structural subunits or these phenomena merely coincide is the subject of current investigations.

In connection with the fact that primary antibody response to endotoxoid-2 is very low, recent results of this laboratory should be mentioned (10). It was shown that while toxic endotoxin is taken up very rapidly by RES cells, endotoxoids are not taken up and remain in the circulation for a longer time. This was measured in normal BRVR mice. If the mice were immunized by toxic endotoxin and the RES uptake of different endotoxoids was measured, a rapid uptake could be observed. This explains the significant effect of the booster injection. This observation is also in agreement with findings reporting that several-times repeated injections of endotoxoid-2 result in an antibody titer comparable to the toxic preparation.

Summary. Several-times repeated injections of heat-killed cells, toxic endotoxins, or CH_3OK detoxified derivative thereof (endotoxid-2) result in a comparable antibody titer after 8–10 injections. The antibody titer produced by toxic preparations shows a rapid initial increase, while endotoxoid-2 produces a lower titer of circulating antibodies in the first 10 days. While toxic preparations produce both 2-Me resistant and sensitive antibodies from the beginning of the immunization, endotoxoid-2 produces almost exclusively 2-Me sensitive antibodies in the first 10 days. Simultaneous with the appearance of 2-Me resistant antibodies, the level of total antibodies rises rapidly. A single injection of toxic endotoxin produces a rapid antibody production which starts to decline after approximately 2 weeks. Booster injections bring this antibody level back to previous highs, but hardly any additional effect can be observed. A single injection of endotoxoid-2 gives a very slow output of antibodies, which seems to remain stable for 1 month. Booster injection of endotoxoid-2 results in a strong increase of antibody production.

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1. Nowotny, A., Nature **197**, 721 (1963).

2. Nowotny, A., in "Bacterial Endotoxins," Landy, M. and Braun, W., p. 29. Rutgers Univ. Press, New Brunswick, New Jersey, 1964.

3. Johnson, A. G. and Nowotny, A., J. Bacteriol. **87**, 809 (1964).

4. Stetson, C. A., Jr., J. Exptl. Med. **94**, 347 (1951).

5. Stetson, C. A., Jr., in "Bacterial Endotoxins," Landy, M. and Braun, W., p. 658. Rutgers Univ. Press, New Brunswick, New Jersey.

6. Farr, R. S., Campbell, D. H., Clark, S. L., Jr., and Proffitt, J. E., Anat. Record **118**, 385 (1954).

7. Farr, R. S., J. Allergy **30**, 268 (1959).

8. Nowotny, A., Cundy, K. R., Neale, N. L., Nowotny, A. M., Radvany, R., Thomas, S. P., and Tripodi, D. J., Ann. N. Y. Acad. Sci. **133**, 586 (1966)

9. Neter, E., Bertram, E. F., and Arbesman, C. E., Proc. Soc. Exptl. Biol. Med. **79**, 255 (1952).

10. Golub, S., Gröschel, D. H. M., and Nowotny, A., Bacteriol. Proc. M113 (1967), Journal of the Reticuloendothelial Society, in press.

11. Kováts, T. G., Nature **190**, 177 (1961).

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The Effect of Reserpine Administration on Vanillylmandelic Acid Excretion in Man (32791)

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Previous studies have shown that the acute administration of reserpine in man is associated with an increase in the urinary excretion of 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, VMA) in both normal and schizophrenic males (1, 2). This finding is consistent with those in animal studies which show that acute reserpine administration induces a depletion of catecholamines in all body tissues (3, 4) and an increase in blood

TABLE I. Effect of Sequential Injections of Reserpine on VMA Excretion.

Time	Reserpine, day 1		Reserpine, day 2	
	μg of VMA/mg of creatinine mean difference from control	SED	μg of VMA/mg of creatinine mean difference from control	SED
8 am– 4 pm	0.856	0.266	—0.166	0.183
4 pm–12 m	0.456	0.186	—0.408	0.226
12 m – 8 am	—0.130	0.179	—0.626	0.220[a]
Composite periods				
8 am–12 m	0.362	0.073[b]	—0.296	0.166
4 pm– 8 am	—0.184	0.157	—0.508	0.151[a]

[a] $p < 0.05$.
[b] $p < 0.01$.

epinephrine and urine catecholamine levels (5, 6) but does not appear to be consistent with a reported decrease in NE excretion in man following acute reserpine administration (7). Chronic administration of reserpine in man is associated with decreased VMA excretion (8), decreased urinary excretion of norepinephrine (NE) (7, 9), and decreased (7) or unchanged (9) excretion of epinephrine (E).

Because both increased and decreased catecholamine and VMA excretion have been reported following reserpine administration the present study has been designed to determine the effect of acute and repeated administration of reserpine on the pattern of VMA excretion in man. In addition the observed pattern of VMA excretion has been compared to known effects of reserpine on catecholamine metabolism.

Methods. Subjects employed in this study consisted of two normal male volunteers and three male schizophrenic subjects. In previous studies it was established that there was no difference in the effect of reserpine on VMA excretion in normal and schizophrenic subjects (2). Their average age was 31 years (range, 25–37) and average body weight was 75.5 kg (range, 67.5–93.5). During the studies smoking, caffeine containing beverages and chocolate were proscribed. The baseline VMA excretory values were obtained while the subjects were receiving reserpine placebo injections at 8-hour intervals for a 24-hour period. Then reserpine was administered, intramuscularly, in 5 doses of 2.5 mg at 8-hour intervals. The placebo or drug was injected at 8 a.m., 4 p.m., and 12 m. All subjects had minimal ambulatory privileges during the day.

Urinary creatinine was determined by the alkaline picrate method (10). Urinary VMA was assayed by the tracer technique described previously (11) with the exception of the use of a longer column of Dowex 1-acetate (21 inches) and the collection of a larger forerun of eluate (250 ml) for the fractionation procedure.

The VMA excretion has been expressed as μg of VMA/mg of creatinine. For each of the 5 subjects VMA excretion during the test period minus VMA excretion during the same period of the placebo day gave a difference. These differences averaged for Table I and the SED was calculated by the equation: SED =

$$\pm \{\Sigma\Delta^2 - [(\Sigma\Delta)^2/N]\}^{1/2}/[N(N-1)]$$

Results. The effect of 5 injections of reserpine given at 8-hour intervals is shown in Fig. 1. Mean control consecutive 8-hour excretion values were 2.11, 2.13, and 2.20 μg of VMA/mg of creatinine. The first injection of reserpine resulted in an increased excretion of VMA during the ensuing 8-hour collection ($p<0.05$). The VMA excretion in the period following the second injection was also increased, though not significantly. However, when the composite excretions for the first two periods were compared with the corresponding placebo periods the significance of difference was increased ($p<0.01$). By the third 8-hour period VMA excretion values

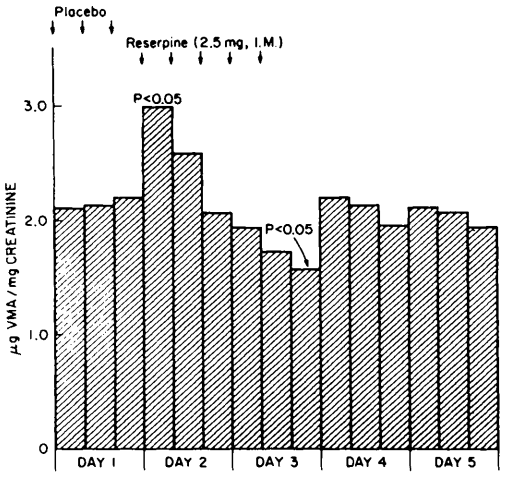

FIG. 1. The effect of repeated injections of reserpine on VMA excretion.

were no different from placebo values, and for the following four consecutive 8-hour periods there was a continued decrease in VMA excretion. During the period from 12 m to 8 a.m. on the second day of reserpine administration, VMA excretion was significantly decreased ($p<0.05$) (Table I). Within 16 hours following the completion of reserpine injections, VMA excretion had returned to prereserpine levels. Reserpine administration did not cause any significant change in creatinine excretion.

Discussion. In man, repeated reserpine administration results in an initial increase and a subsequent decrease in VMA excretion (Fig. 1). Approximately 80–90% of endogenous NE is apparently converted to VMA under normal conditions (1). Thus the average increase of about 40% in VMA excretion observed during the first 16 hours following reserpine administration could not be a consequence of conversion of NE and E to VMA. Furthermore, the subsequent decreased excretion of VMA resulting from reserpine administration contradicts this possible mechanism.

The initial increased VMA excretion does not appear to be due to increased synthesis of catecholamines. Studies of the effect of reserpine on NE synthesis indicate a decrease rather than an increase (12,13). A decrease in NE synthesis would, however, be consistent with the decreased VMA excretion observed after repeated reserpine administration. This decreased VMA excretion is not the consequence of decreased monoamine oxidase activity since reserpine causes increased oxidative deamination of NE (14).

The observed increase in VMA excretion followed by a decrease appears to be best explained by a combination of an initial increased release of stored NE associated with decreased NE synthesis. Such an explanation finds support from the following observations: in animals acute administration of reserpine is associated with depletion of tissue catecholamines (3,4) and increased blood and urine catecholamine leves (6); in man acute administration of reserpine is associated with increased VMA excretion; *in vitro* decreased NE synthesis occurs in the presence of reserpine due to decreased uptake of dopamine (DA) by intracellular storage granules in heart and adrenal tissue (12,13) and thus a decrease in the conversion of DA to NE which is believed to take place in the storage granules. Thus the effect of reserpine on the intracellular storage granule could account for the initial increase in VMA excretion by an increased release of NE and the subsequent decrease in VMA excretion due to inhibition of NE synthesis.

Summary. In man, repeated injections of reserpine result in an initial increase followed by a decrease in the urinary excretion of VMA. Within 16 hours following injection of 2.5 mg of reserpine at 8-hour intervals, increased VMA excretion has ended and there is a continual decrease in the excretory rate. It is suggested that the observed VMA excretory pattern is consistent with a reserpine effect on the intracellular storage granule with an initial release of NE associated with a decreased uptake of dopamine and resultant decreased NE synthesis.

1. McDonald, R. K. and Weise, V. K., J. Pharmacol. Exptl. Therap. **136**, 26 (1962).

2. McDonald, R. K. and Weise, V. K., J. Psychiat. Res. **1**, 173 (1962).

3. Carlsson, A., Rosengren, A., Bertler, A., and Nilsson, J., in "Psychotropic Drugs," Garrattini, S and Ghetti, S., eds., p. 363. Elsevier, Amsterdam, 1956.

4. Brodie, B. B., Olin, J. S., Kuntzman, R. G., and Shore, P. A., Science **125**, 1293 (1957).

5. Muscholl, E. and Vogt, M., Brit. J. Pharmacol. **12**, 532 (1957).

6. Schaepdryver, A. F. de, Arch. Intern. Pharmacol. **121**, 478 (1959).

7. Gaddum, J. H., Krivoy, W. A., and Laverty, G., J. Neurochem. **2**, 249 (1958).

8. Munkvad, I., Randrup, A., in "Neuropsychopharmacology," Vol. 4, p. 289. Bente, D. and Bradley, P. B., eds., Elsevier, Amsterdam, 1965.

9. Carlsson, A., Rasmussen, E. B., and Kristjansen, P., J. Neurochem. **4**, 318 (1959).

10. Reiner, M., "Standard Methods of Clinical Chemistry," Vol. 1, p. 55. Academic Press, New York, 1953.

11. Weise, V. K., McDonald, R. K., and LaBrosse, E. H., Clin. Chim. Acta **6**, 79 (1961).

12. Kirshner, N., Rorie, M., and Kamin, D. L., J. Pharmacol. and Exptl. Therap. **141**, 285 (1963).

13. Weiner, N., Rutledge, C. O., in "Mechanisms of Release of Biogenic Amines," Von Euler, U. Rosell, S., eds., Vol. 5, p. 307. Macmillan (Pergamon) New York.

14. Kopin, I. J. and Gordon, E. K., J. Pharmacol. Exptl. Therap. **138**, 351 (1962).

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Effects of Continuous Light on Hypothalamic FSH-Releasing Factor and Pituitary FSH Levels in Rats* (32792)

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Exposure of female rats to continuous illumination has been reported to induce prolonged or persistent vaginal cornification (1,2). Fiske (3,4) concluded that pituitaries from female rats on constant light had more FSH and less LH activity than rats on constant darkness. Maric *et al.* (5) found that total pituitary gonadotropin content was not changed after 50 or 100 days of constant illumination, whereas pituitary LH content and concentration were markedly reduced. Lawton and Schwartz (6) and Bradshaw and Critchlow (7) also reported decreased LH content in rats with persistent estrus induced by continuous light. It was our purpose to elucidate the role of the FSH-releasing factor (FSH-RF) in the hypothalamus on the pituitary FSH response to constant light.

Materials and Methods. Female Sprague-Dawley rats (Holtzman, Madison, Wisc.) about 3 months old and weighing 200–300 gm each were used in these experiments. Two separate groups of rats were kept under continuous illumination for 21 days. Continuous light was provided by four 100-W white light bulbs, placed 4 feet above the wire-topped cages. Controls were housed under standard light conditions (14 hours of light daily). All rats were maintained on a diet of Wayne Lab Blox pellets (Allied Mills, Chicago, Ill.) and fed *ad libitum*. Vaginal smears were taken daily in both experimental and control groups. After the first 10 days of constant illumination, all rats showed a proestrous or estrous type of smear. Control animals showed normal cycling patterns. On day 22, the rats were killed by guillotine and the hypothalami and anterior pituitaries were removed. The hypothalami were placed in ice cold 0.1 *N* HCl (0.2 ml per hypothalamus) and stored at −20°C. The pituitaries were weighed individually and stored at −20°C. A few days later, the pituitaries were homogenized in saline with a glass homogenizer and assayed for FSH activity. Ovaries and uteri were cleaned, weighed, and processed for histological study. Differences in organ weights were analyzed by Student's *t* test.

Preparation of hypothalamic extracts. Hypothalami were homogenized in chilled 0.1 *N* HCl and centrifuged at 12,000*g* for 40 min at 4°C just prior to use. The supernatants were placed in protein free medium 199 (Difco Labs, Detroit, Mich.) and the pH was adjusted to 7.4 by adding a drop at a time of 1 *N* NaOH and testing with glass electrodes.

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