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THE EFFECT OF PYRROLIZIDINE ALKALOIDS ON LIVER ENZYME SYSTEMS

C. H. GALLAGHER

McMaster Laboratory, Division of Animal Health and Production, CSIRO,
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Abstract—The effect of the pyrrolizidine alkaloids, lasiocarpine and heliotrine, on the activity *in vitro* of mitochondrial enzyme systems has been studied. The alkaloids inhibit enzyme systems which require pyridine nucleotides for electron transfer, do not affect cytochrome oxidase activity and stimulate succinoxidase activity when mitochondria are suspended in 0·25 M sucrose. Neither alkaloid influenced the activity of L-malic dehydrogenase or succinoxidase in water-disrupted mitochondria. Similar results with supinine, sarracine, monocrotaline and platyphylline indicate that the effect on mitochondrial metabolism may be common to pyrrolizidine alkaloids generally.

The effect on mitochondrial metabolism is associated with the nitrogen atom of the cyclic base of the alkaloids, as heliotridine inhibits like the parent alkaloid and the N-oxides do not influence mitochondrial oxidations.

Inhibition of mitochondrial enzyme systems is largely prevented by added DPN, and, to a lesser degree, by EDTA. The alkaloids increase mitochondrial permeability under oxidizing conditions and accelerate the loss of pyridine nucleotides thus inactivating dependent enzyme systems and stimulating succinoxidase activity.

The relevance of the mode of action *in vitro* to the acute hepatoxic effects of the pyrrolizidine alkaloids *in vivo* is discussed.

INTRODUCTION

BULL *et al.*[1] have shown that ingestion by sheep of the plant, *Heliotropium europaeum*, may produce disease of the liver. Pyrrolizidine alkaloids isolated from *H. europaeum*[2-4] were found to be the active principles;[5-7] two major alkaloids, heliotrine and lasiocarpine, possess considerable biological activity. Small doses of heliotrine or lasiocarpine when administered to rats cause chronic liver damage;[6] larger doses cause liver necrosis[5] or rapid death probably due to blockage of impulse transmission at neuromuscular junctions.[8] Heliotrine has been found to be mutagenic[9] and lasiocarpine has been reported to be carcinogenic.[10]

Biochemical disturbances associated with the acute hepatotoxic action of heliotrine have been investigated by Christie.[11] With rats receiving one large dose of heliotrine there was inactivation of enzyme systems requiring pyridine nucleotides for electron transfer and, consequently inactivation of tricarboxylic·acid-cycle activity just before hepatic necrosis became histologically demonstrable.

In the present paper, studies of the effects of pyrrolizidine alkaloids *in vitro* on enzyme preparations from the liver of the normal rat are reported.

* Abbreviations used in this paper: AMP, adenosine-5-monophosphate: DPN, diphosphopyridine nucleotide; GSH, reduced glutathione; EDTA, ethylenediaminetetra-acetic acid; HDP, fructose-1-6-diphosphate.

EXPERIMENTAL

Enzyme preparations. Rats were killed by stunning and decapitation. The liver was removed rapidly and immersed in ice-cold 0·25 M sucrose. Homogenates were prepared in 0·25 M sucrose and fractionated by differential centrifuging at 0–1°.[12] Mitochondria were resuspended in 0·25 M sucrose, except where otherwise indicated.

Reagents. Pyrrolizidine alkaloids were obtained as gifts. The alkaloids were dissolved in an equivalent amount of 1N HCl. AMP, DPN, nicotinamide, GSH, cytochrome *c*, citrate, α-oxoglutarate, L-glutamate, succinate, L-malate, octanoate, oxalo-acetate, L-ascorbic acid, EDTA, ethanol and inorganic reagents were obtained commercially.

Succinic acid was purified by recrystallizing three times from hot water; then dissolved, neutralized with 1N NaOH and precipitated as sodium succinate with ethanol. Sodium succinate was redissolved and crystallized from 80% ethanol. Octanoic acid was purified by distillation *in vacuo*. L-Ascorbic acid and α-oxoglutaric acid were dissolved and neutralized with NaOH just before use. AMP and other substrates were neutralized and stored frozen as sodium salts for 3–4 weeks.

Methods. O_2 uptake and CO_2 evolution were measured by Warburg manometers. Spectrophotometric readings were taken with a Beckman model DU instrument.

RESULTS

Experiments were done in duplicate and repeated at least once. Results are expressed as the means of relevant experiments.

(a) *Effect of lasiocarpine and of heliotrine on oxidative metabolism*

As shown in Tables 1 and 2 both lasiocarpine and heliotrine inhibited oxidations by rat liver mitochondria. The pattern of inhibition with either alkaloid was the same, but lasiocarpine proved inhibitory in lower concentrations than heliotrine. Mitochondrial oxidation of those substrates requiring pyridine nucleotides for electron transfer was inhibited, whereas succinate oxidation was appreciably stimulated. With citrate, oxidation was less inhibited than with all other substrates except succinate. In experiments where alkaloids were added from side-arm compartments of Warburg flasks, inhibition developed rapidly and reached full expression within 15 min.

TABLE 1. EFFECT OF LASIOCARPINE ON OXIDATIONS BY LIVER MITOCHONDRIA

Additions	Oxygen uptake (μl/30 mins)					
	Control	Lasiocarpine				
		33 mM	16·7 mM	6·7 mM	3·3 mM	1·67 mM
Citrate 10 mM	33	3	14	24	29	32
α-Oxoglutarate 10 mM	43	0	3	3	35	40
L-Glutamate 10 mM	30	3	3	2	16	26
Succinate 10 mM	67	91	103	99	83	—
L-Malate 10 mM	42	3	0	4	13	33

System: AMP 1 mM; $MgSO_4$ 6·7 mM; KCl 25 mM; NaK phosphate buffer, pH 7·4, 13·3 mM; cytochrome *c* 10 μM; mitochondria equivalent to 50 mg of fresh rat liver added in 0·25 ml of 0·25 M sucrose; water to 1·5 ml final volume; 0·1 ml of 20% KOH was placed in centre well to absorb CO_2; gas phase, air; temperature, 38 °C; equilibration period 10 min.

Oxidative metabolism by liver homogenates was inhibited by lasiocarpine and heliotrine in a manner similar to inhibition of mitochondrial oxidation. The oxidation of octanoate was found to be inhibited by either alkaloid when assayed with homogenate preparations. Isolated mitochondria were not used.

The activity of L-malic dehydrogenase was assessed in liver homogenates which were subjected to osmotic disruption by preparation in water instead of in 0·25 M sucrose.

TABLE 2. EFFECT OF HELIOTRINE ON OXIDATIONS BY LIVER MITOCHONDRIA

Additions	Oxygen uptake (μl/30 min)					
	Control	Heliotrine				
		67 mM	50 mM	33 mM	16·7 mM	6·7 mM
Citrate 10 mM	41	—	34	45	48	55
α-Oxoglutarate 10 mM	43	—	9	29	43	46
L-Glutamate 10 mM	36	—	5	28	37	32
Succinate 10 mM	91	112	113	111	112	—
L-Malate 10 mM	36	—	2	9	22	34
Oxaloacetate 10 mM + pyruvate 0·67 mM	55	—	4	3	33	48
Pyruvate 10 mM + L-malate 0·67 mM	35	—	10	21	26	33

System: as in Table 1.

The enzyme was assayed in the presence of added DPN. The reaction mixture contained L-glutamate to remove, by transamination, oxaloacetate, whicn inhibits the oxidation of L-malate. Neither 33 mM lasiocarpine nor 50 mM heliotrine had any effect on L-malate oxidation under these conditions, showing that these alkaoloids do not directly inhibit L-malic dehydrogenase.

Neither 33 mM lasiocarpine nor 50 mM heliotrine affected cytochrome oxidase activity of liver homogenates.

N-Oxides of both alkaloids were found to be without effect on mitochondrial or homogenate oxidations at the highest concentrations shown for the parent alkaloid in Tables 1 and 2. Neither substance inhibited nucleotide-dependent enzymes or stimulated the succinoxidase system.

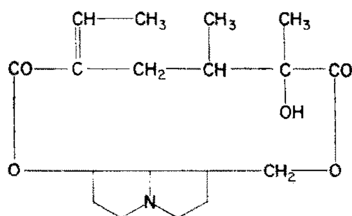
(b) *Effect of lasiocarpine and heliotrine on anaerobic glycolysis*

Anaerobic glycolysis of HDP by liver homogenates was slightly inhibited by 50 mM heliotrine and by 33 mM lasiocarpine.

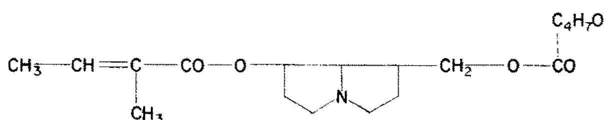
(c) *Effect of other pyrrolizidine alkaloids on mitochondrial oxidations*

Several other pyrrolizidine alkaloids from different plants were tested to determine whether inhibition of pyridine nucleotide-requiring enzyme systems, and stimulation of succinoxidase were properties common to pyrrolizidine alkaloids. The alkaloids, platyphylline (1) and sarracine (2) from *Senecio spp.*, monocrotaline (3) from *Crotalaria spp.* and supinine (4) from *Tournefortia sarmentosa* and *Heliotropium spp.* differ in structure from lasiocarpine (5) and heliotrine (6) as follows:

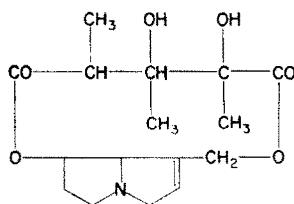
(1) platynecine + senecic acid



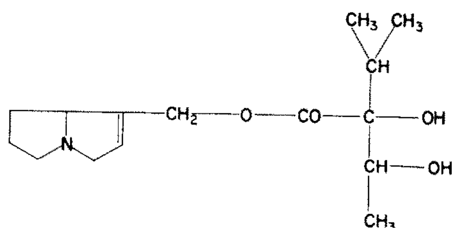
(2) angelic acid + platynecine + sarracenic acid



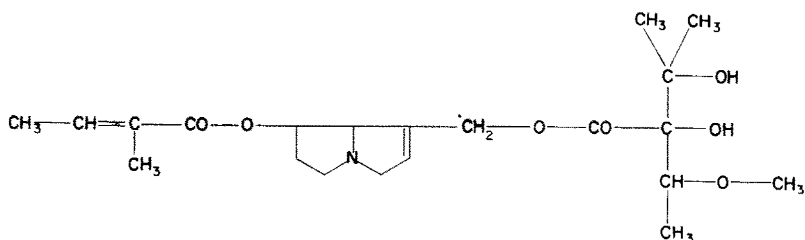
(3) retronecine + monocrotalic acid



(4) supinidine + trachelanthic acid



(5) angelic acid + heliotridine + lasiocarpic acid



(6) heliotridine + heliotric acid

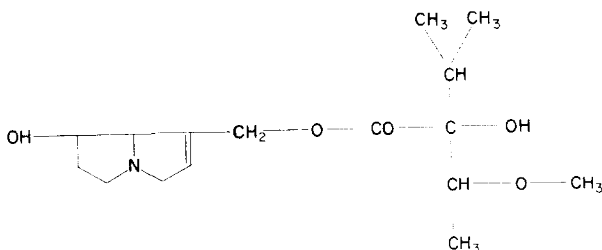


Table 3 shows that a similar effect on mitochondrial metabolism was exerted by each of these alkaloids. Pyridine nucleotide-dependent oxidation of L-malate was inhibited and succinate oxidation was increased or not affected.

(d) *Effect of heliotridine and heliotric acid on L-malate oxidation*

The structural components of heliotrine, heliotridine and heliotric acid were investigated to determine which portion of the alkaloid inhibited enzyme systems.

TABLE 3. EFFECT OF OTHER PYRROLIZIDINE ALKALOIDS ON OXIDATIONS BY MITOCHONDRIA

Additions, mM		Oxygen uptake (μl/30 min)	
		L-Malate 10 mM	Succinate 10 mM
None		31	74
Platyphylline	67	3	80
	33	12	84
	6·7	23	—
Supinine	67	1	78
	33	2	72
	6·7	25	76
Monocrotaline	67	4	74
	33	25	90
	6·7	31	—
Sarracine	67	1	—
	6·7	3	58
	3·3	10	118
	0·67	27	90

System: As in Table 1.

Heliotric acid was found to be without effect on L-malate oxidation but heliotridine was inhibitory in the same order as the complete alkaloid (Table 4).

(e) *Effect of cofactors on inhibition of oxidations*

Inhibition of pyridine nucleotide-requiring enzymes but not of the succinoxidase system suggested that loss or inactivation of mitochondrial nucleotides was the mechanism of inhibition. Addition of 0·5 mM DPN in 40 mM nicotinamide significantly reversed the inhibition of oxidation of citrate, L-glutamate, α-oxoglutarate and L-malate by lasiocarpine or heliotrine. Better protection was afforded by the addition of

TABLE 4. EFFECT OF HELIOTRIDINE AND HELIOTRIC ACID ON L-MALATE OXIDATION

Additions	Oxygen uptake (μl/30 min.)				
	Control	Heliotridine			
		(67 mM)	(50 mM)	(33 mM)	(16·7 mM)
L-Malate (10 mM)	38	4	6	19	35
		Heliotric acid			
		(67 mM)	(50 mM)	(33 mM)	(16·7 mM)
L-Malate (10 mM)	43	41	41	42	43

System: As in Table 1.

0·67 mM GSH to 0·5 mM DPN and 40 mM nicotinamide as shown in Table 5. Similarly, an increase of DPN to 1·5 mM gave greater reversal of inhibition (Table 5).

(f) *Effect of* EDTA

Some reduction of the inhibition by lasiocarpine or heliotrine was obtained by the addition of 1 mM EDTA, although preparations of the alkaloids were free from contamination with calcium or heavy metals. Similarly, the stimulatory effect of the

TABLE 5. EFFECT OF COFACTORS AND EDTA ON INHIBITION OF L-MALATE OXIDATION

Additions	Oxygen uptake (μl/30 min)				
	Control	Cof. 1	Cof. 2	EDTA (1 mM)	EDTA (1 mM) + Cof. 1
None	36	38	33	41	58
Lasiocarpine 6·7 mM	2	23	28	21	50
None	40	54	—	44	50
Heliotrine 33 mM	10	37	—	29	44

System: As in Table 1 + L-malate 10 mM.
Cof. 1 = DPN 0·5 mM, GSH 0·67 mM and nicotinamide 40 mM.
Cof. 2 = DPN 1·5 mM, GSH 0·67 mM and nicotinamide 40 mM.

alkaloids on succinate oxidation was removed by the addition of 1 mM EDTA. Table 5 shows the protective effect of 1 mM EDTA against lasiocarpine or heliotrine inhibition of L-malate oxidation. Greater protection did not result from increasing the EDTA concentration to 2 mM.

Inclusion of 1 mM EDTA, 0·5 mM DPN, 40 mM nicotinamide and 0·67 mM GSH in the reaction mixture almost completely prevented inhibition of L-malate oxidation by either alkaloid (Table 5).

(g) *Effect of pyrrolizidine alkaloids on mitochondrial membrane permeability*

The above data indicated that pyrrolizidine alkaloids inhibited mitochondrial enzyme systems by interfering with electron transport at the pyridine nucleotide level. As the inhibitory effect was reversed by adding excess DPN, the alkaloids appeared to

act either by preventing re-oxidation of reduced pyridine nucleotide or by causing the loss of coenzymes from dependent enzyme systems. In view of the protective action of EDTA against inhibition by the alkaloids and the wellknown stabilizing effect of EDTA on mitochondrial integrity,[13–16] and, in particular its effect on mitochondrial membranes,[17] it seems likely that mitochondrial structure was altered in such a way as to increase membrane permeability with a consequent loss of pyridine nucleotide and other cofactors.

An active measure of mitochondrial permeability may be obtained by following spectrophotometrically at 340 mμ the rate of reduction of DPN which has been added external to intact mitochondria oxidizing a DPN-dependent substrate[18–21] The rate of reduction of DPN is directly proportional to the permeability of mitochondrial membranes to DPN. No effect of lasiocarpine or heliotrine on the rate of DPN reduction coupled to L-malate oxidation could be demonstrated when this system was tested with mitochondria suspended in 0·25 M sucrose or water. These experiments indicated that under the conditions of spectrophotometric as well as manometric experiments the alkaloids do not inhibit L-malate dehydrogenase directly. However, it was realized that the experimental conditions were very different from those obtaining in the system used for respiratory experiments. In particular, the spectrophotometric system contained CN^- to inhibit cytochrome oxidase, semicarbazide to remove oxaloacetate, an excess of added DPN and substrate, a relatively small number of mitochondria, and experiments were carried out at 22 ° as opposed to 38 ° for manometric experiments. Mitochondria re-isolated from a respiratory experiment in which they had been subjected to action of 6·7 mM lasiocarpine were found to be more permeable than incubated control mitochondria to the entry of DPN.

An effect of lasiocarpine on mitochondrial permeability was demonstrated in the following manner. Mitochondria equivalent to 800 mg of fresh rat liver were suspended in 5 ml of 0·25 M sucrose containing, where indicated in Fig. 1, 10 mM L-malate plus 10 mM lasiocarpine, and were placed in an incubator at 37 ° for 30 min. During the incubation the temperature of the reaction mixture rose to 32 °. After adding 35 ml of ice-cold 0·25 M sucrose the mitochondria were re-isolated by centrifuging at 34,000 $\times$ g for 10 min. The mitochondria were then resuspended in 0·25 M sucrose or in water, as required, and permeability was assessed by the spectrophotometric method.[18–20] Fig. 1 shows that incubation of mitochondria in the presence of lasiocarpine increased membrane permeability to the passage of DPN. DPN was reduced much more rapidly by mitochondria which had been pre-treated with lasiocarpine in the presence of L-malate than by mitochondria pre-incubated with L-malate alone. The mitochondria treated with lasiocarpine were apparently even more permeable than those aged by pre-incubation in the absence of a substrate (Fig. 1). Initial differences in optical density shown in the curves (a) and (b) in Fig. 1 were due to reduction of DPN at different rates during the short time between the adding of the enzyme and the taking of the first reading. Lasiocarpine does not absorb light at 340 mμ. The initial optical density at 340 mμ of mitochondria pre-incubated without L-malate was always higher than that of mitochondria pre-incubated with L-malate. Suspension of mitochondria in water instead of in 0·25 M sucrose abolishes selective semipermeability of the membranes. The effect of lasiocarpine was abolished by suspension of mitochondria in water and must therefore have been associated with semipermeability of mitochondrial membranes.

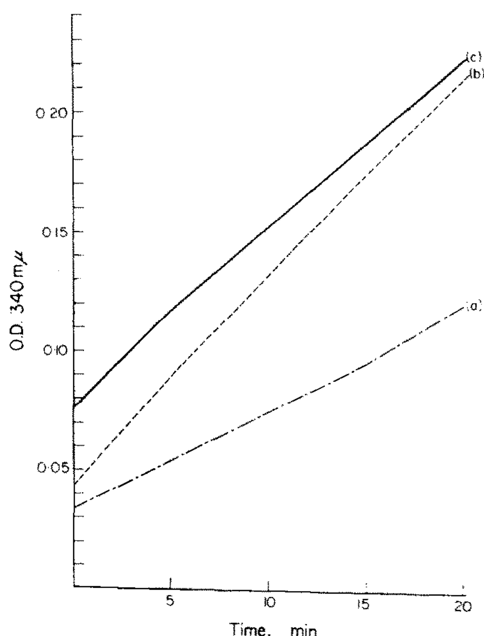


FIG. 1. Mitochondria in 0·25 M sucrose pre-incubated with (a) L-malate 10 mM; (b) L-malate 10 mM + lasiocarpine 10 mM; (c) No addition.
System: NaK phosphate buffer, pH 7·8, 33 mM; KCl 25 mM; $MgSO_4$ 6·7 mM; L-malate 50 mM; semicarbazide HCl (neutralized) 0·17 M; DPN 1·5 mM; KCN 0·2 mM; enzyme, 50 μl 1% mitochondria in 0·25 M sucrose; final volume, 3 ml; gas phase, air; temperature, 22°.

Finally, it was found that mitochondrial permeability increased rapidly under the oxidizing conditions of respiratory experiments. As already described, the alkaloids stimulated succinate oxidation by mitochondria in 0·25 M sucrose. This may be due to the increased permeability of mitochondrial membranes allowing the loss of mitochondrial DPN and thus preventing the production of oxaloacetate which in low concentration inhibits succinic dehydrogenase specifically.[22–24] Lack of any effect of lasiocarpine or heliotrine on succinate oxidation by mitochondria in water provides supporting evidence, as water-disrupted mitochondria would be deficient in DPN and would not be producing oxaloacetate. Further evidence was obtained by adding DPN to mitochondria oxidizing succinate in the presence of 6·7 mM lasiocarpine. DPN added to the suspending fluid enters mitochondria slowly unless membrane permeability is increased.[25] Succinate oxidation by mitochondria in 0·25 M sucrose was slightly inhibited by adding 0·5 mM DPN alone, but was rapidly and greatly inhibited by 0·5 mM DPN plus 6·7 mM lasiocarpine (Table 6). When mitochondria were suspended in water, the addition of DPN alone produced maximum inhibition of succinate oxidation, as shown in Table 6, and lasiocarpine did not further reduce the rate of oxidation when added with or without DPN. Similar results were obtained with heliotrine. The addition of 0·4 mM $CaCl_2$ increased the membrane permeability of mitochondria suspended in 0·25 M sucrose, and produced a pattern of events similar to that resulting from the pyrrolizidine alkaloids. Table 6 shows that Ca^{2+} stimulated succinate oxidation by mitochondria in 0·25 M sucrose and facilitated penetration and

inhibition by DPN. Suspension of mitochondria in water also removed the effect of Ca^{2+} (Table 6). Clearly pyrrolizidine alkaloids, like Ca^{2+}, increased the permeability of mitochondrial membranes to the passage of DPN.[25]

TABLE 6. EFFECT OF LASIOCARPINE, CALCIUM AND DPN ON SUCCINATE OXIDATION

Additions	Oxygen uptake (μl/30 min)			
	Sucrose medium		Water medium	
	No DPN	DPN 0·5 mM	No DPN	DPN 0·5 mM
None	68	62	57	12
Lasiocarpine 6·7 mM	101	47	61	13
$CaCl_2$ 0·4 mM	90	15	68	15
Lasiocarpine 6·7 mM + $CaCl_2$ 0·4 mM	94	19	—	—

System: As in Table 1 + succinate 10 mM. Mitochondria equivalent to 25 mg of fresh rat liver added in 02·5 ml of 0·25 M sucrose or water. Results shown are the means of three experiments.

(h) *Effect of lasiocarpine, and heliotrine, on light absorption at 260 mμ by mitochondrial extracts*

Mitochondria, re-isolated after incubation with lasiocarpine or heliotrine and L-malate in the respiratory system shown in Table 1, were extracted at room temperature for 10 min with 5% trichloracetic acid and the optical densities of the extracts were measured at 260 mμ. Pre-treatment with either alkaloid resulted in significant reduction of the acid-extractable material absorbing light at 260 mμ when compared with similar extracts of incubated control mitochondria. If the extracts were made without incubation, the amount of extractable material absorbing at 260 mμ was the same whether alkaloid had been added or not. At low concentrations of the alkaloids, and for periods of up to 50 min, the loss of absorption at 260 mμ was proportional to alkaloid concentration and incubation time. This evidence suggested an abnormal loss of oxidized pyridine nucleotides from the mitochondria during incubation with the alkaloids, and it fitted the mode of action already postulated. Details of the effects of pyrrolizidine alkaloids on mitochondrial pyridine nucleotides will be presented in a subsequent paper.

DISCUSSION

The pyrrolizidine alkaloids, lasiocarpine and heliotrine, from *Heliotropium europaeum*, were found in these studies to inhibit, *in vitro*, oxidative metabolism of liver mitochondria from normal rats. They inhibited specifically those mitochondrial enzyme systems which require pyridine nucleotides for electron transport. Either alkaloid stimulated succinoxidase activity but neither affected cytochrome oxidase activity. Supinine, which also occurs in *Heliotropium europaeum* and several pyrrolizidine alkaloids from other plants exerted a similar effect on mitochondrial metabolism. It seems likely that this is a property common to all pyrrolizidine alkaloids.

The inhibitory locus of the alkaloids appears to be the nitrogen atom of the cyclic nucleus. Oxidation at the ring nitrogen to form the N-oxide completely removes the effects of the alkaloids on mitochondrial oxidations. From a comparison of the

structure of the pyrrolizidine alkaloids having the same effect on mitochondrial metabolism it seems likely that the pyrrolizidine ring is the inhibitory component, as the esterified organic acids differ greatly. Further, heliotridine the cyclic base of heliotrine influences mitochondrial metabolism in the same way as the parent alkaloid, but heliotric acid is without effect.

The inhibition of pyridine nucleotide-requiring enzyme systems, and the protective effect of excess DPN indicated that electron transport is being blocked at the pyridine nucleotide level, either by interference with re-oxidation of pyridine nucleotides or by loss of coenzymes from mitochondrial enzyme systems. The stimulatory effects of the alkaloids on succinate oxidation by mitochondria in 0·25 M sucrose, and the stabilizing influence of EDTA against this effect and against inhibition of nucleotide-dependent enzymes, indicate that the alkaloids may alter mitochondrial structure in a manner which increases membrane permeability, and so permits the loss of cofactors from the mitochondria. It was in fact shown that mitochondrial membrane permeability to the passage of DPN is increased by lasiocarpine or heliotrine under the oxidizing conditions of respiratory experiments. In the experiments, the loss from mitochondria of acid-extractable material absorbing light at 260 mμ. an absorption which is due in part to oxidized pyridine nucleotides, is accelerated by the addition of the alkaloids. Spectrophotometric estimation of DPN reduction coupled to L-malate oxidation, and manometric assay of L-malate and of succinate oxidation, each with water-disrupted mitochondria, failed to show any direct effect of heliotrine or lasiocarpine on L-malic dehydrogenase or on the succinoxidase system. It is thus clear that pyrrolizidine alkaloids can influence mitochondrial metabolism by increasing the permeability of mitochondrial membranes to pyridine nucleotides and, possibiy, other soluble cofactors of respiration. Loss of pyridine nucleotide inactivates dependent enzyme systems, and stimulates succinate oxidation by suppressing the formation of oxaloacetate.[24] The mechanism by which pyrrolizidine alkaloids cause increased mitochondrial permeability, with loss of pyridine nucleotides, will be discussed in a subsequent paper.

The findings of this study support those of Christie[7,11] who observed that inactivation of pyridine nucleotide-dependent oxidations by liver enzymes preceded the appearance of histological evidence of liver necrosis in rats injected with a single large dose of heliotrine. Christie was able to reverse the inhibitions by adding DPN, and he suggested that heliotrine might disorganize tricarboxylic acid cycle activity by increasing mitochondrial permeability and lead to the loss of respiratory cofactors. Thus it is probable that *in vivo* administration of pyrrolizidine alkaloids influence liver mitochondria in the same way as does direct addition of the alkaloid to mitochondrial preparations *in vitro*.

If a dose of pyrrolizidine alkaloid is considered to be distributed evenly throughout the body, the concentration required to produce acute hepatic necrosis is considerably lower (at least ten times) than that required to inhibit mitochondrial oxidations *in vitro*. However, at least 12 hr must elapse after administration before inhibition of liver mitochondrial metabolism is apparent. Accumulation of alkaloid in the liver during this time could easily result in concentrations of the order necessary to inhibit oxidations *in vitro* by liver mitochondria from normal rats. It seems likely that the delayed onset of measurable mitochondrial dysfunction subsequent to a dose of pyrrolizidine alkaloid is in fact due to the time of accumulation of an inhibitory

concentration in the liver. Finally the different chemical and physical conditions *in vivo* may favour the activity of pyrrolizidine alkaloids at lower concentrations than the conditions of *in vitro* experiments.

Greater potency of lasiocarpine than of heliotrine *in vitro* is in agreement with the relative median lethal doses of these alkaloids established by Bull *et al*[5]. However, in the present study, neither heliotrine N-oxide nor lasiocarpine N-oxide was found to affect oxidative metabolism, although the toxicity of lasiocarpine N-oxide to female rats is relatively high.[5] This difference may be due to reduction of the N-oxide to lasiocarpine after *in vivo* administration. Alternatively lasiocarpine N-oxide may itself affect metabolism in the female rat in a yet undetermined manner.

The acute hepatotoxic action of pyrrolizidine alkaloids would seem to result from loss of pyridine nucleotides from the mitochondria, arising from increased permeability. This loss would inactivate enzyme systems which require pyridine nucleotides for electron transfer, and thus disrupt tricarboxylic acid cycle activity.

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