Effect on phosphatase activity. Both acid and alkaline phosphatase activity in the pupae from diets containing up to 1% of thalidomide showed no difference from those on the control diet.

Department of Biochemistry,
School of Medicine, Laval University,
Quebec, Canada.

K. D. CHAUDHARY
A. LEMONDE*

Acknowledgement—Appreciation and thanks are given to Mr. Serge Ubertelli who separated and counted the insects. This work was supported by a grant from the National Research Council of Canada.

* Medical Research Associate, Medical Research Council, Ottawa, Canada.

REFERENCES

1. K. D. CHAUDHARY and A. LEMONDE, *Canad. J.Zool.* **40**, 375 (1962).
2. W. W. UMBREIT, in *Manometric Techniques*, W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, Eds., p. 1. Burgess, Minneapolis (1959).
3. K. D. CHAUDHARY and A. LEMONDE, *Experienta* **20**, 86 (1964).
4. G. F. SOMERS, *Lancet* **1**, 912 (1962).
5. D. FELISATI, *Lancet* **2**, 724 (1962).
6. M. J. SELLERS, *Lancet* **2**, 249 (1962).
7. C. T. G. KING and F. J. KENDRICK, *Lancet* **2**, 1116 (1962).
8. W. LENZ, *Dtsch. med. Wschr.* **86**, 2555 (1961).
9. W. LENZ, *Lancet* **1**, 45 (1962).
10. F. KEMPER, *Lancet* **2**, 836 (1962).
11. J. VERRETT and J. MCLAUGHLIN, *Fed. Proc.* **22**, 188 (1963).
12. B. SALZGEBER and J. SALAÜN, *C.R. Acad. Sci. (Paris)* **256**, 2719 (1963).
13. C. A. DE BOCK and A. PETERS, *Nature, Lond.* **199**, 1204 (1963).
14. J. F. LUCEY, *Science* **139**, 1295 (1963).
15. G. A. CHRISTIE, *Lancet* **2**, 249 (1962).
16. O. FRANK, H. BAKER, H. ZIPPER, S. AARONSON, S. H. HUNTER and C. M. LEEVY, *Science* **139**, 110 (1963).
17. L. HUOT, G. W. CORRIVAULT and G. BOURBEAU, *Arch. int. Physiol. Biochim.* **68**, 577 (1960).
18. G. CORRIVAULT, L. HUOT and G. BOURBEAU, *Canad. J. Zool.* **40**, 31 (1962).
19. M. S. SHORB, C. SMITH, V. VASAITIS, P. G. LUND and W. POLLARD, *Proc. Soc. exp. Biol. (N.Y.)* **113**, 619 (1963).
20. S. FABRO, H. SCHUMACHER, R. L. SMITH, R. B. L. STAGG and R. T. WILLIAMS, *Biochem. Soc. Agenda Papers* (1963).
21. H. B. TAUSSIG, *Scientific American* **207**, 29 (1962).

Biochemical Pharmacology, 1964, Vol. 13, pp. 943–947. Pergamon Press Ltd., Printed in Great Britain.

Dissociation of reserpine-induced depression of spontaneous motor activity and release of brain serotonin in rats

(*Received* 14 *January* 1964; *accepted* 18 *February* 1964)

THE mechanism of the central depressant action of reserpine is not completely settled after almost ten years of investigation. A great body of evidence has been presented in favor of a concept that reserpine-induced release of serotonin (5-hydroxytryptamine, 5-HT) from its binding sites in the brain causes the tranquilizing action of the drug.[1–15] In opposition to this theory some arguments have been made in support of a role for the release of brain norepinephrine in the depressant action of reserpine.[16, 17]

The available evidence indicates that *if* an indirect mechanism (i.e. amine release) is operative, the most likely system is the uncoupling of serotonin storage in the brain.

Recent evidence[18, 19] has indicated that a role for release of brain serotonin in the depressant action of reserpine is untenable and perhaps may be an oversimplification of the action of reserpine.[20] Gal *et al.*,[18] working with rats made deficient in serotonin with a diet lacking in tryptophan supplemented by a single injection of reserpine, suggested that reserpine-induced sedation is not associated with release of serotonin from the brain. These workers were able to demonstrate a sedative action with reserpine in such serotonin-depleted animals. The effect of reserpine was indistinguisnable from that seen when the drug was administered to control animals on a tryptophan-sufficient diet. These findings have since been corroborated by Boullin[19] using a similar experimental design.

Shore *et al.*[8] indicated that the loss of pharmacologic signs from a single dose of reserpine was related to the recovery of brain serotonin levels. Studies in this laboratory have shown that recovery of spontaneous motor activity (SMA) in rats treated with reserpine does not correlate with the content of serotonin in the brains of these animals.[21] In our experiments it was noted that essentially 100% recovery of SMA occurred 24 hr after a single dose of reserpine in rats, followed by a period of hyperactivity extending for up to 3 days, with no concomitant recovery of the brain serotonin content. The present communication describes these experiments and the effect of a repeated dose of reserpine on the parameters under investigation.

Male Sprague-Dawley rats (av. wt., 165 g) were used. Reserpine (2 mg/kg, i.p.) was administered in the solvent described by Martindale.[22] Control animals in both groups received a proportional volume of the vehicle (1 ml/kg). Spontaneous motor activity was measured in a commercial six-photocell device* for a 10-min period at appropriate times after injection of the drug or placebo. Since environmental factors can influence the responsiveness of animals in this type of experiment[23] several precautions were taken accordingly. Each animal was used only once in the photocell unit to eliminate the possibility of environmental conditioning. Activity was measured only in the daylight hours and hence the drug and placebo were administered at times appropriate for such a measure. The photocell unit was operated under quiet laboratory conditions and away from direct overhead lighting. Food,

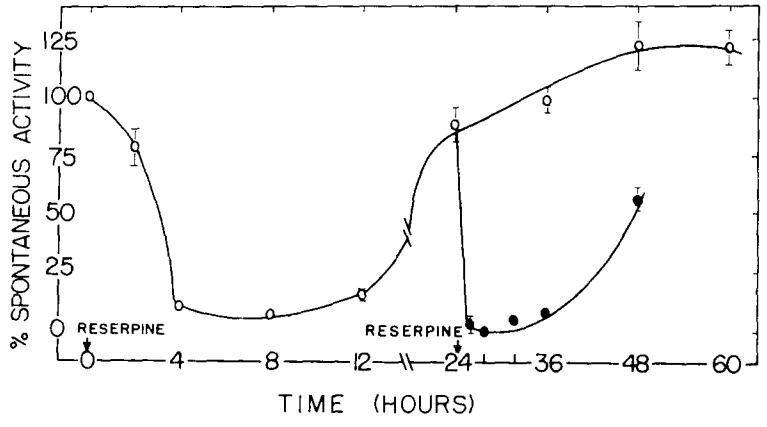

FIG. 1. The effect of sequential 24-hr doses of reserpine (2 mg/kg; arrows) on the spontaneous activity of the male rat. The circles represent 32 normal animals at zero time (100% activity = 38 cpm) and 16 animals at each other time period. The open circles represent the effect of a single dose of reserpine; closed circles represent the effect of a second dose of the drug. Vertical bars (where they are longer than the symbols representing activity) are standard errors of the mean.

but not water, was removed from the animals 12 hr prior to measurement of SMA. Preliminary experiments showed that differences in weight did not affect the observed SMA (Pearson's correlation coefficient: r = 0·086). Administration of the placebo showed a slight (10%) depression of SMA only in the 2-hr control group representing the animals given the first dose of reserpine. This effect

* Woodard Research Corp., Herndon, Va.

was not seen in the other (reserpine pretreated) control group. Brain serotonin was estimated fluorometrically* after extraction into butanol as described previously.[24]

The effect of 24-hr sequential administration of reserpine on the SMA of rats is illustrated in Fig. 1. It will be seen that a plateau of near immobility was reached 4 hr after the first dose of the drug and continued for about 8 hr, following to some extent the time course of depletion of brain serotonin (8 hr) (Fig. 2). However, it can be seen that SMA recovered in 24 hr and was not significantly different fromnormal ($0{\cdot}3 < P < 0{\cdot}4$).† A period of hyperactivity ($0{\cdot}02 < P < 0{\cdot}05$)† was seen 48 and 60 hr after administration of the drug. Examination of the content of serotonin in the brains of these animals revealed no apparent recovery of the amine levels (Fig. 2). When a second dose of reserpine (2 mg/kg) was administered to a group of animals treated with the drug 24 hr previously, an effect on SMA strikingly similar to that obtained with the first dose was seen (Fig. 1). The brains of these animals given a second dose of the drug showed no alteration in the already decreased level of serotonin (Fig. 2). This agrees with an early report by Shore *et al.*[2] who indicated that body depots of serotonin in the dog are maximally depleted with the first of two successive (26-hr) *intravenous* doses of reserpine (5 mg/kg), although the total depletion of brain serotonin obtained with a cumulative *intraperitoneal* dose of 4 mg/kg in the present experiments was to the

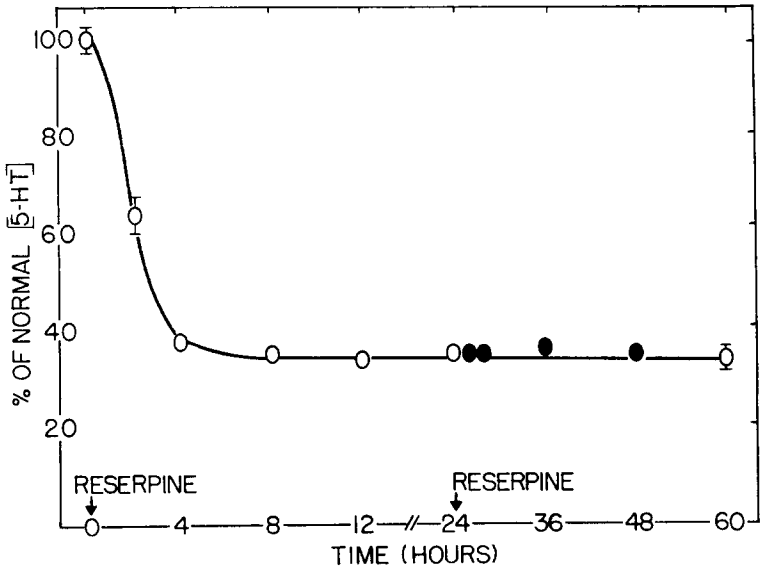

FIG. 2. The effect of sequential 24-hr doses of reserpine (2 mg/kg; arrows) on the serotonin (5-HT) content of the male rat brain. Open circles represent analyses of 16 whole brains at each time interval indicated after administration of a single dose of the drug. The closed circles represent the level of serotonin after a second equivalent dose of the drug and are the mean of 4 analyses at each time interval. Vertical bars (where they are longer than the symbols) are standard errors of the mean. Normal level of brain 5-HT was 0·47 μg/g fresh tissue (100%).

extent of only 63%.‡ These findings show that reserpine is able to depress SMA in the serotonin-depleted rat and suggest that release of brain serotonin is not necessarily coupled to this effect.

Correlation of drug-induced changes in SMA with altered central nervous activity necessarily assumes a single site of action of the drug—i.e. in the brain centers. Although such a relationship is

* Aminco-Bowman spectrophotofluorometer.

† Student's "t" test; two-tailed distribution.

‡ Westermann[25] has indicated that the serotonin-releasing potency of reserpine is to some extent dependent on the route of administration, intravenous being more effective than the intraperitoneal route for a given dose.

often assumed in drug-screening procedures, it remains to be proven that this assumption is valid. It must therefore be emphasized that the observed lack of causal relationship between brain serotonin release and SMA depression is to be taken at face value and that these data do not enable us to state unequivocally that the central depressant action of reserpine is not caused by brain serotonin release. Such an extrapolation from the present experiments would also be invalidated by the strong possibility that the sudden environmental change to which the animals are subjected when placed in the photocell unit is a stimulus capable of arousing them from the drug-induced stupor. It is therefore noteworthy to mention that the blepharospastic signs characteristic of reserpine were seen in these animals during the period of hyperactivity. Nevertheless, reserpine or perhaps a metabolite was able to depress the recovered SMA by an apparently direct action.

The precise significance of this finding must await the outcome of further experiments on this phenomenon. In this regard earlier experiments showed that reserpine potentiated the depressant action of methyldopa on rat SMA.[21] Under these conditions methyldopa pretreatment effectively blocks the serotonin-releasing action of reserpine.[26] Furthermore, that release of brain serotonin and SMA depression may not be causally related has been shown in experiments with guanethidine, which demonstrate that the drug releases serotonin (and norepinephrine[27]) from the rat brain[28] and, in mice, does not cause a depression of SMA.[27] Freedman and Giarman[29] have recently cited evidence that dissociation of amine levels and behavior is operant in various other testing procedures and in humans.[30]

Acknowledgements—This work was supported in part by grants from the Medical Research Council of Canada and Warner-Lambert Co. The reserpine used in these studies was generously supplied by Dr. C. W. Murphy of Ciba Ltd., Dorval, Que., Canada. The authors wish to extend their appreciation to Dr. N. J. Giarman for his assistance in preparation of this manuscript.

Faculty of Pharmacy,
University of Alberta,
Edmonton, Alta., Canada

RAYMOND D. MAGUS*
FRED W. KRAUSE
BERNARD E. RIEDEL

* Present address: Department of Pharmacology, Yale University School of Medicine, 333 Cedar St., New Haven, Conn., U.S.A., 06511.

REFERENCES

1. P. A. SHORE, S. L. SILVER and B. B. BRODIE, *Experientia (Basel)* **11**, 272 (1955).
2. P. A. SHORE, S. L. SILVER and B. B. BRODIE, *Science* **122**, 284 (1955).
3. B. B. BRODIE, A. PLETSCHER and P. A. SHORE, *Science* **122**, 968 (1955).
4. Q. DEMING, D. F. BOGDANSKI, S. UDENFRIEND, P. A. SHORE and B. B. BRODIE, *Fed. Proc.* **15**, 284 (1956).
5. A. PLETSCHER, P. A. SHORE and B. B. BRODIE, *J. Pharmacol. exp. Ther.* **116**, 84 (1956).
6. S. M. HESS, P. A. SHORE and B. B. BRODIE, *J. Pharmacol. exp. Ther*, **118**, 84 (1956).
7. B. B. BRODIE and P. A. SHORE, in *Hormones, Brain Function, and Behavior*, H. HOAGLAND, Ed., p. 161. Academic Press, New York (1957).
8. P. A. SHORE, A. PLETSCHER, E. G. TOMICH, A. CARLSSON, R. KUNTZMAN and B. B. BRODIE, *Ann. N.Y. Acad. Sci.* **66**, 609 (1957).
9. P. A. SHORE and B. B. BRODIE, in *Psychotropic Drugs*, S. GARATTINI and V. GHETTI, Eds., p. 423. Elsevier, Amsterdam (1957).
10. B. B. BRODIE, in *5-Hydroxytryptamine*, G. P. LEWIS, Ed., p. 64. Pergamon Press, New York (1958).
11. S. GARATTINI and L. VALZELLI, *Science* **128**, 1278 (1958).
12. B. B. BRODIE, K. F. FINGER, F. B. ORLANS, G. P. QUINN and F. SULSER, *J. Pharmacol. exp. Ther.* **129**, 250 (1960).
13. F. SULSER and B. B. BRODIE, *Science* **131**, 1440 (1960).
14. J. J. BURNS and P. A. SHORE, *Ann. Rev. Pharmacol.* **1**, 79 (1961).
15. B. B. BRODIE and E. COSTA, in *Monoamines et Systéme Nerveux Central*, J. DE AJURIAGUERRA, Ed., p. 13. Georg, Geneva (1962). (*Psychopharmacology Service Center Bulletin (Washington)* **2**, 1 (1962)).
16. N. T. KÄRKI and M. K. PAASONEN, *J. Neurochem.* **3**, 352 (1959).

17. A. PLETSCHER, H. BESENDORF and K. F. GEY, *Science* **129**, 844 (1959).
18. E. M. GAL, P. A. DREWES and C. A. BARRACLOUGH, *Proc. First Int. Pharmacol. Meeting*, W. D. M. PATON, Ed., vol. 8, p. 118. Pergamon Press, Oxford (1962).
19. D. J. BOULLIN, *Psychopharmacologia (Berl.)* **5**, 28 (1963).
20. N. J. GIARMAN, D. X. FREEDMAN and S. M. SCHANBERG, in *Progress in Brain Research*, H. E. HIMWICH and W. A. HIMWICH, Eds. Elsevier, Amsterdam (1964). In press.
21. R. D. MAGUS, The Role of Serotonin Release in the Effect of Reserpine on the Central Nervous System and the Thyroid Gland. M.Sc. Thesis, University of Alberta (1963).
22. W. MARTINDALE, *The Extra Pharmacopeia*, vol. 1, p. 745. Pharmaceutical Press, London (1958).
23. L. COOK and R. T. KELLEHER, *Ann. Rev. Pharmacol.* **3**, 205 (1963).
24. R. D. MAGUS, F. W. KRAUSE and B. E. RIEDEL, *Biochem. Pharmacol.* **13**, 115 (1964).
25. E. O. WESTERMANN, *Proc. First Int. Pharmacol. Meeting*, B. B. BRODIE and B. UVNAS, Eds., vol. 6, p. 205. Pergamon Press, Oxford (1962).
26. R. D. MAGUS, J. R. FERGUSON, F. W. KRAUSE and B. E. RIEDEL. In preparation (1964).
27. A. K. PFEIFER, E. S. VIZI and E. SATORY, *Biochem. Pharmacol.* **11**, 397 (1962).
28. R. FERRINI and A. GLÄSSER, *J. Pharm. Pharmacol.* **15**, 772 (1963).
29. D. X. FREEDMAN and N. J. GIARMAN, in *EEG and Behavior*, G. H. GLASER, Ed., p. 198. Basic Books, New York (1963).
30. D. X. FREEDMAN and A. J. BENTON, *New Engl. J. Med.* **264**, 529 (1961).