fection results in reversal. 2. NaCl when added late, although it stimulates virus growth, does not increase tissue proliferation. Glucose 0.14 M in 25%BSS permits almost normal tissue proliferation, but does not significantly stimulate virus synthesis during the first 24 hours. 3. Loss of virus from heavily infected tissue occurs on exposure to 50, 35, or 25%BSS. This is prevented by adjusting the solution to near normal osmolarity with glucose. Tissue respiration is markedly inhibited by 25%BSS but not by 50%BSS. 4. The relative roles of electrolytes and osmotic properties of the solution in virus attachment, penetration, and synthesis and on tissue respiration are considered.

1. Levine, A. S., Bond, P. H., Scala, A. R., and Eaton, M. D., *J. Immunol.*, 1956, in press.

2. Lowell, F. C., and Buckingham, M., *ibid.*, 1948, v58, 229.

3. Burnet, F. M., and Edney, M., *Australian J. Exp. Biol. and Med. Sci.*, 1952, v30, 105.

4. Gohd, R. S., Doctoral Thesis, Faculty of Arts and Sciences, Div. Medical Sciences, Harvard Univ., 1952.

5. Davenport, F. M., and Horsfall, F. L., *J. Exp. Med.*, 1948, v88, 621.

6. Puck, T., and Sagik, B., *ibid.*, 1953, v97, 807.

7. Eaton, M. D., Adler, L. T., and Perry, M. E., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 57.

8. Eaton, M. D., Adler, L. T., Scala, A. R., and Bond, P., *J. Inf. Dis.*, in press.

9. White, P. R., *Growth*, 1946, v10, 231.

10. Garen, A., and Puck, R., *J. Exp. Med.*, 1951, v94, 177.

11. Hogeboom, G. H., Schneider, W. C., and Palade, G. E., *J. Biol. Chem.*, 1948, v172, 619.

12. Harman, J. W., and Feigelson, M., *Exp. Cell Res.*, 1952, v3, 47, 509.

Received April 30, 1956. P.S.E.B.M., 1956, v92.

Anesthesia: LIV. Effects of Viadril and Some Water-Soluble Steroids on Brain Oxidative Phosphorylation.* (22455)

EDWARD B. TRUITT, JR., FREDERICK K. BELL, AND JOHN C. KRANTZ, JR.
(With assistance of Ann M. Morgan and M. Joseph Rehak.)
Department of Pharmacology, University of Maryland School of Medicine, Baltimore.

Although claims of "anesthesia" produced by steroids have been made previously(1,2-5), Viadril, a new water-soluble steroid, represents the first compound of this type to evoke anesthesia with a safety margin adequate for human use(6). A survey of the effects of various hypnotic and anesthetic drugs on the coupling between oxidative phosphorylation and respiration in rat brain mitochondria(7) prompted the extension of this study to Viadril. Other water-soluble steroids lacking anesthetic action were tested for comparison.

Methods. The methods used for measurement of the ratio of oxidative phosphorylation to respiration have been described more fully elsewhere(7,8). Briefly, the ratio is measured as the inorganic phosphorus uptake in micromoles of phosphorus divided by the microatoms of oxygen consumed by rat brain mitochondria which were isolated in 0.25 M sucrose containing 0.001 M disodium Versenate. The final concentrations in the medium used were: pH 7.4 phosphate buffer 0.020 M; potassium chloride 0.050 M; potassium fumarate 0.002 M; sodium pyruvate 0.013 M; ATP 0.0025 M; glucose 0.028 M; hexokinase 6.25 mg/cc; disodium Versenate 0.001 M; sodium fluoride 0.012 M; magnesium chloride 0.008 M; and was incubated at 20° for 30 minutes following a 10 minute equilibration. Viadril (21-hydroxy pregnanedione), hydrocortisone, and pregnenolone were all employed in the form of their sodium succinate salts. The sodium salt of dehydrocholic acid was used.†

* Supported by grant from Ohio Chemical & Surgical Equipment Co., (Division of Air Reduction Co., Inc.), New York City.

† These compounds were kindly supplied by Dr. Michael Carlozzi of Pfizer Laboratories.

TABLE I. Effect of Steroid Derivatives on Oxidative Phosphorylation of Rat Brain Mitochondria.

Drug	Conc., mM	Avg control P	O	P/O	Avg experimental P	O	P/O	P/O % change	No. of flasks
Viadril	.05	15.4	4.99	3.09	13.7	5.41	2.54	−17.8	3
	.10	14.4	4.59	3.12	11.5	4.59	2.51	−19.6	5
	.25	14.9	4.48	3.33	10.0	4.04	2.48	−25.6	2
	.50	15.4	4.85	3.18	1.0	2.50	.40	−87.5	3
Hydrocortisone sodium succinate	.104	11.5	3.99	2.89	13.7	4.78	2.87	− .07	5
	.208	11.5	3.99	2.89	14.2	5.36	2.65	− 8.3	6
	.416	11.5	3.99	2.89	14.1	4.77	2.96	+ .24	5
Pregnenolone sodium succinate	.114	14.4	4.81	3.0	10.6	5.20	2.04	−32.0	3
	.228	14.4	4.81	3.0	3.9	3.51	1.11	−63.0	3
	.455	14.4	4.81	3.0	2.8	4.49	.62	−79.5	3
Dehydrocholic acid	.125	11.3	3.65	3.10	9.75	3.29	2.96	− 4.5	2
	.25	11.3	3.65	3.10	10.15	3.18	3.20	+ 3.2	2
	.50	11.3	3.65	3.10	5.5	2.55	2.16	−30.4	2

Results. Table I sets forth the data obtained for several concentrations of each agent. Both Viadril and a closely-related pregnenolone derivative devoid of anesthetic activity produced a substantial uncoupling of oxidative phosphorylation from respiration. High concentrations of dehydrocholic acid exhibited a slight effect and hydrocortisone no action.

Discussion. Pregnenolone produced uncoupling of oxidative phosphorylation and is devoid of anesthetic activity. This lack of correlation occurs in the relationship of uncoupling to anesthetic or hypnotic activity for other narcotic drugs(7,8). In this group of drugs the most potent uncoupling agents were Viadril and pregnenolone, of which only Viadril exerts anesthetic action. Murphy *et al.* (9) reported the range of anesthetic doses in the majority of patients to be 1.0-1.5 g. Using the upper figure, an estimation of the expected concentration in extracellular body water would be 10 mg % (0.22 mM) for a 75 kg person. This plasma level should produce significant depression of P/O ratio *in vitro* as indicated by the results in Table I.

Difficulties of solubility discourage the extension of these studies to other steroids claimed by Selye to have central depressant action. A previous paper from this laboratory questions the suitability of the word "anesthesia" for the effects of the steroids used by Selye(10).

Conclusion. 21-Hydroxypregnanedione sodium succinate and pregnenolone sodium succinate produce significant uncoupling of oxidative phosphorylation from oxygen consumption. Dehydrocholic acid was less effective. Hydrocortisone sodium succinate was ineffective.

1. Cashin, M. F., and Moravek, V., *Am. J. Physiol.*, 1927, v82, 294.

2. Selye, H., *J. Pharmacol. and Exp. Therap.*, 1941, v73, 127.

3. ———, PROC. SOC. EXP. BIOL. AND MED., 1941, v46, 116.

4. ———, *Endocrinology*, 1942, v30, 437.

5. ———, *Anes. and Anal.*, 1943, v22, 105.

6. Laubach, G. D., P'an, S. Y., and Rudel, H. W., *Science*, 1955, v122, 78.

7. Wolpert, A., Truitt, Jr., E. B., Bell, F. K., and Krantz, Jr., J. C., *J. Pharmacol. and Exp. Therap.*, in press.

8. Truitt, Jr., E. B., Bell, F. K., and Krantz, Jr., J. C., *Anesthesiology*, in press.

9. Murphy, E. J., Guadagni, N. P., and De Bon, F., *J.A.M.A.*, 1955, v158, 1412.

10. Farson, de C. B., Carr, C. J., and Krantz, Jr., J. C., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 70.

Received April 30, 1956. P.S.E.B.M., 1956, v92.