physeal system produce, when chronically injected in normal rats, a significant enlargement of the adrenal glands and a conspicuous increase of the adrenal sudanophilic material. 2) The hormones do not produce modification in hypophysectomized animals. 3) An inactivated preparation of antidiuretic hormone is completely ineffective. 4) These results are consistent with the hypothesis that antidiuretic and oxytocic hormones of the hypothalamo-posthypophyseal system may act as neurohumoral agents linking the hypothalamus to the anterior pituitary through the portal blood flow.

1. Scharrer, E., and Scharrer, B., *Recent Progr. Horm. Res.*, 1954, v10, 183.

2. Fraja, A., and Martini, L., *Boll. Soc. It. Biol. Sper.*, 1952, v28, 407.

3. Bertelli, A., and Martini, L., *Atti Soc. Lomb. Sc. Med. Biol.*, 1952, v7, 430.

4. Martini, L., and Morpurgo, C., *Nature, Lond*, 1955, v175, 1127.

5. Martini, L., and De Poli, A., *J. Endocrin.*, 1956, in press.

6. Martini, L., *Ann. Endocr., Paris*, 1955, v16, 670.

7. Van Dyke, H. B., Chow, B. F., Greep, R. O., and Rothen, A., *J. Pharmacol.*, 1942, v74, 190.

8. Moehlig, R. C., and Osius, E. A., *Ann. Intern. Med.*, 1931, v4, 578.

9. Hantschmann, L., *Klin. Wschr.*, 1937, v16/1, 378.

10. Heinbecker, P., *Ann. Surg.*, 1946, v124, 252.

11. Saffran, M. A., Schally, A. V., and Benfey, B. G., *Endocrinology*, 1955, v57, 439.

12. Harris, G. W., *Brit. Med. J.*, 1951, v2, 627.

13. Green, J. D., *Anat. Rec.*, 1947, v99, 21.

14. Palay, S. L., *Am. J. Anat.*, 1950, v47, 338.

15. Rothballer, A. B., *Anat. Rec.*, 1953, v115, 21.

16. Barrnett, R. J., *Endocrinology*, 1954, v55, 484.

17. Mirsky, I. A., Stein, M., and Paulisch, G., *ibid.*, 1954, v55, 28.

18. McCann, S. M., and Brobeck, J. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 318.

19. Sobel, H., Levy, R. S., Marmorston, J., Schapiro, S., and Rosenfeld, S., *ibid.*, 1955, v89, 10.

Received February 28, 1956. P.S.E.B.M., 1956, v91.

Actions of Reserpine and Chlorpromazine Hydrochloride on Rat Brain Oxidative Phosphorylation and Adenosinetriphosphatase.* (22304)

BERNARD CENTURY AND M. K. HORWITT.
Biochemical Research Laboratory, Elgin State Hospital, Elgin, Ill.

The use of chlorpromazine and reserpine in treating psychiatric disorders has been discussed by several investigators(1-8). Chlorpromazine is widely used as a tranquilizing agent for mentally disturbed patients and is regarded as a general central nervous system depressant capable of potentiating certain other depressant drugs(9-11). The sedative and hypotensive actions of reserpine have been attributed to a central inhibition of the sympathetic nervous system(12-14). Although chlorpromazine and reserpine are similar in their clinical actions, a number of opposing pharmacological characteristics have been reported. In contrast to chlorpromazine, reserpine facilitates production of convulsions by caffeine or metrazol and antagonizes actions of barbiturates and anti-convulsive drugs(15-17). Chlorpromazine-HCl, at concentrations of 10 mg% (2.8×10^{-4} M) or greater, has been reported to depress oxygen uptake by brain slices(18).

In the present investigation, effects of chlorpromazine and reserpine upon two important high energy phosphate enzymatic reactions were studied. These compounds were tested separately and in combinations in order to detect possible synergistic or antagonistic actions. For comparison chlorpromazine was tested in combinations with sodium pentobarbital, the latter drug also being known to uncouple oxidative phosphorylation(19).

Materials and methods. Adult Sprague-Dawley albino rats were used. Fifteen percent brain homogenates were prepared in isotonic sucrose with a Potter-Elvehjem type

* Supported in part by grant from the Mental Health Fund of the State of Illinois.

TABLE I. Effect of Reserpine on Oxidative Phosphorylation in Rat Brain Homogenates.

Reserpine, μg/ml	Δ P		Δ O		P : O	
	μM	% ± S.E.	μatoms	% ± S.E.	Ratio	% ± S.E.
0	27.2	100	10.11	100	2.70	100
10	27.0	99.2 ± 1.6	10.07	99.6 ± 1.1	2.68	99.5 ± .8
25	25.5	93.4 ± 1.2†	9.70	95.9 ± 1.6*	2.63	97.6 ± 1.7
50	23.9	87.8 ± 1.8†	9.05	89.6 ± 1.8†	2.64	98.2 ± 3.1

Δ P = μmoles inorganic phosphate esterified. Δ O = μatoms oxygen uptake.
* p <.05 and † <.01 that differences from controls are due to chance.

homogenizer. The components added to each Warburg flask for the study of oxidative phosphorylation were as follows: in the main compartment, 100 μM of KCl, 25 μM of glycylglycine, 40 μM of K_2HPO_4, 3 μM of KOH, 0.03 μM of cytochrome c, 26 μM of Na-pyruvate, 4 μM of malic acid, 2.5 μM of K-ATP (neutralized with KOH), 16 μM of $MgCl_2$, 20 μM of NaF, and 75 mg of rat brain homogenate in a total of 1.8 ml of triple distilled water; in the sidearm, 5 mg of hexokinase (Pabst) and 50 μM of dextrose in a total of 0.2 ml; and in the center well, 0.2 ml of 10% KOH. Additions of drugs† are indicated in appropriate tables. Because of its insolubility in water, reserpine was dissolved in 10% polyethylene glycol containing 0.04 malic acid and 0.26 M Na-pyruvate, and all concentrations of the drug were added to the flasks in a total of 0.1 ml containing the substrates, so that in experiments with reserpine all flasks including controls contained 0.01 ml of polyethylene glycol as well as the components mentioned above. The vessel contents when mixed was pH 7.4. All steps directly involving the homogenate were carried out in an ice water bath until the vessels were attached to the manometers and then placed in the incubating bath. The sidearms were tipped after 6 minutes, and zero time readings were taken exactly 3 minutes after tipping each flask. Zero time flasks were removed and 0.2 ml aliquots were quickly pipetted into 2.8 ml of 0.32 M trichloracetic acid. Oxygen uptake of the experimental flasks was measured for 25 minutes at 25°C, after which 0.2 ml aliquots were also added to trichloracetic acid. Inorganic phosphate uptake was determined for each set of experimental conditions(20) and P:O ratios were calculated. The method of DuBois and Potter(21) was employed for ATPase determinations. In experiments with reserpine, the drug was dissolved in 10% polyethylene glycol containing 0.02 M malic acid, and all test amounts were added in 0.05 ml. All flasks including controls,

† Chlorpromazine hydrochloride and reserpine were kindly supplied by Smith, Kline and French Laboratories, Philadelphia, Pa., and Ciba Pharmaceutical Co., Summit, N. J., respectively.

TABLE II. Effects of Combinations of Reserpine and Chlorpromazine on Oxidative Phosphorylation in Rat Brain Homogenates.

Chlorpromazine, M × 10^4	Reserpine, μg/ml	Δ P			Δ O			P : O		
		μM	% inhibition by RSP ± S.E.	p*	μatoms	% inhibition by RSP ± S.E.	p*	Ratio	% inhibition by RSP ± S.E.	p*
0	0	26.3			9.50			2.77		
	50	24.2	7.9 ± 1.3	—	8.42	11.2 ± 2.0	—	2.88	−4.0 ± 1.4	—
2.5	0	23.5			8.56			2.75		
	50	18.7	20.4 ± 1.4	<.001	7.22	15.6 ± 2.7	>.1	2.60	5.2 ± 2.4	<.01
5.0	0	16.3			6.78			2.41		
	50	9.7	41.0 ± 2.0	"	4.61	31.5 ± 3.9	<.001	2.11	12.5 ± 3.5	<.01
7.5	0	6.8			4.14			1.64		
	50	2.9	57 ± 4	"	2.59	37.1 ± 3.4	"	1.10	32.3 ± 5.3	<.001

RSP = Reserpine.
* p values indicate significance of inhibition by reserpine in the presence of various concentrations of chlorpromazine as compared with reserpine inhibition in the absence of chlorpromazine.

TABLE III. Effects of Combinations of Sodium Pentobarbital and Chlorpromazine on Oxidative Phosphorylation in Rat Brain Homogenates.

Chlorpromazine, M × 10^4	Pentobarbital, M × 10^4	△ P μM	△ P % inhibition by PTB ± S.E.	△ P p*	△ O μatoms	△ O % inhibition by PTB ± S.E.	△ O p*	P:O Ratio	P:O % inhibition by PTB ± S.E.	P:O p*
0	0	28.4			10.59			2.69		
	2.5	15.1	46.9 ± .9	—	5.92	44.0 ± .9	—	2.55	5.0 ± 1.5	—
2.5	0	23.6			9.32			2.53		
	2.5	12.2	48.3 ± 1.4	>.1	5.00	46.4 ± 1.0	>.1	2.44	3.5 ± 3.1	>.1
5.0	0	14.9			7.22			2.06		
	2.5	7.4	50.5 ± 1.6	>.05	3.76	47.9 ± 2.1	"	1.98	3.6 ± 5.3	"
7.5	0	6.5			4.56			1.43		
	2.5	2.9	55.3 ± 3.3	"	2.67	41.0 ± 2.9	"	1.10	23.1 ± 6.7	<.05

PTB = Pentobarbital.

* p values indicate significance of inhibition by pentobarbital in the presence of various concentrations of chlorpromazine as compared with pentobarbital inhibition in the absence of chlorpromazine.

therefore, contained 0.005 ml of polyethylene glycol and 1 μM of malic acid as well as the other necessary components. Sufficient KOH was added to the stock solution of ATP to bring the reaction mixture to pH 7.45.

Results. All of the studies represent determinations from 6 or more animals each. At flask concentrations of 25 and 50 μg/ml of reserpine, small but significant decreases in phosphate and oxygen uptake were observed (Table I), but no significant changes were found in P:O ratios(22).

The depression of oxidative phosphorylation of brain homogenate systems by chlorpromazine is shown in Tables II and III. Effective concentrations are similar to those reported by Finkelstein, Spencer, and Ridgeway(18), but are 10 times as high as those reported by Abood who used mitochondrial preparations(23). Reserpine at 8.22 x 10^{-5} M (50 μg/ml) more effectively depressed phosphate and oxygen uptake and P:O ratios in the presence of increasing amounts of chlorpromazine than in the absence of the latter drug (Table II). On the other hand, very little difference was observed in the percent depression of P, O, and P:O values by 2.5 x 10^{-4} M Na-pentobarbital in the absence and presence of increasing amounts of chlorpromazine (Table III).

Six rats were given 10 mg/kg of chlorpromazine-HCl subcutaneously and 6 control animals were given water injections. All were sacrificed after 1 hour. Animals receiving the drug were severely depressed and incontinent but were not unconscious. Oxidative phosphorylation by brain homogenates was determined in the absence and presence of Na-pentobarbital in the flasks. Small but significant increases in oxygen uptake were obtained with homogenates from animals treated with chlorpromazine in the absence and presence of 2.5 x 10^{-4} M pentobarbital in the flasks (Table IV). No changes were seen in organic phosphate uptake or P:O ratios.

Chlorpromazine up to 6.25 x 10^{-4} M resulted in little or no depression of hexokinase activity(24) so that the decrease in phosphate uptake was not due to depression of the hexokinase trapping system.

TABLE IV. *In Vivo* Chlorpromazine on Oxidative Phosphorylation in Rat Brain Homogenates.

Inj. chlorpromazine, mg/kg	Pentobarbital in flasks, M × 10^4	△ P μM ± S.E.	△ O μatoms ± S.E.	P:O Ratio ± S.E.
0	0	28.3 ± .4	10.19 ± .14	2.78 ± .03
	2.5	13.1 ± .7	5.37 ± .09	2.44 ± .11
10	0	28.5 ± .4	10.74 ± .07*	2.69 ± .04
	2.5	14.1 ± .4	5.78 ± .07*	2.44 ± .08

* p <.01 that increases over corresponding values from untreated animals are due to chance.

Reserpine up to 50 μg/ml (8.22 x 10^{-5} M) had no significant effect on rat brain adenosinetriphosphatase activity, and chlorpromazine at 1.2 x 10^{-4} M and 1.5 x 10^{-4} M only slightly depressed ATPase.

Discussion. Both chlorpromazine and reserpine are capable of depressing oxygen and inorganic phosphate uptake by rat brain homogenates, but at concentrations much higher than ordinary pharmacological or therapeutic doses. It is highly improbable that reserpine depresses phosphorylation *in vivo*, since single effective pharmacological doses of 1 mg/kg in animals and daily sedative doses of as little as 0.5 mg represent much lower concentrations than the 25 mg/l needed to depress phosphate and oxygen uptake in rat brain. It is interesting to note that in effective concentrations, reserpine acts synergistically with chlorpromazine in depressing phosphate and oxygen uptake and P:O ratios in brain homogenates in contrast to similar combinations of pentobarbital and chlorpromazine. Although the concentration of chlorpromazine (2.5 x 10^{-4} M or 89 mg/l) needed to depress oxidative phosphorylation in rat brain was much greater than the physiologically depressing dose of 10 mg/kg, small increases in oxygen uptake by brain homogenates from treated animals are suggestive that the drug may have some effect on oxidative processes *in vivo*. Small increases in rat brain oxygen uptake were reported to be insignificant by Grenell, Mendelson, and McElroy (26). However, increases in oxygen uptake have been reported by Abood(23) with mitochondria using 2 x 10^{-5} M (7.1 mg/l) chlorpromazine *in vitro*. This concentration is comparable to the usual pharmacological doses.

From the data presented here and in recent reports(18,19,23), it appears that mitochondrial preparations are more sensitive to certain depressant drugs than whole homogenates or slices. Studies by Johnson and Ackerman (25) showing that nuclei potentiate mitochondrial oxidative phosphorylation suggest the possibility that nuclei and perhaps other cell fractions may protect mitochondria from the actions of uncoupling drugs. Suitable studies of this subject are now being undertaken.

Summary. Oxidative phosphorylation by rat brain homogenates was depressed by chlorpromazine at 2.5 x 10^{-4} M or greater, while reserpine at 25 and 50 μg/ml only slightly depressed oxygen uptake and phosphorylation. Effective concentrations of reserpine appeared to depress phosphorylation synergistically with chlorpromazine. Oxygen uptake was slightly increased with brain homogenates from animals treated with 10 mg/kg or chlorpromazine. Reserpine up to 50 mg/l had no effect on enzymatic ATP breakdown, while chlorpromazine, at 1.2 x 10^{-4} M and 1.5 x 10^{-4} M depressed rat brain adenosinetriphosphatase slightly.

1. Anton-Stephen, D., *J. Ment. Sci.*, 1954, v100, 543.
2. Winkelman, N. W., *J.A.M.A.*, 1954, v155, 18.
3. Bleuler, M., and Stoll, W. A., *Ann. N. Y. Acad. Sci.*, 1955, v61, 167.
4. Kinross-Wright, V., *ibid.*, 1955, v61, 174.
5. Noce, R. H., Williams, D. B., and Rapaport, W., *J.A.M.A.*, 1954, v156, 821.
6. Kline, N. S., and Stanley, A. M., *Ann. N. Y. Acad. Sci.*, 1955, v61, 85.
7. Hollister, L. E., Krieger, G. E., Kringel, A., and Roberts, R. H., *ibid.*, 1955, v61, 92.
8. Tasher, D. C., and Chermak, M. W., *ibid.*, 1955, v61, 108.
9. Brand, E. D., Harris, T. D., Borison, H. L., and Goodman, L. S., *J. Pharmacol. and Exp. Therap.*, 1954, v110, 86.
10. Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P., *Arch. Int. Pharmacodyn.*, 1953, v92, 305.
11. Zipf, H. F., *Arch. exp. Pathol. Pharmakol.*, 1954, v221, 76.
12. Plummer, A. J., Earl, A., Schneider, J. A., Trapold, J., and Barrett, W., *Ann. N. Y. Acad. Sci.*, 1954, v59, 8.
13. Winsor, T., *ibid.*, 1954, v59, 61.
14. Bein, H. J., *ibid.*, 1955, v61, 4.
15. Schneider, J. A., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 614.
16. Chen, G., and Ensor, C. R., *ibid.*, 1954, v87, 602.
17. Chen, G., Ensor, C. R., and Bohner, B., *ibid.*, 1954, v86, 507.
18. Finkelstein, M., Spencer, W. A., and Ridgeway, E. R., *ibid.*, 1954, v87, 343.
19. Brody, T. M., and Bain, J. A., *J. Pharmacol.*

and Exp. Therap., 1954, v110, 148.

20. Lowry, O. M., and Lopez, J. A., *J. Biol. Chem.*, 1946, v162, 421.

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

22. Youden, W. J., *Statistical Methods for Chemists*, John Wiley & Sons Inc., 1951, p24.

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

24. Berger, L., Slein, M. W., Colowick, S. P., and Cori, C. F., *J. Gen. Physiol.*, 1946, v29, 379.

25. Johnson, R. B., and Ackerman, W. W., *J. Biol. Chem.*, 1953, v200, 263.

26. Grenell, R. G., Mendelson, J., and McElroy, W. D., *Arch. Neurol. and Psychiat.*, 1955, v73, 347.

Received November 2, 1955. P.S.E.B.M., 1956, v91.

Transmethylation Reactions *in vivo* and *in vitro* in the Young Calf.* (22305)

JOHN H. HOPPER† AND B. CONNOR JOHNSON.

Division of Animal Nutrition, University of Illinois, Urbana.

Johnson *et al.*(1) have shown that the young calf on a "normal" methionine intake requires a dietary source of choline and the present investigation was undertaken to determine whether this was replaceable by methionine. Earlier work has shown that rat liver slices and homogenates methylate homocysteine to form methionine with either choline or betaine as methyl donor. In studies by Borsook and Dubnoff(2), guanidoacetic acid was shown to be methylated by methionine in guinea pig liver homogenates. Bernheim and Bernheim(3) demonstrated that rat liver homogenates could oxidize acetylcholine, but found guinea pig liver to be inactive in this oxidation. Kensler and Langemann(4) reported that human and rabbit liver resembled guinea pig liver in this respect. We have now studied these enzyme systems in the calf.

Methods. Methionine-choline interrelationship. Male calves of various dairy breeds, 24 hours old, were fed a synthetic milk diet previously described by Hopper and Johnson (5) except that alpha protein was used as the protein source in the diet. Various levels of DL-methionine were added to the diet at time of feeding to give the total dietary methionine values indicated in Table I. The vitamin premix contained all the vitamins, except choline, which was a variable in the experiment. When choline was administered, it was a constituent of the vitamin premix. All animals were fed *ad lib.* twice daily from nipple pails. At the end of the trials, all animals were sacrificed and liver samples obtained for ether extract determinations. Post mortem examinations of all animals were conducted for the detection of histological lesions.

In vitro studies using calf liver homogenates. Liver samples were obtained from calves which had been fed the complete synthetic milk diet and compared with liver samples from 175-200 g, male rats fed commercial laboratory pellets. These samples were handled and the study of the methylation of homocysteine by betaine was conducted by

* The authors are grateful to Dr. J. O. Alberts and Dr. D. A. Willegan, College of Veterinary Medicine, for autopsy of animals, and to Dr. K. E. Gardner, Department of Dairy Science, for securing the calves.

This investigation was supported in part by funds donated by Merck and Co., Rahway, N. J., and Armour and Co., Chicago, Ill. Thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid, choline and vit. B_{12} were generously supplied by Merck and Co. through courtesy of Dr. L. Michaud. Folic acid was generously supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., through courtesy of Dr. T. H. Jukes. Lard emulsion in feeding the animals was generously supplied by Armour and Co., Chicago, Ill. through the courtesy of Mr. B. M. Shinn.

† This paper is taken from a thesis submitted by the senior author to the graduate college, University of Illinois in partial fulfillment of requirements for the Degree of Doctor of Philosophy in Animal Nutrition. Present address: Department of Animal Husbandry, University of Nebraska, Lincoln.