1. Annegers, J. H., Am. J. Physiol. **206**, 1095 (1964).
2. Csaky, T. Z. and Ho, P. M., Proc. Soc. Exptl. Biol. Med. **120**, 403 (1965).
3. Crane, R. K., Biochim. Biophys. Acta **45**, 477 (1960).
4. Tracey, M. V., Biochem. J. **47**, 433 (1950).
5. Wilson, D. W. and Ball, E. G., J. Biol. Chem. **79**, 221 (1928).
6. Annegers, J. H., Am. J. Physiol. **26**, 701 (1966).
7. Snedecor, G. W., "Statistical Methods," Iowa State Univ. Press, Ames, Iowa, 1956.

Received July 25, 1967. P.S.E.B.M., 1968, Vol. 127.

The Influence of Serotonin on Oxidative Metabolism of Brain Mitochondria (32874)

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It has been stated that serotonin functions as a neurohormone in the brain(1,2). Although this role is not well defined the concept is strengthened by the findings which show that serotonin potentiates hexobarbital hypnosis (3,4). Subsequent reports have verified this but found that the metabolism of serotonin rather than its concentration in the nervous system was the cause of this potentiation(5). It was further determined that the succinoxidase activity of rat brain homogenates was inhibited by the carbonyl derivative of serotonin (6). From this it is suggested that the serotonin metabolites which depress nervous tissue metabolism might be a contributing factor in the potentiation of hypnosis. Additional studies of the effect of serotonin and its metabolites on energy transformation, particularly of the brain mitochondria, might prove helpful in determining the mechanism of action of serotonin.

Various reports have dealt with the effect of drugs and chemicals on mitochondrial function because of mitochondria's role in bioenergetics. Drugs such as thyroxine, dinitrophenol (DNP), and salicylates have been reported to uncouple phosphorylation from oxidation(7–11). The DNP also stimulates mitochondrial adenosine triphosphatase(12–15); as do salicylates(16). Mitochondrial swelling has been reported to be caused by thyroxine and Ca^{2+}(8), by tris (hydroxymethyl) amino methane(17) and by salicylates (11). Thus it can be seen that a variety of agents that influence physiological functions have an effect on mitochondria. Therefore, this study has been carried out to determine what effect serotonin and its metabolites have on oxidative phosphorylation and the function of electron transport by brain mitochondria since this could have a net effect on physiological function of the nervous system.

Methods. Mitochondria were prepared from adult Sprague-Dawley rats by homogenization of the brain in 0.25 *M* sucrose (1:10 w/v) using a glass homogenizer with a teflon pestle (Arthur H. Thomas Co., Philadelphia, Pa.). The homogenate was centrifuged 10 min at 800*g* and the major portion of the supernatant was removed without disturbing the precipitate. The precipitate was washed with sucrose (1:5 w/v) and recentrifuged. The combined supernatants were centrifuged at 10,000*g* for 10 min. This supernatant was removed by decanting and the inverted tube, was allowed to drain momentarily. The mitochondria adhering to the bottom of the tube were resuspended in sucrose solution, at a concentration equivalent to 500 mg original weight per ml of sucrose solution. The entire process was carried out at 0–4°C.

The quality of the mitochondrial preparation was determined by evaluation of their adenosine triphosphatase activity according to the method of Recknagel and Anthony (15), with the addition of KCl 0.075 *M*(12). Only those mitochondria showing a high re-

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TABLE I. Inhibition of Oxidative Phosphorylation[a] of Rat Brain Mitochondria by Serotonin.

Serotonin (M)[e]	μM P ± SE[b]	n[d]	μA O ± SE[c]	n	P/O ratio
		Normal animals			
None	5.03 (0.48)	6	2.08 (0.22)	6	2.42
4.1×10^{-5}	4.11 (0.38)	9	1.07 (0.15)	9	3.84
None	6.93 (0.53)	15	2.98 (0.15)	17	2.41
4.1×10^{-4}	3.13 (0.34)	16	1.97 (0.10)	18	1.59
		Iproniazid pretreated[f]			
None	5.58 (0.48)	8	2.19 (0.14)	8	2.54
4.1×10^{-4}	5.19 (0.35)	14	1.75 (0.11)	12	2.96

[a] Substrate was α-ketoglutarate added from flask sidearm after 5 min equilibration.
[b] Average μM of P uptake per 20 min at 30°C ± SE.
[c] Average μatoms of oxygen uptake per 20 min at 30°C ± SE.
[d] n = the no. of values per average.
[e] M = serotonin final molar concentration.
[f] Animals given 100 mg/kg of iproniazid-PO_4, im, 18 hours prior to sacrifice.

sponse to DNP stimulation and a low adenosine triphosphatase response to Mg^{2+} were included in the oxidative-phosphorylation studies.

Protein content of the mitochondrial suspension was determined according to the method of Cleland and Slater(18) as modified by Jobsis(19).

Oxidative-phosphorylation studies of intact mitochondria were made using the procedure described by Umbreit, *et al.*(20). The test was carried out by incubation at 30°C; air was used as the gas phase. The flasks were equilibrated 5 min in the Warburg bath before the α-ketoglutarate, glucose, and hexokinase were added to the main compartment from the sidearm. Oxygen uptake was measured during the following 20 min after which the reraction was stopped by addition of 0.2 ml of 20% trichloroacetic acid. When succinate served as substrate the equilibration period varied up to 120 min, and oxygen uptake was measured for 15 min. Inorganic phosphorus (P_i) uptake was measured spectrophotometrically by the method of Griswold, *et al.*(21).

Oxidative phosphorylation of mitochondrial fragments was studied by the procedure of Miyahara and Karler(22). The measurement of oxygen and phosphorus uptake was essentially the same as described for intact mitochondria.

Mitochondrial fragmentation was performed according to the procedure of McMurray, *et al.*(23) which employs sonification. The mitochondrial suspension in water was exposed to three periods of sonification of 10 sec each at step 6 intensity using a Bronson Sonifier (Heat Systems Co., Great Neck, N. Y.). The suspension was kept cool by means of an ice bath during the sonification process. This suspension of fragmented mitochondria was used without centrifugation for oxidative-phosphorylation studies.

All chemicals used were analytical grade reagents. The ATP (Disodium), NADH (nicotinamide adenine dinucleotide, reduced form), and hexokinase (Type III) were obtained from Sigma Chemical Co., St. Louis, Mo. The α-ketoglutarate (α-KG) and serotonin (creatinine sulfate complex) were obtained from Calbiochem. Inc., Los Angeles, Calif. The 3IAAL(3-indole-acetaldehyde sodium bisulfite) was purchased from Mann Res. Lab., New York, N. Y. The succinate was prepared by titrating analytical grade succinic acid (Fisher Chem. Co., Pittsburgh, Pa.) with KOH to a pH of 7.4. Iproniazid phosphate was provided by Hoffmann-La-Roche Inc., Nutley, N. J.

Results. The effect of serotonin on oxidative phosphorylation of brain mitochondria was studied using either α-ketoglutarate or succinate as substrate. In this way we were able to study the influence on energy transformation arising from entrance to the electron

TABLE II. The Inhibition of Succinate Oxidative Phosphorylation of Rat Brain Mitochondria by Serotonin.

Serotonin (M)[e]	μM P ± SE[a]	n[d]	μA O ± SE[b]	n	P/O ratio
	5 min equilibration[c]				
None	9.80 0.74	6	4.66 0.14	6	2.12
4.1×10^{-4}	7.13 0.49	5	4.67 0.19	5	1.98
	30 min equilibration				
None	9.80 0.74	6	4.66 0.14	6	2.12
4.1×10^{-4}	7.13 0.49	5	3.42 0.13	5	2.09
	120 min equilibration				
None	7.71 0.10	7	5.78 0.05	7	1.33
4.1×10^{-4}	1.75 0.43	8	3.46 0.10	9	0.50
	Iproniazid pretreated[f] 120 min equilibration				
None	6.14 0.96	8	5.18 0.06	8	1.19
4.1×10^{-4}	6.91 0.48	8	5.33 0.05	8	1.30

[a] Average μM P is μMoles of phosphorous uptake per 15 min at 30°C ± SE.

[b] Average μA O is μatom of oxygen uptake per 15 min at 30°C ± SE.

[c] Equilibration time refers to time of exposure of mitochondria to serotonin before addition of substrate (succinate).

[d] n = the number of experiments per average.

[e] M = serotonin final concentration in reaction flask.

[f] Animals given 100 mg/kg of iproniazid-PO_4, im, 18 hours prior to sacrifice.

chain at two different sites. This may be useful in locating the site of action of serotonin on mitochondrial activity.

When mitochondria were exposed to serotonin at 4.1×10^{-5} M concentration there was an increase in the P/O ratio (Table I). However, upon closer examination one sees that both phosphorus fixation and oxygen consumption were decreased. The resulting increase in P/O ratio was the result of a greater reduction in oxygen consumption relative to the decrease in phosphorus fixation at this concentration of serotonin. When the serotonin concentration was made 10^{-4} M, there was a more decided reduction of phosphorus fixation and a reduction of the P/O ratio. This could be interpreted as uncoupling of phosphorylation. These effects of serotonin were not evident when the mitochondria were prepared from animals whose monoamine oxidase (MAO) system had been previously blocked by pretreatment with iproniazid (Table I).

The P_i and oxygen uptake from the oxidation of succinate was similarly studied. However, the succinoxidase enzymes are much more durable under *in vitro* conditions than those involved in oxidation of α-ketoglutarate. Hence, it was possible to expose this system to serotonin for a longer time in order to bring out inhibition by serotonin. Reasons for the difference in the onset of inhibition by serotonin could be the penetration of the mitochondria by serotonin. This could be significant since the enzyme systems involved have different loci(24) in the case of beef heart mitochondria. However, as with α-ketoglutarate, serotonin at 4.1×10^{-4} M inhibited succinate oxidation. The uptake of oxygen but not P_i was inhibited after a 30 min equilibration before adding succinate. Both oxygen and P_i uptake were inhibited by serotonin after 120 min equilibration. This inhibition was not evident in mitochondria with MAO blockage (Table II). A concentration of 10^{-5} M serotonin was not used in this experiment but possibly would have an intermediate effect on P_i uptake, since there was such a large reduction at $10^{-4}M$ serotonin.

The results given above indicate that oxidative-phosphorylation is decreased by a pro-

TABLE III. The Effect of Indole-3-acetaldehyde on Oxidation Phosphorylation by Rat Brain Mitochondria.[a]

Aldehyde (M)	μM P ± SE[b]	n	μA O ± SE[c]	n	P/O ratio
—	5.41 0.44	12	2.32 0.30	11	2.34
4.1×10^{-4}	3.72 0.41	12	2.31 0.30	12	1.61

[a] Substrate was α-KG, added from flask sidearm after 5 min equilibration.
[b] Average μM P uptake per 20 min at 30°C ± SE.
[c] Average μatoms oxygen uptake per 20 min at 30°C ± SE.

duct of monoamine oxidation of serotinin rather than serotonin per se. This could have been verified if the carbonyl derivative 5-hydroxyindole acetaldehyde had been available. Since it was not, 3-indole-acetaldehyde (3IAAL) in the bisulfite form, which is commercially available, was tested for its influence on mitochondrial oxidative phosphorylation. We found, using the aldehyde at a concentration of 4.1×10^{-4} M, that the uptake of P_i was decreased, but oxygen uptake was unchanged in the oxidation of α-ketoglutarate by intact brain mitochondria (Table III). This resulted in a reduction of the P/O ratio similar to the effect of serotonin (Table I).

The effect of this aldehyde on the oxidation of succinate was also studied. The difference between the experimental groups was that the aldehyde was added 120, 60, or 30 min before the succinate. The aldehyde under these conditions inhibited oxygen uptake at 30 min but inhibition of P_i uptake at 30 min is doubtful. Both P_i and oxygen uptake were inhibited by 60- and 120-min exposures to the aldehyde before succinate addition. The decrease in the P/O ratio became more pronounced as the time of exposure to aldehyde increased (Table IV). These two experiments (Tables III and IV) with 3-indole-acetaldehyde add substance to the hypothesis that a carbonyl derivative of serotonin is most likely the cause of the inhibition of oxidative phosphorylation shown by addition of serotonin.

There is a noticeable reduction in the control P/O ratio when the incubation time was extended to 120 min (Tables II, IV). This reduction could conceivably be the result of mitochondrial degeneration. Nevertheless, both serotonin as well as 3-indole-acetaldehyde blocked P_i uptake to a greater extent than oxygen consumption.

We desired to determine more specifically the locus of action of serotonin. Therefore, a study was made using fragmented mitochondria, with which a comparison was made of succinate and NADH as substrate. As can be seen in Table V the addition of the aldehyde caused a decrease in P_i uptake with only a small decrease in oxygen uptake. A reduction in the P/O ratio is evident. These results differ from those obtained from whole mitochondria (Table IV) where both oxygen and P_i uptake were inhibited. A significant feature of this experiment with fragmented mitochondria is that no more than a minimal 5 min equilibration period was needed for the aldehyde to produce inhibition (Table V); while in the experiments with intact mitochondria 60–120 min exposure to aldehyde was required to inhibit oxidative phospho-

TABLE IV. Effect of Indole-3-acetaldehyde on Oxidative Phosphorylation from Succinate by Rat Brain Mitochondria.[e]

	μM P ± SE[a]	μA O ± SE[b]	P/O ratio
Control	6.44 0.76	4.40 0.21	1.46
Aldehyde[c]			
30 min[d]	4.64 0.65	3.06 0.15	1.52
60 min	2.23 0.49	2.13 0.10	1.05
120 min	0.68 0.41	0.95 0.13	0.71

[a] Average μM of phosphorous uptake/15 min at 30°C ± SE.
[b] Average μatoms of oxygen uptake/15 min at 30°C ± SE.
[c] 3-Indole-acetaldehyde final concentration 4.1×10^{-4} M.
[d] All flasks were at 30°C 120 min before substrate (succinate was added; aldehyde added at 30, 60, and 120 min before succinate).
[e] All values based on average of 6 determinations

TABLE V. The Influence of 3-Indole-acetaldehyde on Oxidative Phosphorylation by Rat Brain Mitochondrial Fragments.

Aldehyde (M)[a]	Substrate	μM P ± SE[b]	n[c]	μA O ± SE	n	P/O ratio
—	Succinate	1.55 0.20	16	1.59 0.18	16	0.98
4.1×10^{-4}		0.48 0.27	16	1.18 0.16	15	0.40
—	NADH	2.56 0.43	16	2.89 0.18	16	0.89
4.1×10^{-4}		1.29 0.32	16	2.85 0.13	16	0.45

[a] M = final molarity of aldehyde.

[b] μM P ± SE and μA O ± SE are average μatoms uptake per 15 min at 30°C of inorganic phosphorus and oxygen, respectively, ± their SE.

[c] n = the no. of experiments per average.

rylation with succinate as substrate (Table IV). An additional study was carried out with fragmented mitochondria. In this case NADH was used as substrate rather than α-ketoglutarate because of the lack of activity from α-ketoglutarate as substrate. The results resembled those obtained from the oxidation of succinate under similar conditions (Table V). The aldehyde caused a decrease in P_i uptake without a change in oxygen uptake and resulted in a lowered P/O ratio. In both circumstances the aldehyde interfered with P_i fixation rather than electron transport. Since oxygen uptake was not significantly inhibited the aldehyde may be considered to have caused uncoupling of phosphorylation from oxidation.

Discussion. The importance of glycolysis and aerobic oxidation by the brain cannot be overemphasized. Interference with this metabolic scheme will cause impaired nervous function because of the major dependence of brain on carbohydrate metabolism. The studies presented here have been designed to explore the possible role of serotonin in the brain in terms of its influence on the metabolic pathway and bioenergetics of the brain. This has been done by studying the effect of serotonin or its metabolites on mitochondrial enzymatic pathway which are involved in oxidative phosphorylation.

The effect of serotonin is to reduce phosphorus uptake of mitochondria to a greater extent than it reduces oxygen uptake (Tables I and II). This is an uncoupling of phosphorylation as indicated by a decrease in the P/O ratio. This is similar to the effect produced by salicylates (9–11,22,25) as well as thyroxine(8) and DNP(7).

The inhibition demonstrated by serotonin can be eliminated by MAO blockade (Tables I and II). This is an indication that the carbonyl derivative of serotonin oxidation is the responsible factor in blocking brain mitochondrial oxidative phosphorylation. It has been shown previously that the carbonyl derivative of serotonin has an inhibitory action with regard to the succinoxidase system of rat brain homogenates(6). Further support implicating the aldehyde derivative of serotonin is obtained by the experiments which show that 3-indole-acetaldehyde can substitute for serotonin in causing a reduction of oxidative phosphorylation (Tables III and IV).

Indications from these studies are that the phosphorus uptake is affected to a greater degree than is the oxygen uptake of intact mitochondria. This was also the case for mitochondrial fragments when succinate or NADH served as substrate (Table V). Here as before 3-indole-acetaldehyde caused a reduction in oxidative phosphorylation mainly at the expense of phosphorus uptake.

Evidence thus far points to phorphorus fixation as being most affected by serotonin in the systems tested. This would indicate that enzymes involved with entrance into the electron transport system were not the primary site of action.

Summary. The effect of serotonin on oxidative phosphorylation by rat brain mitochondria was studied. It was found that both phosphorus and oxygen uptake were inhibited by the addition of serotonin. However, the uptake of phosphorus was more sensitive to serotonin inhibition. The addition of serotonin

caused an uncoupling of phosphorylation from oxidation as evidenced by a reduced P/O ratio. This effect of serotonin was removed by monoamine oxidase blockade. Indication that the effect of serotonin is mediated via its carbonyl derivative was further strengthened by the use of 3-indole-acetaldehyde which gave similar inhibition of phosphorus and oxygen uptake. These findings support the hypothesis that serotonin could impede brain function by inhibition of energy producing reactions.

1. Woolley, D. W. and Shaw, E., Proc. Natl. Acad. Sci. U. S. **40**, 228 (1954).

2. Brodie, B. B., Pletscher, A., and Shore, P. A., Science **122**, 968 (1955).

3. Holtz, P., Bolzer, ,H., Westerman E., and Wezler, E., Arch. Exptl. Pathol. Pharmakol. **231**, 333 (1957).

4. Shore, P. A., Silver, S. L., and Brodie, B. B., Experientia, **11**, 272 (1955).

5. Mahler, D. J. and Humoller, F. L., J. Neuropsychiat. **5**, 252 (1964).

6. Mahler, D. J. and Humoller, F. L., Int. J. Neuropsychiat. **3**, 229 (1967).

7. Loomis, W. F. and Lipmann, F., J. Biol. Chem., **173**, 807 (1948).

8. Karler, R., Sulkowski, T. S., and Miyahara, J. T., Biochem. Pharmacol. **14**, 1025 (1965).

9. Whitehouse, M. W., Biochem. Pharmacol. **13**, 319 (1964).

10. Penniall, R., Biochim. Biophys. Acta. **30**, 247 (1958).

11. Jeffrey, S. W. and Smith, M. J. H., Biochem. J. **72**, 462 (1959).

12. Meyers, D. K. and Slater, E. C., Biochem. J. **67**, 558 (1957).

13. Potter, V. R., Siekevitz, P., and Simonison, H., J. Biol. Chem. **205**, 893 (1953).

14. Lardy, H. A. and Wellman, H., J. Biol. Chem. **201**, 357 (1953).

15. Recknagel, R. O. and Anthony, D. D., J. Biol. Chem. **234**, 1052 (1959).

16. Charnock, J. S. and Opit, L. J., Biochem. J. **83**, 596 (1962).

17. Childress, C. C. and Sactor, B., Science **154**, 268 (1966).

18. Cleland, K. W. and Slater, E. C., Biochem. J. **53**, 547 (1953).

19. Jobsis, F. F., Biochim. Biophys. Acta **74**, 60 (1964).

20. Umbriet, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques," 4th ed. Burgess, Minneapolis, Minnesota, 1964.

21. Griswold, B. L. and Humoller, F. L., Anal. Chem. **23**, 192 (1951).

22. Miyahara, J. T. and Karler, R., Biochem. J. **97**, 194 (1965).

23. McMurray, W. C., Maley, G. F., and Lardy, H., J. Biol. Chem. **230**, 219 (1958).

24. Green, D. E., Bachmann, E., and Allmann, D. W., Arch. Biochem. Biophys. **115**, 172 (1966).

25. Brody, T. M., J. Pharmacol. Exptl. Therap. **117**, 39 (1956).

Received Sept. 5, 1967. P.S.E.B.M., 1968, Vol. 127.

Experimental Infection of Chimpanzees with Human Rhinovirus Types 14 and 43* (32875)

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Judging from findings in etiologic studies of upper respiratory illness, rhinoviruses are associated with more "cold-like" illness than any other group of viral agents (1–3). Experimentation on the pathogenesis, prevention, and cure of human rhinovirus infections has been handicapped by the lack of an experimental animal other than man. In 1930, Dochez and his associates (4) found that

* Gratitude is expressed to the entire staff of the Delta Regional Primate Center; particularly helpful were Dr. Arthur Riopelle, Director, Dr. Charles Hill, Chief of Cooperative Research, Dr. William Greer, Primate Veterinarian, and Mr. Michael Brady, Research Specialist in Large Primates. Gratitude is also expressed to Miss Becky Blumer and Mr. Stanley Williams of Wisconsin for excellent virologic work. This research was supported in part by USPHS Grant E 1299 and was presented at the annual meeting of the American Society for Microbiology in New York, May, 1967.

[1] Career Research Development Awardee of the National Institute of Allergy and Infectious Diseases.