Using the thin layer estimation system described, DEN added directly to the *in vitro* incubation system was also found to inhibit hexobarbital oxidation. It is evident from Fig. 1 that relatively high concentrations were required, 50 per cent inhibition occurring in the presence of 5×10^{-3} M DEN. The possibility was considered that the reduced ability (Table 1) of livers from DEN-treated rats to oxidise hexobarbital *in vitro* might result from their content of unmetabolised DEN. However, estimation of DEN by a polarographic technique[12] showed the DEN content of livers from treated rats to be extremely low, being less than 5 μg DEN/g liver 2 days after the last of the three injections of the carcinogen. Since the concentration arising *in vitro* from this liver content is of the order of 10^{-6} M this possibility is extremely unlikely. Preliminary experiments, using short time incubations indicate that *in vitro* DEN itself and not a metabolite is the inhibitory species. This is again in contrast with the inhibitory agent SKF 525-A, whose inhibitory effects *in vitro* on drug metabolising enzymes appear to be due to a metabolite formed during incubation.[13]

DEN is obviously a compound which appreciably affects heptatic drug metabolising enzymes in the rat. Administration of this agent can apparently produce a stimulation of the microsomal drug metabolising enzyme UDP-glucuronyltransferase. Isolated liver microsomes from the same animals had a reduced capacity to oxidise hexobarbital however. The results indicate that this reduced hexobarbital oxidation is not due to a direct inhibition of the microsomal oxidase by DEN. It would appear more likely that other mechanisms such as repression of enzyme synthesis might be involved

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REFERENCES

1. A. H. Conney and J. J. Burns, in *Advances in Pharmacology* (Eds. S. Garattini and P. A. Shore), vol. 1, p. 31. Academic Press, New York (1962).
2. J. R. Fouts, Ann. *N.Y. Acad. Sci.* **104**, 875 (1963).
3. D. T. Greenwood and I. H. Stevenson, *Biochem. J.* **96**, 37 P (1965).
4. G. J. Dutton, *Proc. 1st Int. Pharmac. Meet.* **6**, 39 (1962).
5. E. F. McLuen and J. R. Fouts, *J. Pharmac. exp. Ther.* **131**, 7 (1961).
6. J. R. Cooper and B. B. Brodie, *J. Pharmac. exp. Ther.* **114**, 409 (1955).
7. I. Sunshine, *Clin. Chim. Acta* **9**, 321 (1963).
8. J. R. Fouts and L. A. Rogers, *J. Pharmac. exp. Ther.* **147**, 112 (1965).
9. T. Gram, L. A. Rogers and J. R. Fouts, *J. Pharmac. exp. Ther.* **155**, 479 (1967).
10. P. Emmelot and E. L. Benedetti, *Biophys. biochem. Cytol.* **7**, 393 (1960).
11. E. A. Smuckler, E. Arrhenius and T. Hultin, *Biochem. J.* **103**, 55 (1967).
12. D. F. Heath and J. A. Jarvis, *Analyst* **80**, 613 (1959).
13. J. R. Gillette and H. A. Sasame, *Fedn Proc.* **23**, 537 (1964).

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The effects of 1-(1-phenylcyclohexyl)piperidine·HCl (phencyclidine, Sernyl) on respiration and related reactions of liver mitochondria *in vitro**

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Previous work showed that 1-(1-phenylcyclohexyl)piperidine-HCl (phencyclidine, Sernyl) stimulated mitochondrial respiration and inhibited phosphorylation coupled to the oxidation of succinate,

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α-ketoglutarate, β-hydroxybutyrate, or ferrocytochrome *c*.[1] With succinate as the substrate, these effects of phencyclidine and those of chlorpromazine, imipramine and laurylamine, which are similar, were greater at an alkaline pH, whereas uncoupling by dinitrophenol, laurate and lauryl sulfate was greater at an acid pH.[2, 3] To further define the effects of phencyclidine, studies on respiration, ATPase activity, and the P_i–ATP exchange reaction are reported here.

All procedures were carried out with rat liver mitochondria as already described.[4] Phencyclidine was donated by Parke, Davis & Co.

The effects of phencyclidine on respiration with succinate were measured polarographically (Table 1); previous work[1] had been done with a manometric technique. As the concentration of

TABLE 1. EFFECT OF PHENCYCLIDINE ON RESPIRATION WITH AND WITHOUT INORGANIC PHOSPHATE OR WITH OLIGOMYCIN ADDED*

Expt. No.	Concn phencyclidine (mM)	O_2 uptake (μatoms/min)			ADP:O	Initial O_2 uptake	
		Initial	With ADP	After ADP		Without Pi	With 6 μg oligomycin
1	None	0·038	0·178	0·038	1·73	0·028	
	0·15	0·071	0·186	0·061	1·39	0·050	
	0·30	0·077	0·188	0·081	1·16	0·060	
	0·50	0·099	0·198	0·091	1·02	0·072	
	0·80	0·113	0·175	0·094	0·89	0·086	
2	None	0·045					0·039
	0·50	0·091					0·097
	0·80	0·094					0·096

* Medium contained in 1·4 ml: 87 μmoles KCl, 10 μmole potassium phosphate, 10 μmole $MgCl_2$, 33 μmole Tris–HCl buffer, 33 μmole potassium succinate, and mitochondria (2·1 mg protein) in 0·2 ml of 0·25 M sucrose; ADP added was 0·2 μmole. The pH was 7·5, temperature 22°.

phencyclidine was increased respiration was stimulated, particularly the initial rate and after ADP was used, and the acceptor control and ADP:O ratios were decreased. Without inorganic phosphate added or in the presence of oligomycin, respiration was also stimulated by the amine. Respiration with ADP added tended to decrease when the amine was increased to 0·8 mM. However, high levels do cause clumping of the mitochondria, which interferes with the electrode. When the manometric technique (with shaking) was employed, the oxygen uptake was depressed below control levels only by 6–9 mM phencyclidine with succinate or α-ketoglutarate as substrate (ATP added) and mitochondria at 3–4 mg protein in 2·5 ml (data not shown). Though marked clumping occurred at these concentrations, it has also been observed when the amines have stimulated respiration, so it is not likely the cause of the inhibition.

ATPase activity was somewhat stimulated by phencyclidine (Fig. 1). When ATPase activity was induced by 0·02 mM 2,4-dinitrophenol (DNP), phencyclidine was inhibitory at concentrations over 0·8 mM, but lower levels (0·1 to 0·4 mM) caused some further stimulation (about 8 per cent). ATPase produced by 0·1 mM dinitrophenol was inhibited with phencyclidine as low as 0·2 mM.

Phencyclidine inhibited the P_i–ATP exchange reaction (Fig. 1).

These results suggest tht the site influenced by the amine is independent of phosphate and closer to the respiratory chain than that sensitive to oligomycin (Lardy[5]); it could be the same as that affected by DNP though probably not in the same way, considering the differences in concentration required and opposite effects of pH with the two compounds. The inhibition of DNP-stimulated ATPase activity does not seem to be due simply to an interference with the access of DNP to sites on the mitochondria, since lower concentrations of the amine interfere only at higher concentrations of dinitrophenol. The effects of the phenol plus the amine may be additive to cause the inhibition, since dinitrophenol has an optimal concentration for the stimulation of ATPase, beyond which the rate drops off.[6]

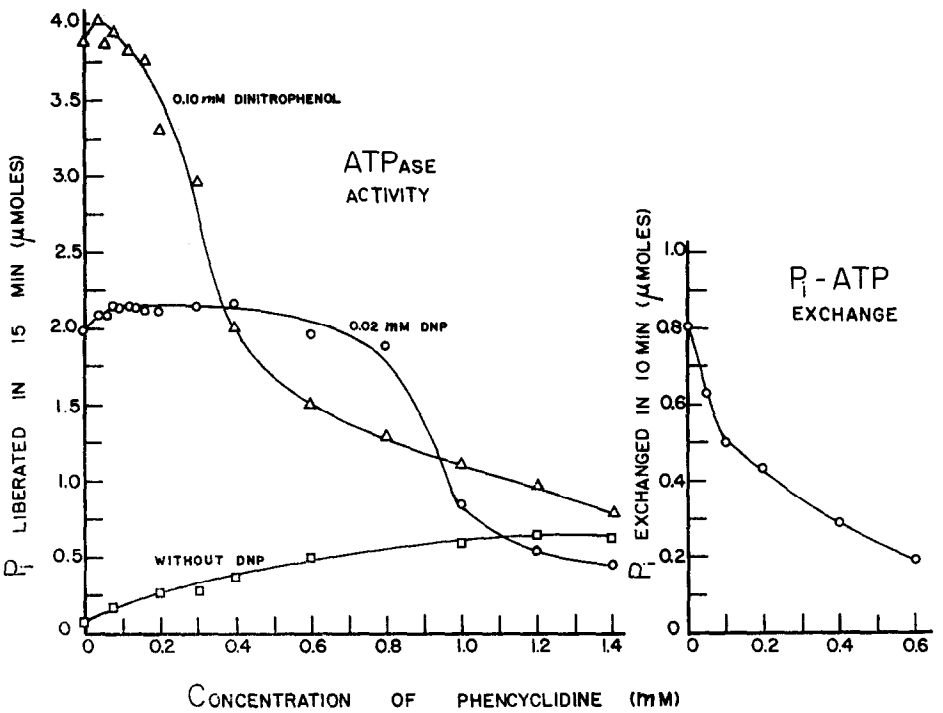

FIG. 1. The effects of phencyclidine on ATPase activity and on the P_i–ATP exchange reaction. For ATPase activity the medium contained in 1·5 ml: 112 μmole KCl, 75 μmole Tris-HCl buffer, 0·8 μmole EDTA, 10 μmole ATP, and mitochondria (1·2 mg protein) in 0·5 ml of 0·25 M sucrose. The pH was 7·5, temperature 25°. After 15 min of incubation, 0·5 ml of 20% trichloroacetic acid was added. An aliquot of the supernatant was analyzed for inorganic phosphate; initial values were subtracted. For the P_i–ATP exchange reaction, the medium contained in 1 ml: 5 μmole potassium orthophosphate labeled with ^{32}P (500,000 cpm), 5 μmole ATP, 25 μmole Tris-HCl buffer, and mitochondria (1·0 mg protein) in 0·2 ml of 0·25 M sucrose; pH was 7·5. After 10 min of incubation at 22°, 0·2 ml of 30% trichloroacetic acid was added.

The effects of phencyclidine on respiration, ATPase activity and the P_i–ATP exchange reaction appear to be similar to those of laurylamine[4] and chlorpromazine,[3, 4, 7–9] except that somewhat higher levels of phencyclidine are required and, perhaps even more important, only at much higher concentrations does it significantly inhibit respiration. Interestingly, ATPase activity induced by low levels of dinitrophenol can be stimulated to a greater extent by low levels of chlorpromazine[7] and laurylamine[4] than by phencyclidine. It is suggested that with respect to the stimulation of respiration and uncoupling of oxidative phosphorylation (at least at lower concentrations), the three amines and imipramine, which appears to act much like chlorpromazine,[10–12] probably affect mitochondria in the same way. That these effects are similarly influenced by pH[2, 3] lends further support to this. All four amines uncouple oxidative phosphorylation with ferrocytochrome *c* or ascorbate-tetramethyl-*p*-phenylenediamine as substrate[1, 4, 13–15] (unpublished data on imipramine). However, with chlorpromazine Berger[13] and Dawkins, Judah and Rees[14] could find no evidence of uncoupling between α-ketoglutarate or β-hydroxybutyrate and cytochrome *c*. Coupling site III may be the most senstive, a point that is under investigation.

The inhibition of respiration caused by higher levels of these amines could be a separate effect and not simply the result of more compound further affecting the site(s) uncoupled by lower concentrations. Yagi, Ozawa and Nagatsu[16] showed that chlorpromazine can complex with flavins, and Löw[7] and Dawkins *et al.*[8] have pointed out that it may inhibit respiration by interfering with the flavoproteins.

Cytochrome oxidase activity is inhibited by chlorpromazine[14, 17] and imipramine[10, 13] (also by laurylamine and phencyclidine, unpublished work), but this could occur in a different manner, e.g. by limiting access of cytochrome *c*, which is cationic around pH 7. In general, the optimal concentration for a stimulatory effect by the four amines on succinoxidase activity has been 0·1 to 0·2 mM for laurylamine and chlorpromazine (depending on the concentration of mitochondria, usually 3–4 mg protein/2·5 ml), and 0·4 mM for imipramine,[3] and about 1 mM for phencyclidine; marked inhibition has been obtained at 0·2 to 0·3 mM with the first two compounds, at 0·8 mM imipramine (unpublished results), and at 7 mM phencyclidine. Thus, in proportion to the amounts that stimulate respiration, a much greater amount of phencyclidine is required for an inhibition of respiration. This suggests, that the sites that influence the stimulatory and inhibitory effects have differing affinities for the amines which would imply that the sites are different.

The various observations discussed here and the previous finding that differences in pH, which influenced respiration, had little or no effect on the amount of amine bound[3] seem to indicate that the amines interact with specific groups on the mitochondria. However, this still does not rule out suggestions that chlorpromazine may act primarily by affecting the mitochondrial membranes (Spirtes and Guth[18]) or structural organization (Løvtrup[12]). Weinbach and Garbus[19] have even suggested that dinitrophenol may bind to an enzyme or structural protein to cause a configurational or structural change rather than at the active site.

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REFERENCES

1. H. LEES, *Biochem. Pharmac.* **11**, 1115 (1962).
2. H. LEES, *Biochim. biophys. Acta* **105**, 187 (1965).
3. H. LEES and K. LONCHARICH, *Biochim. biophys. Acta* **113**, 181 (1966).
4. H. LEES, *Biochim. biophys. Acta* **131**, 310 (1967).
5. H. LARDY, *First Int. Symp. IUB/IUBS, Biological Structure and Function*, (Stockholm, 1960) vol. 2, p. 265. Academic Press, New York (1961).
6. H. C. HEMKER, *Biochim. biophys. Acta* **73**, 311 (1963).
7. H. LÖW, *Biochim. biophys. Acta* **32**, 11 (1959).
8. M. J. R. DAWKINS, J. D. JUDAH and K. R. REES, *Biochem. J.* **76**, 200 (1960).
9. M. J. R. DAWKINS, J. D. JUDAH and K. R. REES, *Biochem. J.* **73**, 16 (1959).
10. P. N. ABADOM, K. AHMED and P. G. SCHOLEFIELD, *Can. J. Biochem. Physiol.* **39**, 551 (1961).
11. S. LØVTRUP, *J. Neurochem.* **10**, 471 (1963).
12. S. LØVTRUP, *J. Neurochem.* **11**, 377 (1964).
13. M. BERGER, *J. Neurochem.* **2**, 30 (1957).
14. M. J. R. DAWKINS, J. D. JUDAH and K. R. REES, *Biochem. J.* **72**, 204 (1959).
15. A. ANDREWJEW and A. J. ROSENBERG, *C.r. Séanc. Soc. Biol.* **150**, 639 (1956).
16. K. YAGI, T. OZAWA and T. NAGATSU, *Biochim. biophys. Acta* **43**, 310 (1960).
17. J. BERNSOHN, I. NAMAJUSKA and L. S. G. COCHRANE, *Archs Biochem. Biophys.* **62**, 274 (1956).
18. M. A. SPIRTES and P. S. GUTH, *Nature, Lond.* **190**, 274 (1961).
19. E. C. WEINBACH and J. GARBUS, *J. biol. Chem.* **240**, 1811 (1965).

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Observations on the release of lysosomal enzymes from the isolated bovine adrenal gland

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STIMULATION of the isolated bovine adrenal gland by acetylcholine or carbachol causes the release of catecholamines,[1–3] of adenine nucleotides and their metabolites,[4, 5] of chromogranin A,[3, 6, 7] the main component of the soluble protein of chromaffin granules,[8] as well as the other soluble proteins