation. Minor increases in lactate excretion appeared at a concentration of $2{\cdot}0 \times 10^{-5}$ M, but this was the highest concentration that could be used in tissue culture without severely depressing growth. At sublethal doses (60 mg/kg) in mice no effect on liver ALA synthetase was demonstrable.

Meperidine hydrochloride inhibited purified NADH oxidase by 50 per cent at $5{\cdot}0 \times 10^{-4}$ M. In this same concentration only a minor increase in lactate excretion was detectable, and above this range the drug became too toxic to growing tissue culture cells to show any further effects on terminal oxidation. In short-term oxygen uptake measurements by cells, 30 per cent inhibition was demonstrable at levels between $1{\cdot}0 \times 10^{-3}$ M and $5{\cdot}0 \times 10^{-3}$ M. Thus, this drug also departs from the pattern observed in tissue culture for the other NADH oxidase inhibitors. In mice 45 mg/kg as a single dose administered intraperitoneally did not increase the activity of liver ALA synthetase.

The compounds tested in this investigation were not routinely evaluated for their possible effect on succinate oxidase, a branch of the terminal electron transport system. However, in a number of earlier reports[6, 17, 18] representative compounds from the different groups were found not to inhibit succinate oxidation in those concentrations sufficient to inhibit NADH oxidase.

Drug-induced porphyrinogenesis is primarily centered in the liver, a tissue which was not employed here. In other studies,* however, liver mitochondria were inhibited in the oxidation of NADH-linked substrates by AIA, secobarbitol, and Sedormid. Since subcellular fractions from beef heart and rat liver behave similarly toward these drugs, porphyrinogenesis in the heart might be expected. That this is not known, suggests that porphyrinogenic drugs either do not accumulate in heart tissue in sufficient concentration or the ALA synthetase in this tissue is not inducible as it is in liver. The excessive formation of lactate is not unique to the particular cells used: (1) porphyria patients excreted elevated amounts of lactate;† (2) the lactate/pyruvate ratio was increased 3-fold in porphyric mouse liver;‡ and (3) total tissue lactate concentration in porphyric chick embryos was increased.‡

Considering the two systems employed in the present study, a purified particulate

* Personal communication, Dr. Tsoo E. King, Oregon State University, Corvallis, Ore.
† Personal communication, Dr. James M. Orten, Wayne State University, Detroit, Mich.
‡ Unpublished result.

enzyme from beef heart and intact mouse fibroblast cells, one would hardly expect an exact parallel between the two parameters measured and molecular structure. Yet similarities emerged and significant deviations were the exception rather than the rule. In general, the inhibition of NADH oxidase correlated well with the increased lactate formation, regardless of the molecular species being tested. This conclusion is made apparent by plotting the effect of any given compound on NADH oxidase versus its effect on lactate. In order to establish a basis for comparison, the per cent inhibition of NADH oxidase activity and the per cent increase in lactate excretion by L cells were divided by the concentration of the compound in those instances where the data were available on both parameters. The quotients were then plotted (Fig. 1). A linear

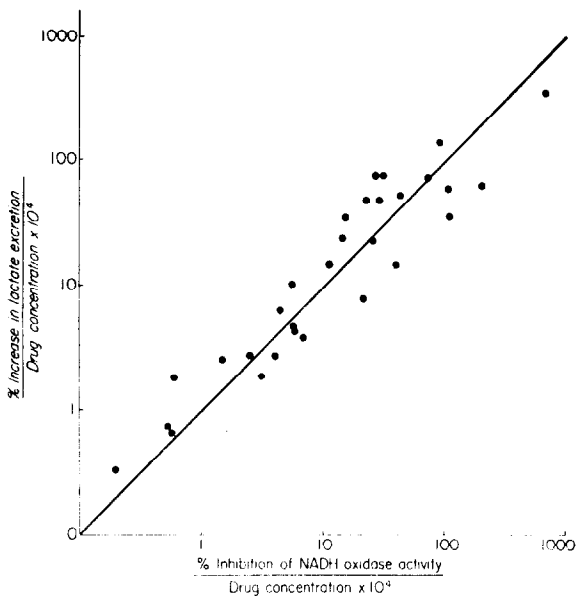

FIG. 1. Relationship of NADH oxidase inhibition to lactate production increase as affected by various compounds. In those instances where the inhibition of purified beef heart NADH oxidase and the increase in lactate production by L-929 cells were measured at the same drug concentration, the ratios of per cent inhibition/drug concentration and per cent increase in lactate/drug concentration were calculated. These ratios were then plotted versus one another.

correlation over a very wide range of values appeared. This result is consistent with the conclusion that control of glycolysis through the Pasteur effect resides at the first phosphorylation site in the oxidation of NADH[32], that is, in the region of the flavin where the porphyrinogenic drugs are presumed to act.

An attempt to correlate impaired oxidation with an overproduction of porphyrins is made difficult by the variety of biological systems in which porphyrinogenesis has been studied. Perhaps the most acceptable conclusion is that all drugs which are porphyrinogenic also inhibit NADH oxidation. An exception might be cited for malonate, a classical inhibitor of succinate dehydrogenase, which was also recently

reported to be porphyrinogenic.[33] However, even in this case an apparent decrease in NADH oxidation exists since the NADH/NAD ratio was increased in rat liver,[34] probably due to reversed electron flow.

The results described suggest two pertinent factors regarding diseases of porphyrin metabolism. First, a wide variety of drugs, it now becomes clear, must be used with caution in the treatment of porphyrias; no longer are these restricted to the barbiturates. Second, the very wide variety of molecular species which will precipitate symptoms in patients or induce porphyria in experimental systems as well as inhibit NADH oxidase supports the hypothesis that the metabolic lesion in acute intermittent porphyria is a defect in terminal oxidation. Experimental evidence establishing a direct metabolic link between terminal electron transport and the overproduction of porphyrins is becoming available. The succinyl-CoA utilized in heme biosynthesis under the stress of drug treatment may arise via a reductive dicarboxylic acid pathway originating with pyruvate carboxylation.[35] Intermediates would include malate, fumarate, and succinate, which is converted into succinyl-CoA by an inducible form of succinyl-CoA synthetase.[36]

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