The results presented in this paper have been obtained in the course of several different investigations in which a number of people have been involved. We wish to thank them all, and particularly Professor R. A. McCance for his help with the presentation of the results. The method for the estimation of inulin in tissues was developed by Dr W. I. M. Holman, and the figures for the fowl were given us by Mr J. W. T. Dickerson.

REFERENCES

Bacon, J. S. D. & Bell, D. J. (1948). *Biochem. J.* **42**, 397.
Barnes, B. A., Gordon, E. B. & Cope, O. (1957). *J. clin. Invest.* **36**, 1239.
Bernstein, R. E. (1952). *Biochim. biophys. Acta*, **9**, 576.
Cotlove, E. (1952). *Fed. Proc.* **11**, 28.
Crismon, J. M. (1948). *Fed. Proc.* **7**, 24.
Darrow, D. C., Harrison, H. E. & Taffel, M. (1939). *J. biol. Chem.* **130**, 487.
Gamble, J. L. jun. & Robertson, J. S. (1952). *Amer. J. Physiol.* **171**, 659.
Gaudino, M., Schwartz, J. L. & Levitt, M. F. (1948). *Proc. Soc. exp. Biol., N.Y.*, **68**, 507.
Hines, H. M. & Knowlton, G. C. (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 133.
Kerpel-Fronius, E. (1937). *Z. Kinderheilk.* **58**, 726.
King, E. J. (1932). *Biochem. J.* **26**, 292.
Lowry, O. H., Hastings, A. B., Hull, T. Z. & Brown, A. N. (1942). *J. biol. Chem.* **143**, 271.
McCance, R. A. & Shipp, H. L. (1933). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 187.
McCance, R. A., Widdowson, E. M. & Shackleton, L. R. B. (1936). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 213.
Manery, J. F. & Hastings, A. B. (1939). *J. biol. Chem.* **127**, 657.
Mokotoff, R., Ross, G. & Leiter, L. (1952). *J. clin. Invest.* **31**, 291.
Mudge, G. H. & Vislocky, K. (1949). *J. clin. Invest.* **28**, 482.
Nichols, G. jun., Nichols, N., Weil, W. B. & Wallace, W. M. (1953). *J. clin. Invest.* **32**, 1299.
Sendroy, J. (1937). *J. biol. Chem.* **120**, 405.
Talso, P. J., Spafford, N. & Blaw, M. (1953). *J. Lab. clin. Med.* **41**, 405.
White, H. L. & Rolf, D. (1955). *Amer. J. Physiol.* **180**, 287.
White, H. L. & Rolf, D. (1956). *Amer. J. Physiol.* **185**, 152.
Wilde, W. S. (1945). *Amer. J. Physiol.* **143**, 666.
Wilson, A. O. (1955). *Brit. J. Surg.* **43**, 71.

The Effect of Chlorpromazine on the Respiratory Chain

CYTOCHROME OXIDASE

By M. J. R. DAWKINS,* J. D. JUDAH and K. R. REES
Department of Morbid Anatomy, University College Hospital Medical School and Biochemistry Department, University College London

(*Received* 17 *September* 1958)

The phenothiazine 'tranquillizer' chlorpromazine [10(3-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride] has a powerful depressant action on the central nervous system. In view of the inhibitory effects of the barbiturates on electron transport (Ernster, 1956), we have investigated the effects of chlorpromazine on the respiratory chain. The work of Berger, Strecker & Waelsch (1956) has shown that chlorpromazine inhibited the phosphorylation coupled to the oxidation of cytochrome *c*. This paper is concerned with a detailed analysis of this inhibition.

METHODS

Wistar albino rats fed on an unrestricted M.R.C. diet no. 41 (Bruce, 1950) were used throughout the investigation. Mitochondria were prepared from liver by the method of Schneider (1948) and from brain as described by Christie, Judah & Rees (1953). Mitochondria were depleted of pyridine nucleotide by pre-incubation in 0·25 M-sucrose–0·025 M-sodium orthophosphate–HCl buffer, pH 7·2, for 10 min. at 30° before use, as described by Hunter & Ford (1955). The phosphorylation coupled to the oxidation of ferrocytochrome *c* was studied with mitochondria suspended in 0·25 M-sucrose as described by Judah (1951) or suspended in 0·075 M-sucrose as described by Lehninger, ul Hassan & Sudduth (1954). Phosphorylation coupled to the reduction of cytochrome *c* by L-glutamate and β-hydroxybutyrate was studied by the method of Borgstrom, Sudduth & Lehninger (1955). The reduction of cytochrome *c* was followed spectrophotometrically with the Unicam SP. 500 spectrophotometer by measuring the increase in absorption at 550 mμ. Phosphorylation was measured by the method described by Nielsen & Lehninger (1955).

Oxidative phosphorylation with oxygen as electron acceptor was studied as described by Judah (1951). The final concentrations of the components were: adenosine triphosphate (ATP), 1·7 mM; $MgSO_4$, 6·7 mM; KCl, 25 mM; sodium orthophosphate–HCl buffer, pH 7·2, 10 mM; cytochrome *c*, 10 μM; glucose, 30 mM; purified yeast hexokinase added in 0·05 ml.; substrate 10 mM; NaF, 13 mM. Mitochondria were added in 0·25 M-sucrose as indicated in the text. Final volume was 3 ml. Incubation temperatures are indicated for each experiment. The gas phase was air.

Ethylenediaminetetra-acetate (EDTA) was added as indicated in all experiments with cytochrome oxidase in

* Stothert Research Fellow of the Royal Society.

order to decrease the metal-catalysed auto-oxidation of ascorbate. All oxygen uptakes quoted are corrected for this. EDTA was also included in experiments in which small quantities of enzyme were used, e.g. spectrophotometric experiments.

MATERIALS

All nucleotides and cytochrome *c* were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Radioactive phosphate was obtained from The Radiochemical Centre, Amersham, Bucks, and purified as described by Kennedy (1953). Chlorpromazine was a gift from May and Baker Ltd., Dagenham, Essex. Yeast hexokinase was prepared by the method of Berger, Slein, Colowick & Cori (1945). It was purified to the stage of fraction 3 of their procedure. The conditions for autolysis of baker's yeast described by Berger *et al.* (1945) were found to be unsatisfactory and those described by Bailey & Webb (1948) were used instead.

RESULTS

Action on phosphorylating system. When chlorpromazine at concentrations of 0·2 mM is added to suspensions of respiring liver mitochondria, with β-hydroxybutyrate and succinate as substrates, reduction of the efficiency of the coupled phosphorylation invariably occurs (Table 1). In about 30% of the experiments with β-hydroxybutyrate as substrate, the oxygen uptake was inhibited 50% or more. In these experiments, the P/O ratios were the same as in those in which no respiratory inhibition was produced by chlorpromazine. The reduction of phosphorylation is far greater with succinate as substrate. This result suggests that chlorpromazine may be acting at a single phosphorylating site. The only one common to both these substrates is that involved in the oxidation of ferrocytochrome *c*. Inhibition of this step would have a disproportionately greater effect on the P/O ratio for succinate (P/O 2) than on the P/O ratio for diphosphopyridine nucleotide (DPN)-linked substrates (P/O 3).

We found that 0·1 mM-chlorpromazine inhibited phosphorylation coupled to the oxidation of ferrocytochrome *c* by upwards of 70%. Table 2 shows that this result could be obtained when the mitochondria were suspended in 0·25 M-sucrose or 0·075 M-sucrose and at concentrations of cytochrome *c* between 0·01 and 0·06 mM. The small uptakes of inorganic phosphate occasionally observed in the presence of 0·2 mM-chlorpromazine are not considered significant. It is clear that the phosphorylation is essentially nil.

We also tested the effect of chlorpromazine on mitochondria reducing cytochrome *c* in the presence of β-hydroxybutyrate and L-glutamate. The method of Borgstrom *et al.* (1955), which we used, requires a pretreatment of the mitochondria with hypotonic sucrose solution in order to make the added cytochrome *c* available to the electron-transport chain

Table 1. *Effect of chlorpromazine on phosphorylation coupled to oxidation of β-hydroxybutyrate and succinate*

Contents of the flasks were as described in Methods. Mitochondria equivalent to 1 mg. of N were added to each flask in 0·5 ml. of 0·25 M-sucrose. Temperature of the bath was 38° and the final volume of the flask contents was 3 ml.

Substrate	Concn. of chlorpromazine (mM)	O_2 uptake (μatoms)	P uptake (μmoles)	P/O ratio	Inhibition of P/O ratio (%)
Succinate	Nil	12·5	21	1·7	Nil
Succinate	0·2	15·0	12	0·8	53
β-Hydroxybutyrate	Nil	5·3	15·5	2·9	Nil
β-Hydroxybutyrate	0·2	5·3	12	2·3	21

Table 2. *Action of chlorpromazine on phosphorylation coupled to oxidation of ferrocytochrome* c

Flasks contained: cytochrome *c*, as indicated; ascorbate, 10 mM; neutralized to pH 7·0; EDTA, final concn. 0·3 mM; other components were as described in Methods except that no substrate other than ascorbate and cytochrome *c* was added. Final volume, 3 ml. Mitochondria equivalent to 2 mg. of N were added. Temperature of the bath was 20°. The results are corrected for blank values in the absence of cytochrome *c*. They are representative of 10 such experiments.

Mitochondria in	Concn. of cytochrome *c* (μM)	Concn. of chlorpromazine (mM)	O_2 uptake (μatoms)	P uptake (μmoles)	P/O	Inhibition of P/O ratio (%)
0·25 M-Sucrose	60	Nil	11·2	4	0·36	Nil
	60	0·1	10·8	1	0·09	75
	20	Nil	7·8	3·5	0·45	Nil
	20	0·1	8·2	Nil	—	100
0·075 M-Sucrose	10	Nil	10·9	6·5	0·6	Nil
	10	0·1	9·0	1·5	0·17	72

Table 3. *Effect of chlorpromazine on reduction of cytochrome* c *and coupled phosphorylation*

Components were as described in Methods. EDTA (0·3 mM) was added; mitochondria in 0·075 M-sucrose, equivalent to 0·04 mg. of N, were added to the cuvettes to start the reaction. Final volume, 3 ml. Temperature, 20°. Figures in the table are corrected for the appropriate blanks without added substrate. The expression P/2E is defined as the ratio of μm-moles of inorganic orthophosphate esterified per pair of μm-moles of cytochrome *c* reduced, since this is a one-electron transfer.

Substrate	Concn. of chlorpromazine (mM)	Time of incubation (min.)	Cytochrome *c* reduced (μm-moles)	Inorganic P esterified (μm-moles)	P/2E	Inhibition of reduction (%)
L-Glutamate	Nil	11	27·0	21·5	1·6	—
	0·1	16	20·0	15·8	1·58	50
β-Hydroxybutyrate	Nil	10	12·0	7·8	1·3	—
	0·1	15	8·2	6·5	1·58	54

Table 4. *Effect of chlorpromazine on phosphate-treated mitochondria oxidizing succinate in the presence and the absence of cytochrome* c

Components were as described in Methods. Mitochondria equivalent to 0·5 mg. of N were added in 0·5 ml. of 0·25 M-sucrose–0·02 M-sodium orthophosphate–HCl buffer (pH 7·15). Final volume, 3 ml. Temperature of the bath, 38°. These results are typical of four such experiments.

Cytochrome *c* (20 μM)	Concn. of chlorpromazine (mM)	O_2 uptake (μatoms)	P uptake (μmoles)	P/O	Inhibition of P/O ratio (%)
−	Nil	6·0	9·0	1·5	Nil
+	Nil	15·6	24	1·5	Nil
−	0·1	6·3	7	1·1	27
+	0·1	16·3	14·0	0·8	43

of the phosphorylating system. Chlorpromazine was added at a concentration of 0·1 mM, since preliminary experiment showed that under these conditions gross inhibitions of electron transport were encountered at higher concentrations. Table 3 shows that 0·1 mM-chlorpromazine gave some 50 % inhibition of the rate of cytochrome *c* reduction. The inhibited systems were allowed to run longer to achieve reasonably comparable figures, since under the conditions of these experiments the reduction of cytochrome *c* and the esterification of inorganic phosphate were linear with time. There was no reduction in the efficiency of the phosphorylating process, which is expressed in terms of moles of inorganic orthophosphate esterified per pair of electrons passing to cytochrome *c*. It may be concluded therefore that the inhibitor is relatively specific for the phosphorylation coupled to the terminal step of electron transport.

Further information on the action of chlorpromazine was obtained from a study of phosphate-treated mitochondria. Hunter & Ford (1955) described this technique for depleting mitochondria of DPN. Such mitochondria show pronounced requirements for DPN and are able to carry out oxidative phosphorylation only upon addition of substrate and DPN. We investigated these phosphate-treated particles and found that they also showed considerable stimulation of oxygen uptake with succinate as substrate, on the addition of cytochrome *c*. The extra oxygen uptake is coupled to phosphorylation, the P/O ratios being similar

Table 5. *Effect of adenosine triphosphate, magnesium ions, potassium ions and cytochrome* c *on ferrocytochrome* c *oxidation*

Flasks contained: sodium phosphate buffer (10 mM), pH 7·2; ascorbate, 10 mM, pH 7·0; EDTA, 0·3 mM; mitochondria equivalent to 1 mg. of N were added in 0·25 M-sucrose. Temperature of the bath, 38°. Final volume of the flask contents was 3 ml.

Additions	O_2 uptake (μl./10 min.)
Cytochrome *c* (20 μM)	68
Cytochrome *c* (40 μM)	136
Cytochrome *c* (40 μM) + ATP (1·7 mM)	78
Cytochrome *c* (40 μM) + ATP (1·7 mM) + KCl (25 mM) + Mg^{2+} (6·7 mM)	17
Cytochrome *c* (40 μM) + KCl (25 mM) + Mg^{2+} (6·7 mM)	60

both in the presence and the absence of added cytochrome *c*. Table 4 shows that chlorpromazine inhibits both systems, but the inhibition in the presence of added cytochrome *c* is 43 % and only 27 % in its absence, suggesting that the inhibitor acts less effectively upon the bound as opposed to the added protein.

Action of chlorpromazine on ferrocytochrome c *oxidation.* In the systems used for studying the coupled phosphorylation, the oxidation of ferrocytochrome *c* does not proceed at maximal rates. This is due to several factors, amongst which are the low levels of cytochrome *c* added, and probably the accessibility of the protein to the oxidase system. Table 5 shows the importance of these

points. Thus the level of cytochrome *c* usually employed in the phosphorylating system (0·02 mM) permits very much less than the maximal rate, and addition of ATP, Mg^{2+} and K^+ ions reduces the rate still further. If the ATP is hydrolysed in N-HCl for 10 min. and then neutralized its inhibitory action is removed; this suggests that its action is due to maintenance of the normal mitochondrial permeability, which thus prevents access of added cytochrome *c*.

We therefore tested the effect of 0·2 mM-chlorpromazine in mitochondrial systems where such barriers do not exist. This was achieved by the use of water suspensions of mitochondria where no coupled phosphorylation took place. Table 6 shows that an 89% inhibition of the cytochrome oxidase system occurred under these conditions. The inhibition was dependent upon the concentration of added cytochrome *c*, falling to 18% when the cytochrome *c* was increased to 0·2 mM.

This result may be due to binding of the inhibitor by the protein, and in order to exclude this possibility the three following tests were performed.

Nature of the inhibition of cytochrome oxidase. Analysis by the method of Lineweaver & Burk (1934) was carried out with three concentrations of chlorpromazine and four concentrations of cytochrome *c*. Fig. 1 shows the results of one such experiment, which is typical of several. It will be observed that curves are obtained which meet at a common intercept when extrapolated to infinite substrate concentration. This indicates competitive inhibition, and makes it unlikely that simple binding is the cause of the reversal of chlorpromazine inhibition by added cytochrome *c*. Nevertheless, further experiments were performed to confirm this point. Use was made of two other properties of chlorpromazine, one being that the substance at a concentration of 0·1 mM will inhibit powerfully the oxidation of β-hydroxybutyrate, provided that the mitochondria have been subjected to hypotonic treatment in order to permit access of the inhibitor. The other function tested was the inhibition of oxidative phosphorylation with succinate as substrate. In this latter test, phosphate-treated mitochondria were again used, this time to ensure a one-step oxidation of succinate, since the successive oxidations cannot occur in mitochondria depleted of DPN. Neither of these actions of chlorpromazine was in the least affected by the presence of cytochrome *c* (up to 0·2 mM), and it may therefore be concluded that simple binding of the inhibitor by the protein does not occur.

Action of chlorpromazine on succinate oxidation. Elsewhere we have noted that both succinate and β-hydroxybutyrate oxidations may be powerfully inhibited by chlorpromazine (Dawkins, Judah & Rees, 1959, unpublished work). However, we have observed that water suspensions of mitochondria oxidizing succinate may show considerable acceleration of respiration in the presence of 0·1 mM-chlorpromazine. Table 7 shows this effect on succinate

Table 6. *Inhibition of cytochrome oxidase by chlorpromazine*

Flasks contained: sodium phosphate buffer (16 mM), pH 7·2; ascorbate, 10 mM; EDTA, 0·3 mM; mitochondria in water equivalent to 0·2 mg. of N were added to each flask. Temperature of the bath, 38°. Final volume of the flask contents was 3 ml.

Concn. of cytochrome *c* (μM)	O_2 uptake (μl./5 min.) Control	Chlorpromazine (0·2 mM)	Inhibition by chlorpromazine (%)
20	45	5	89
67	72	23	68
130	79	52	34
200	79	65	18

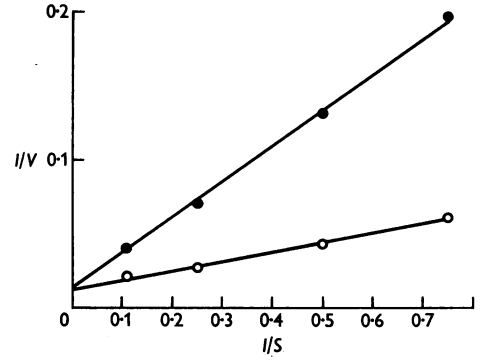

Fig. 1. Demonstration of competitive inhibition between chlorpromazine and cytochrome *c*. The cytochrome *c* concentrations (*S*) were 200, 89, 40 and 30 μM. The conditions of the reaction were as described in Table 6. Concentrations of chlorpromazine were 0·15 mM (○) and 0·2 mM (●). *V* is the oxygen uptake in the first 10 min. Temperature was 38°.

Table 7. *Acceleration of succinate oxidation by chlorpromazine*

Flasks contained: sodium phosphate buffer (17 mM), pH 7·2; cytochrome *c*, as indicated. Mitochondria in water equivalent to 0·2 mg. of N were added. Final volume of the flask contents was 3 ml. Temperature of the bath was 38° and the time of incubation 20 min.

	O_2 uptake (μl.)		
Concn. of cytochrome *c* (μM) ...	20	100	200
Additions			
Succinate (33 mM)	82	82	88
Succinate (33 mM) + chlorpromazine 0·1 (mM)	117	121	122

Table 8. *Action of chlorpromazine on brain mitochondria*

The succinate system was as described in Methods. Mitochondria were suspended in 0·25 M-sucrose and the incubation temperature was 38°. The ferrocytochrome *c* system was as described in Table 2. Mitochondria were suspended in 0·075 M-sucrose and the incubation temperature was 20°. Final volume of flask contents was 3 ml.

Substrate	Concn. of chlorpromazine (mM)	O_2 uptake (μatoms)	P uptake (μmoles)	P/O	Inhibition of P/O (%)
Succinate	Nil	8·6	10·0	1·2	Nil
Succinate	0·2	6·1	3·5	0·57	53
Ferrocytochrome *c*	Nil	5·0	4	0·80	Nil
Ferrocytochrome *c*	0·1	6·2	3·0	0·48	40
Ferrocytochrome *c*	0·2	3·3	0·0	—	100

oxidation at three concentrations of cytochrome *c*. It seems that both control and accelerated rates are not influenced by variation in cytochrome concentration. Quite probably this result reflects a structural effect on the particles, since it is difficult to see how such an acceleration could be brought about by other means in this system.

Action of chlorpromazine on brain systems. Berger *et al.* (1956) were unable to detect any uncoupling effect of chlorpromazine on brain preparations. Table 8 shows typical effects of chlorpromazine on brain mitochondria. Thus there is 50 % inhibition of the P/O ratio in the presence of 0·2 mM-chlorpromazine when succinate is the substrate. In addition, there is a very distinct inhibition of cytochrome oxidase, though this is not so marked as with liver preparations. Finally, there is a clear-cut inhibition of the phosphorylation coupled to ferrocytochrome *c* oxidation.

It thus appears that there is no special difference between brain and liver preparations in their sensitivity to chlorpromazine, but it is true that brain-mitochondrial preparations are far from homogeneous, and it may be that the results of Berger *et al.* (1956) were due to the presence of extraneous matter which might well bind the inhibitor.

DISCUSSION

The fact that Berger *et al.* (1956) observed no inhibition of respiration by chlorpromazine may be ascribed to several factors which are pertinent to this discussion. In the first place, the systems they used for determining the phosphorylation coupled to the oxidation of ferrocytochrome *c* do not absorb oxygen at maximum rate. We have found in investigating this point that rates of the order of 20 % of maximal are in fact observed. This in turn is due to several factors. The concentration of added cytochrome *c* used by Berger *et al.* was kept below the optimum; and it also happens that the presence of ATP, K^+ ions and Mg^{2+} ions exert a powerful inhibitory effect, presumably by influencing mitochondrial permeability to cytochrome *c*. Our results show that powerful inhibitions of the oxidase system may be obtained under the correct conditions. The nature of the inhibition remains in doubt. However, the apparent competitive nature of the inhibition does suggest that interaction between ferrocytochrome *c* and the next component, probably cytochrome *a*, is being disturbed, in which case one would expect reduction of cytochrome *c* and oxidation of cytochromes *a* and a_3 to occur during chlorpromazine inhibition of the system.

The fact that inhibition of the phosphorylation is not relieved by added cytochrome *c* is of interest, since it suggests that the inhibitor is acting at two sites both related to cytochrome *c*. One possibility that should be borne in mind is that some non-phosphorylating pathway for the oxidation of ferrocytochrome *c* is being brought into play by chlorpromazine.

Our experiments with phosphate-treated mitochondria oxidizing succinate in the presence and the absence of added cytochrome *c* showed that the inhibition of phosphorylation by chlorpromazine in the former case was some 50 %, whereas in the latter it was only some 20 %. This system made it possible for us to perform an experiment in which added cytochrome *c* induced a large excess of oxygen uptake, which was coupled to a phosphorylation, and in which the behaviour of the internally bound cytochrome *c* could also be studied, and it would appear that chlorpromazine is a more effective uncoupler of phosphorylation accompanying the oxidation of added ferrocytochrome *c* than of the phosphorylation associated with the oxidation of the endogenous protein.

The apparent specificity of chlorpromazine in inhibiting the phosphorylation coupled to ferrocytochrome *c* oxidation needs some discussion. We have shown that a concentration of chlorpromazine which brings about 50 % inhibition of electron transport between substrate and cytochrome *c* (Table 3) reduces the phosphorylation coupled with this process by the same amount, leaving the P/O ratio unaltered. Such a result does not necessarily mean that one phosphorylation is basically different from the others, it may merely

be more sensitive. Lehninger *et al.* (1954) noted that the phosphorylation coupled to the oxidation of ferrocytochrome *c* was rather more sensitive to common uncoupling agents than other steps.

We think that the specificity of chlorpromazine is a true one, since studies of other phenothiazines reveal a similar action to that of chlorpromazine on the phosphorylation coupled to the oxidation of ferrocytochrome *c*. In addition, at least one compound also uncouples the phosphorylation coupled to reduction of cytochrome *c* by succinate, but has no effect on the efficiency of the phosphorylation coupled to the reduction of cytochrome *c* by β-hydroxybutyrate (Dawkins, Judah & Rees, 1959, unpublished work).

A question which clearly must be raised is whether these actions of chlorpromazine can account for its biological effect? The inhibition of oxidative phosphorylation is, at its maximum, rather small—it cannot be greater than some 33% overall—and it is hard to see how this could bring about any pronounced effect on the organism. Conceivably, the inhibition of electron transport might be of importance. In this connexion, our results with brain mitochondria, though they are similar to those with liver, do not indicate any special sensitivity to the drug. If anything, there is rather less effect on brain mitochondria.

The concentration of cytochrome *c* in whole cells is probably lower than in our artificial systems. An increase in the concentration of cytochrome *c* reduces the inhibition of cytochrome oxidase by chlorpromazine. Thus the high (by pharmacological standards) concentrations of chlorpromazine which we found necessary to inhibit cytochrome oxidase may only reflect the high concentrations of cytochrome *c* in our test system. Possibly there is some sensitive site *in vivo* which specifically responds to low concentrations of chlorpromazine. Investigations are now proceeding into other agents of the phenothiazine group and into related compounds to see whether they affect tissues in the same way as chlorpromazine.

SUMMARY

1. Chlorpromazine at a concentration of 0·1 mM has been shown to uncouple the phosphorylation coupled to the oxidation of reduced cytochrome *c*. It does not affect the phosphorylations coupled to the previous steps in the electron-transport chain.

2. Chlorpromazine inhibits cytochrome oxidase; this inhibition is dependent on both chlorpromazine and cytochrome *c* concentrations.

3. Analysis of the nature of the inhibition suggests that it is competitive.

4. These effects were found in mitochondria isolated both from brain and liver.

We should like to thank Sir Roy Cameron, F.R.S., for his constant interest and Dr J. F. Smith for helpful discussions. This work was supported by grants to one of us (J.D.J.) by the Medical Research Council and by the Graham Research Fund of the University of London.

REFERENCES

Bailey, K. & Webb, E. C. (1948). *Biochem. J.* **42**, 60.
Berger, L., Slein, M. W., Colowick, S. P. & Cori, C. F. (1945). *J. gen. Physiol.* **29**, 379.
Berger, M., Strecker, H. J. & Waelsch, H. (1956). *Nature, Lond.*, **177**, 1234.
Borgstrom, B., Sudduth, H. C. & Lehninger, A. L. (1955). *J. biol. Chem.* **215**, 571.
Bruce, H. M. (1950). *J. Hyg., Camb.*, **48**, 171.
Christie, G. S., Judah, J. D. & Rees, K. R. (1953). *Proc. Roy. Soc.* B, **141**, 523.
Ernster, L. (1956). *Exp. cell. Res.* **10**, 721.
Hunter, F. E. & Ford, L. (1955). *J. biol. Chem.* **216**, 357.
Judah, J. D. (1951). *Biochem. J.* **49**, 271.
Kennedy, E. P. (1953). *J. biol. Chem.* **201**, 399.
Lehninger, A. L., ul Hassan, M. & Sudduth, H. C. (1954). *J. biol. Chem.* **210**, 910.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
Nielsen, S. O. & Lehninger, A. L. (1955). *J. biol. Chem.* **215**, 555.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.