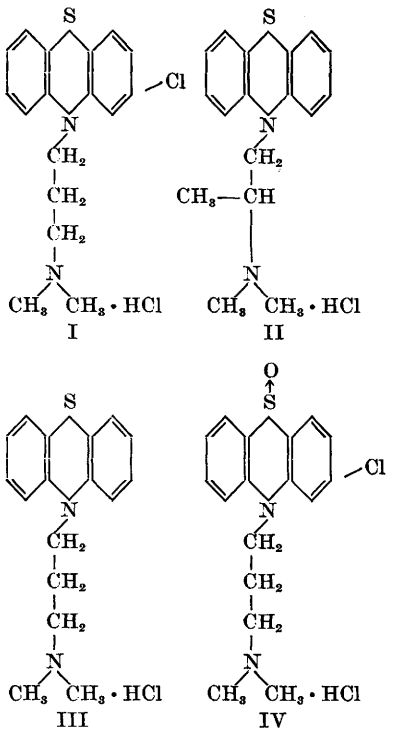
Inhibition of Brain Cytochrome Oxidase and ATP-ase by Chlorpromazine Analogues.* (22427)

JOSEPH BERNSOHN, IZOLINA NAMAJUSKA, AND L. S. G. COCHRANE.
(Introduced by Robert Schrek.)
Veterans Administration Hospital, Hines, Ill., and Northwestern University Medical School, Chicago.

It has been previously reported that chlorpromazine inhibits brain cytochrome oxidase and ATP-ase systems *in vitro*(1,2). Apart from any mechanism of drug action which might be postulated by this inhibitory effect, it is of interest that 2 enzyme systems apparently unrelated, one involved in electron transport, the other a phosphatase, should both be similarly affected by the compound. Either the groupings involved on each of these enzymes bear a relationship to one another and allow them to react with the same unit on the chlorpromazine molecule, or 2 different groupings on the drug react with 2 differing centers on the enzymes.



* This investigation was supported in part by grant of Mental Health Fund of Illinois Department of Welfare.

To determine whether information on this problem could be elicited, 3 analogues of chlorpromazine (I), namely, promethazine (II), promazine (III), and chlorpromazine sulfoxide (IV) were studied in relation to their effect on the enzyme systems involved. Since the pharmacological activity of the drugs has been reported, some correlation between these activities and their inhibitory properties has also been explored.

Methods. Adult, albino, male rats, 200-300 g in weight, were exsanguinated and the brains removed and homogenized in 0.25 M sucrose without delay. A "mitochondrial" preparation was obtained by Procedure I of the method of Brody and Bain(3), and assayed for cytochrome oxidase and ATP-ase activity. The manometric assay of Schneider and Potter(4), was used for determining cytochrome oxidase using the standard Warburg procedure. ATP-ase levels were determined according to DuBois and Potter(5), using a Dubnoff metabolic incubator. ATP-ase activity was measured by analyzing for the phosphorus liberated during the experimental period by the method of Gomori(6). The Mg^{++}-activated enzyme was studied throughout. For cytochrome oxidase assays the inhibitor was placed in the main compartment and the enzyme tipped in from the sidearm, since previous studies had indicated that pre-incubation of enzyme and inhibitor did not alter the results. In the ATP-ase studies the enzyme was the final substance added to the reaction mixture. Cytochrome c was obtained from Sigma Chemical Company, and K_2ATP from the Pabst Laboratories. Chlorpromazine, promethazine and chlorpromazine sulfoxide were furnished by Dr. Leonard Cook of Smith, Kline and French Laboratories and promazine was donated by Dr. Daniel L. Shaw, Jr., of Wyeth Laboratories. The compounds were used as the hydrochlor-

TABLE I. Effect of Chlorpromazine Analogues on Cytochrome Oxidase Activity.

	Chlorpromazine		Promethazine		Promazine		Chlorpromazine sulfoxide	
Conc., M	μmoles/hr*	% inhibition	μmoles/hr	% inhibition	μmoles/hr	% inhibition	μmoles/hr	% inhibition
0	44.1		45.0		43.5		44.5	
1×10^{-4}	34.1	22.7	50.8	+12.9	48.8	+12.2		
5×10^{-4}	1.2	97.3	25.9	42.4	22.6	48.0	44.0	1.2
1×10^{-3}	0	100.0	7.8	82.7	4.1	90.5	41.8	7.2

* O_2 uptake/100 mg equivalent fresh tissue.
Reaction mixture: Cytochrome C, 8×10^{-5} M; Na ascorbate, 1.1×10^{-2} M; $AlCl_3$, 4×10^{-4} M; phosphate buffer, .04 M, pH 7.4; mitochondria equivalent to 20 mg fresh tissue. Inc. = 30 min.; F.V. = 3.0 ml.

ide. Experimental conditions for the assay are noted in the tables and the results for the analogues are averages of three experiments, while the chlorpromazine data are the averages of a number of determinations.

Results. The inhibitory properties of chlorpromazine toward cytochrome oxidase and ATP-ase are apparent from the data (Table I). In the cytochrome oxidase system, both promazine and promethazine evidence an inhibitory effect, though the two drugs are not as potent as chlorpromazine in this respect. The concentration of drug required to produce a 50% inhibition of this enzyme system is 2.5×10^{-4} M for chlorpromazine and 6.2 and 6.5×10^{-4} M for promazine and promethazine respectively. Chlorpromazine sulfoxide, on the other hand has no marked inhibitory effect on the system, the oxidation of the sulfur atom abolishing the cytochrome oxidase inhibition. The small activating effect of promazine and promethazine has also been observed for chlorpromazine at lower concentrations, but the significance of this finding is undetermined.

The inhibition of the ATP-ase system parallels the results obtained in the cytochrome oxidase assays (Table II). Chlorpromazine, promazine and promethazine all exert an effect and the concentration required to produce a 50% inhibition of this system is 4.1×10^{-4} M, 8.8×10^{-4} M and 10.7×10^{-4} M for the 3 drugs respectively. Again, as in the cytochrome oxidase system, the sulfoxide had no inhibitory effect. A comparison of the data shows that the cytochrome oxidase system is more sensitive to the action of the drugs studied, since the 50% inhibition values are lower in all cases for this enzyme system.

Discussion. Absence of any inhibitory effect of chlorpromazine sulfoxide toward ATP-ase and cytochrome oxidase systems, in contradistinction to other compounds studied, affords an opportunity to evaluate this mechanism as a factor in the pharmacological activity of the drugs. While some positive correlation is only suggestive, a negative relationship would eliminate this inhibitory mechanism as an explanation for the mode of action of the drugs. The use of chlorpromazine, promethazine and promazine as hypotensive agents(7), as potentiators of barbiturates(8), in sedation(9), and as ataractic drugs(10), has been well documented.

TABLE II. Effect of Chlorpromazine Analogues on ATP-ase Activity.

	Chlorpromazine		Promethazine		Promazine		Chlorpromazine sulfoxide	
Conc., M	μmoles/hr*	% inhibition	μmoles/hr	% inhibition	μmoles/hr	% inhibition	μmoles/hr	% inhibition
0	40.8		37.6		33.2		40.8	
1×10^{-4}	25.6	37.3	32.8	12.8	33.2	0		
5×10^{-4}	19.2	53.0	26.0	30.9	29.2	12.0	40.0	2.0
1×10^{-3}	10.8	73.5	16.4	56.4	17.2	48.2	39.6	2.9

* P liberated/100 mg equivalent fresh tissue.
Reaction mixture: K_2ATP, 3×10^{-3} M; $MgSO_4$, 3×10^{-3} M; veronal buffer, .023 M, pH 7.4; mitochondria equivalent to 40 mg fresh tissue. Inc. = 15 min.; F.V. = 2.6 ml.

Chlorpromazine sulfoxide is reported to have minimal sedation and potentiation effects in mice, rats and rabbits and only one-eighth the action of chlorpromazine in dogs. In addition its hypotensive properties in dogs are of a low order of magnitude(11). Effects of sulfoxide on mental state have not been reported. Thus it is evident that the sulfoxide derivative does not possess clinical properties of chlorpromazine, promazine and promethazine in areas which have been tested, and this parallels the findings on inhibition of cytochrome oxidase and ATP-ase *in vitro*. Since sulfoxide is a major metabolite of chlorpromazine(11), it seems that chlorpromazine is the active principle and does not act through its conversion to sulfoxide.

The prerequisite of a divalent sulfur in the ring might imply that other phenothiazine derivatives with an unsubstituted sulfur atom may also have the inhibitory properties reported. Collier and Allenby(12), have reported that neither phenothiazine, phenothiazine sulfoxide, phenothiazone nor thional were inhibitory to rat-liver cytochrome oxidase. Thus it would appear that substitution on the N atom of the ring might also be necessary to produce inhibition of this enzyme system.

Summary. 1. Inhibitory properties of 3 chlorpromazine analogues show that oxidation at the sulfur atom, abolishes inhibition of ATP-ase and cytochrome oxidase systems by chlorpromazine. 2. Removal of chlorine atom from the ring *viz*: promazine, and alteration of the N-substituted sidechain *viz*: promethazine, result only in a small diminution of the inhibitory properties. 3. Inhibitory findings are correlated with known pharmacological effects of the drugs.

1. Abood, L. G., PROC. SOC. EXP. BIOL. AND MED, 1955, v88, 688.

2. Bernsohn, J., Namajuska, I., and Cochrane, L. S. G., *Arch. Biochem. Biophys.*, in press.

3. Brody, T. M., and Bain, J. A., *J. Biol. Chem.*, 1952, v195, 685.

4. Schneider, W. C., and Potter, V. R., *ibid.*, 1945, v149, 217.

5. DuBois, K. P., and Potter, V. R., *ibid.*, 1943, v150, 185.

6. Gomori, C., *J. Lab. Clin. Med.*, 1942, v27, 955.

7. Rea, E. L., Shea, J., and Fazekas, J. F., *J.A.M.A.*, 1954, v156, 1249.

8. Kopera, J., and Armitage, A. K., *Brit. J. Pharm.*, 1954, v9, 392.

9. Baxter, R. W., Bolster, J. A., and McKechnie, S., *Anesthesia*, 1954, v9, 79.

10. Wyeth Laboratories, personal communication.

11. Salzman, N. P., Moran, N. C., and Brodie, B. B., *Nature*, 1955, v178, 1122.

12. Collier, H. B., and Allenby, G. M., *Can. J. Med. Sci.*, 1952, v30, 443.

Received April 12, 1956. P.S.E.B.M., 1956, v92.

Erythropoiesis. II. Assay of Erythropoietin in Hypophysectomized Rats.* (22428)

W. FRIED, L. PLZAK, L. O. JACOBSON, AND E. GOLDWASSER.

Argonne Cancer Resesarch Hospital and the Departments of Medicine and Biochemistry, University of Chicago, Ill..

Experimental evidence supporting the theory that a humoral factor in the blood plasma mediates red cell production has been reviewed by several investigators(1,2). This factor, which has been referred to as erythropoietin by Bonsdorff and Jalavisto(3) and others(4), has not been isolated nor is it known whether it is a single substance or several. The method utilized to determine the presence of erythropoietin in the plasma of anemic animals has been confined largely to evidence of increased erythrocyte production in normal animals that have received injections of plasma from animals made anemic by repeated phlebotomy or injections of phenylhydrazine. The hematologic technics for determining increased

* Number I in this series of papers appears in *J. Lab. Clin. Med.*, 1955, v46, 671.