3. ———, PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 387.

4. ———, *Nature*, 1959, v184, 371.

5. Jacobson, L. O., Goldwasser, E., Field, W., Plzak, L., *ibid.*, 1957, v179, 633.

6. Gordon, A. S., Piliero, S. J., Medici, P. T., Siegel, C. D., Tannenbaum, M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 598.

7. Piliero, S. J., Medici, P. T., Gordon, A. S., unpublished observations.

8. Rambach, W. A., Alt, H. L., Cooper, J. A. D., *Blood*, 1957, v12, 1101.

9. Fried, W., Plzak, L., Jacobson, L. O., Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.

10. Crosby, W. H., Munn, J. I., Furth, F. W., *U. S. Armed Forces Med. J.*, 1954, v5, 693.

11. Peters, T., Giovanniello, T. J., Apt, L., Ross, J. F., *J. Lab. Clin. Med.*, 1956, v48, 280.

12. Hodgson, G., Eskuche, I., Yudilevich, D., Hernandez, P., Tcha, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 826.

Received September 25, 1959. P.S.E.B.M., 1960, v103.

Further Studies on Effects of Tranquilizing Drugs on Mammary Involution in the Rat.* (25436)

G. K. BENSON (Introduced by R. Gaunt)

Nat. Inst. for Research in Dairying, Shinfield, Reading, Berkshire, England

Oxytocin administration inhibits mammary involution in rats after removal of the litter at fourth day of lactation, and it was suggested that oxytocin elicits prolactin release under these conditions(1,2). Later, following reports of its lactogenic effects in several species (3-7), it was shown that reserpine resembles oxytocin in showing a striking effect in retarding mammary involution in the rat(8). It was of interest to study effects on mammary involution of other tranquilizing drugs, and the present paper describes experiments carried out with chlorpromazine, reported to cause galactorrhoea in women(9,10), and syrosingopine (Singoserp), an analogue of reserpine possessing hypotensive properties similar to those of reserpine, but with a considerably reduced central sedative action(11,25). To throw light on the mechanism of action of reserpine, the effects of hypophysectomy and of adrenalectomy on reserpine-induced parenchyma maintenance have been investigated. Further, in view of claims that the pharmacological action of reserpine may be due to altering the serotonin-binding capacity of brain cells(12,13), the effects of serotonin on mammary involution have been studied; and since reserpine has been reported to release histamine from blood platelets(14), the effect of this substance on mammary involution was also investigated.

Materials and methods. Hooded Norway rats were used, 2-3 months old, undergoing first lactation. Stock colony diet(15) was fed *ad lib.*; daily food and water consumption was recorded. After parturition all litters were reduced to 9 young on first day of lactation, and to 8 on second day. Litters were weighed daily to check lactational performance of the mother, and mothers were also weighed daily as in previous experiments. Litters were removed on 4th day of lactation when treatment began and when operations, if any, were carried out. Substances administered during treatments were a) Reserpine (Serpasil†), b) Syrosingopine (Singoserp‡), c) Chlorpromazine (Largactil, May & Baker Ltd.), d) 9 α fluorocortisol (FC) supplied as acetate by E. R. Squibb & Sons, N. J.; this was administered in suspension in 0.5 ml carboxy-methylcellulose (CMC) solution, e) Serotonin, supplied as creatinine sulphate by May & Baker Ltd., f) Histamine, supplied as acid phosphate by British Drug Houses Ltd. Full details of experimental groups, dosages,

* The author is indebted to Ciba Inc., N. J., through courtesy of Dr. R. Gaunt, for grant to cover cost of publication.

† Kindly supplied by Mr. C. W. S. Taylor, Ciba Labs., Horsham, Sussex.

‡ Kindly supplied by Dr. R. Gaunt, Ciba Inc., Summit, N. J.

etc. are included in Table I. At end of treatment period, which except where otherwise stated lasted 9 days, mammary tissue was studied histologically, alveolar maintenance estimated by determination of percentage parenchyma(2). Various other organs were removed, weighed, and preserved for histology. Where appropriate, brain and pituitary region, suitably trimmed, was sectioned after decalcification, and checked for pituitary remnants.

Results. Reserpine and Syrosingopine. In this experiment both drugs were made in citric acid-glycol vehicle, as follows:—citric acid anhydrous 0.25%; benzyl alcohol 2%; polyethylene glycol 10%; distilled water q.s. 100%. Stock solutions were diluted to enable necessary quantities to be injected in 0.1 ml of the vehicle.

Mammary gland involution was retarded to a similar degree by all treatments except lower dose of syrosingopine, where maintenance was less well-marked (Table I). The control group showed some maintenance, quantitatively similar to that previously found for intact animals receiving ACTH(16,17), and may therefore be due to a non-specific stress effect of the vehicle. As in previous experiments(8) a significant decrease in uterine weight was observed as a result of reserpine treatment, though not after syrosingopine treatment (Table I), and histological examination indicated that in the control group, weaning was followed by return to normal ovarian cyclic activity. This finding was borne out by absence of mucification in the control vaginae; the vaginae of 9 of reserpine-treated animals and 7 of syrosingopine-treated animals were mucified. As before(8) thymus weights were depressed by reserpine at both dose levels, particularly the higher, and also, by syrosingopine at higher dosage. Adrenal weights were increased by higher dose of reserpine but surprisingly a slight decrease in mean adrenal weight was observed in the lower dosage group. Syrosingopine had no effect.

Depression of body weight previously noted after administration of 100 μg reserpine daily (8) was again observed, but all other treatments resulted in significant gains in body weight. The higher dose of reserpine was obviously in the toxic range, since animals so treated exhibited severe diarrhoea and deterioration in coat condition. Syrosingopine did not produce effects of this nature.

Chlorpromazine. Dosages were higher than those reported by Sulman & Winnik(18) to depress cyclic ovarian activity in rats. However, chlorpromazine was much less effective than reserpine in retarding mammary involution, though at the higher dose level it was somewhat more potent than the lower dose level of syrosingopine (Table I). Ovaries of the treated groups were significantly lighter than those of control group, but no significant differences in uterine weight were apparent. Histological examination showed evidence of ovarian cyclic activity in some treated animals, as in all control animals. Treated animals not showing resumption of ovarian cyclic activity had mucified vaginae. Ovaries of the control group contained many very large corpora lutea. Little influence on adrenal and thymus weights was apparent, though the lower dose level appeared to have depressed adrenal weight below the control value. The depressive effect on body weight after reserpine treatment (Table I) was not a feature of chlorpromazine administration.

Reserpine in hypophysectomized animals. All hypophysectomized and sham operated animals lost weight rapidly during treatment period. By the 6th day after operation, 3 hypophysectomized animals were in a parlous condition, and were therefore killed, 3 controls were killed on equivalent day to match them. The other 2 hypophysectomized animals survived to the 13th day after parturition, when they and remaining controls were killed. No residual fragments of anterior pituitary were detected in hypophysectomized animals in sections of brain.

Involution of mammary tissue in hypophysectomized animals was quite clearly unaffected by reserpine. Even in rats killed on 10th day of lactation, marked involution had occurred (Table I). In contrast, administration of reserpine to the control group again sustained integrity of the parenchyma to a marked degree. In these sham-operated animals percentage parenchyma values indicated no fall-off in degree of maintenance between 10th and 13th day of lactation. In hypophy-

TABLE I. Effects of Various Treatments on Percentage Parenchyma, Body Weight, Organ Weights and Food and Water Consumption in Lactating Rats Following Removal of Litters on 4th Day of Lactation, When Treatments Commenced.

Treatment	Daily dosage and route of admin.§	Day of lactation on which animals were killed	No. of animals	Mean % parenchyma	Mean % change in body wt over treatment period	Mean daily food consumption over treatment period (g)	Mean daily water intake over treatment period (ml)	Mean wt (mg)/100 g body wt				
								Uterus	Ovaries	Thyroids	Thymus	Adrenals
Controls, vehicle only	0.1 ml s.c.	13	5	30.5 ± 1.6	− 2.1 ± .6	10.6 ± .5	19.3 ± 1.4	195.7 ± 15.0	30.0 ± 2.7	14.4 ± 1.7	127.2 ± 9.7	32.5 ± 1.8
Reserpine	100 μg "	13	5	53.6 ± 6.0†	−11.6 ± 2.4†	10.6 ± .7	20.0 ± 1.1	109.8 ± 9.0‡	31.4 ± 2.4	12.4 ± .9	52.5 ± 2.1‡	41.2 ± 2.2†
"	50 μg "	13	5	49.7 ± 4.3‡	+ 7.2 ± .9‡	16.6 ± 2.7*	27.9 ± 1.2‡	129.0 ± 15.0†	26.1 ± 1.5	13.4 ± 1.6	97.6 ± 5.8*	27.1 ± 1.7*
Syrosingopine	100 μg "	13	5	57.6 ± 2.6‡	+ 7.6 ± 4.0*	12.4 ± .9	21.4 ± 3.3	160.8 ± 27.1	23.6 ± 1.1*	12.1 ± 1.6	85.9 ± 12.2*	30.8 ± 1.9
"	50 μg "	13	5	33.3 ± 7.5	+ 9.6 ± 2.8‡	13.9 ± .6†	27.8 ± .8‡	186.9 ± 19.8	28.2 ± 1.2	12.2 ± .3	106.4 ± 11.6	28.2 ± .6
Saline	.5 ml "	13	6	24.9 ± 5.7	− 1.1 ± .4	13.5 ± .5	21.3 ± 2.2	190.9 ± 15.4	47.7 ± 4.0	13.9 ± .7	133.5 ± 7.2	34.1 ± 1.7
Chlorpromazine	2.5 mg "	13	7	25.5 ± 5.1	− 0.8 ± .4	16.0 ± .7‡	19.9 ± .9	182.2 ± 9.9	31.0 ± 1.1‡	13.8 ± .4	139.3 ± 7.2	28.3 ± 1.9*
"	5.0 mg "	13	7	41.8 ± 7.1*	− 3.6 ± .6‡	15.4 ± .7†	19.6 ± 1.4	166.3 ± 18.3	33.0 ± 1.7†	13.7 ± .9	136.0 ± 15.4	30.8 ± 1.6
Hypophysectomy + reserpine	100 μg "	10	3	24.8 ± 4.3†	−19.1 ± .9*	7.5 ± .7	39.2 ± 6.6	84.3 ± 6.2†	31.2 ± 2.5	10.9 ± .4	83.0 ± 3.1	24.1 ± 1.6†
		13	2	21.6 / 25.6 } 23.6	−24.5 / −26.9 } −25.7	8.9 / 8.8 } 8.8	44.8 / 37.1 } 40.9	82.6 / 62.7 } 72.6	23.4 / 26.7 } 25.0	9.4 / 7.1 } 8.2	116.4 / 74.7 } 95.5	20.4 / 16.9 } 18.6
Sham-operation + reserpine	100 μg "	10	3	55.7 ± 7.7	−10.1 ± 3.1	12.3 ± 2.6	26.9 ± 2.5	110.4 ± 4.9	27.9 ± 1.7	11.0 ± .8	77.6 ± 18.7	33.4 ± 2.0
		13	3	70.3 ± 8.9	−26.5 ± 1.1	8.7 ± 1.1	16.7 ± 2.0	109.8 ± 2.6	24.9 ± 1.9	10.0 ± .6	56.4 ± 9.9	37.9 ± 3.1
Adrx. + saline	.5 ml "	9	4	37.8 ± 5.7	−15.0 ± 2.7	9.3 ± 1.2	36.0 ± 4.0	175.1 ± 40.4	33.3 ± 2.8	12.1 ± .9	115.4 ± 20.3	
		13	2	12.2 / 19.5 } 15.8	−12.3 / −15.8 } −14.0	8.1 / 8.7 } 8.4	33.7 / 39.7 } 36.7	167.9 / 130.2 } 149.0	38.9 / 37.6 } 38.2	11.3 / 11.4 } 11.3	128.5 / 210.2 } 169.3	
Adrx. + reserpine	100 μg "	9	4	50.7 ± 2.2*	− 7.0 ± 2.4*	10.4 ± 1.1	20.0 ± 1.5†	104.9 ± 10.1	30.1 ± 3.5	12.7 ± 1.3	96.4 ± 25.5	
		13	2	29.5 / 34.3 } 31.9	−25.8 / −24.1 } −24.9	9.5 / 10.1 } 9.8	14.8 / 18.8 } 16.8	217.9 / 115.7 } 166.8	37.9 / 34.1 } 36.0	9.7 / 14.8 } 12.2	96.4 / 132.7 } 114.5	
Adrx. + CMC sol.	.5 ml "	13	6	20.0 ± 3.3	−15.2 ± 1.2	8.3 ± .4	26.7 ± .4	195.2 ± 13.1	39.3 ± 1.2	15.4 ± .8	162.1 ± 6.9	
Adrx. + reserpine + FC	100 μg " 100 μg "	13	5	65.3 ± 5.3‡	−29.7 ± .7‡	6.7 ± .2*	10.3 ± .6‡	110.2 ± 4.8‡	33.8 ± 1.6†	14.8 ± .6	52.8 ± 3.7‡	
Saline 3×	0.5 ml i.p.	13	5	25.7 ± 4.4	+ 2.3 ± 3.0	12.1 ± .5	25.7 ± 1.0	226.5 ± 26.7	32.3 ± 2.6	14.1 ± 2.2	120.1 ± 13.8	28.6 ± 2.5
Serotonin "	250 μg "	13	5	24.8 ± 1.9	− 0.5 ± 4.1	12.6 ± 1.0	25.5 ± 1.7	218.1 ± 25.6	36.2 ± 1.4	14.6 ± 1.0	105.6 ± 7.7	34.5 ± 1.4*
Histamine "	1.0 mg "	13	5	31.7 ± 7.3	+ 4.7 ± 2.5	11.3 ± .7	22.5 ± 2.0	198.1 ± 31.5	30.6 ± 2.6	12.8 ± 1.0	103.6 ± 15.0	27.0 ± 1.2

Values of P for differences between treatment and control means: * $.02 < P < .05$; † $.001 < P < .01$; ‡ $P < .001$.

§ s.c. = subcutaneous; i.p. = intraperitoneal.

sectomized animals, reduced organ weights presumably reflected absence of trophic hormones. In controls, reserpine greatly depressed thymus weights, and some elevation of adrenal weight was apparent. Depressed ovarian and uterine weights in these sham-operated animals expressed lack of cyclic function, borne out by atrophic appearance of these organs when examined histologically. Vaginae of several sham-operated groups were mucified. Over the 6-day period, body weight of hypophysectomized animals decreased at significantly higher rate than did controls, but at 13 days, loss of body weight in both groups was about equal, and similar to that previously reported after reserpine administration(8). Food consumption did not vary significantly over the 6- or 9-day period and was low throughout both groups. Water consumption was generally higher in hypophysectomized animals.

Reserpine in adrenalectomized animals. Dosage of FC in this experiment has previously been shown to give a considerable degree of lactation maintenance in adrenalectomized rats(19). Combined effects of the operation and reserpine treatment resulted in rapid loss of condition in a number of the group receiving reserpine alone, confirming findings of Gaunt *et al.*(20) and 4 of these animals were either dead at 9th day of lactation or were killed because of their condition. Four of control saline-injected group were therefore killed on 9th day of lactation, thus leaving 2 animals in each of these 2 groups, all of which survived to 13th day (Table I). All animals receiving corticoid in addition to reserpine and controls receiving CMC solution survived to 13th day.

Over 5-day and 9-day treatment reserpine alone had some effect in maintaining the mammary gland in absence of adrenals (Table I), though degree of maintenance was by no means as marked as in intact animals already described(8). However, when FC was administered in addition to reserpine a very marked degree of maintenance was achieved (Table I). Little significant information can be derived from organ weights which differed widely within groups. Histological examination of uteri, ovaries and vaginae revealed little sign of cyclic activity within reserpine treated groups, vaginae generally being mucified. In control groups, however, some uteri and ovaries showed clear evidence of a return to normal ovarian cycles following weaning.

Serotonin. The dosage used (250 μg 3 $\times$ daily) was referred to the 5 H-T base, and each dose was contained in 0.5 ml 0.9% saline. No marked effects were observed with respect to maintenance of mammary parenchyma, either qualitatively or quantitatively (Table I). There were no significant effects on organ weights, except for an increase in adrenal weight after serotonin administration. Body weights and food and water intakes were not affected.

Histamine. Each dose (1 mg 3 $\times$ daily) was contained in 0.5 ml saline. No effects of mammary involution were observed (Table I), and there were no effects on organ weights, body weight, or food and water intakes.

Discussion. That reserpine retards mammary involution in rats(8) has been amply confirmed in this experiment. This finding, together with histological studies on uteri and vaginae, supports the view, first suggested by lactogenic responses(3-7, 21), that reserpine released prolactin. Syrosingopine was less effective in retarding mammary involution than was reserpine. In view of the reduced central sedative action of this analogue the finding lends support to the theory that prolactin release by these drugs is achieved by suppression of a center in the hypothalamus which normally inhibits prolactin release.

It is interesting that the retarding action of chlorpromazine on mammary involution was less than one-fiftieth of that of reserpine on a weight for weight basis, although dosages used were larger than those previously reported to depress anterior-pituitary function in rats (18).

The possibility that the effects on mammary involution are due to a direct effect of reserpine on the mammary gland receives no support from the experiment with hypophysectomized animals, retardation of mammary involution being obtained only in sham-operated rats. This finding, together with supplementary observations on uteri and vaginae,

supports the inference that the pituitary gland is involved, as in experiments with oxytocin (2). However, whether or not this is due to a "permissive action" of anterior-pituitary hormones cannot be determined on the basis of this work.

Unlike hypophysectomy, adrenalectomy does not totally impair the effect of reserpine on retardation of mammary involution, though it markedly diminishes it, the full reserpine effect being restored by corticoid administration. Reserpine appears to stimulate ACTH release, indicated here by changes in thymus and adrenal weight, and also reported elsewhere(20,22). It might be thought that its effects in these experiments could be attributed to ACTH release in view of the established importance of this hormone in lactation(23). However, both ACTH and adrenal corticoids, including FC, have previously been shown in similar experiments to possess only a relatively slight effect in retarding mammary involution in intact animals(16, 17,24). It therefore seems unlikely that the whole of the reserpine effect can be attributed to stress-induced release of ACTH. The comparative failure of reserpine to retard mammary involution in adrenalectomized animals, unless corticoids are administered, may be explained in terms similar to those suggested in connection with oxytocin-induced release of prolactin in adrenalectomized animals(17), *i.e.*, in absence of adequate levels of adrenal steroids, secretion of prolactin by the anterior lobe is reduced.

The general lack of effect of syrosingopine on adrenal and thymus weights in these experiments confirms the findings of Chart, Renzi & Gaunt (personal communication) who also observed no adrenal weight increase following chronic administration of this substance, and is in harmony with their observations that reserpine is 5 or more times as potent as syrosingopine in causing adrenal ascorbic acid depletion. The results reported here do not confirm the findings of Meites *et al.*(26) that serotonin retarded mammary involution in the rat, and they lend no support to the possibility that action of reserpine in retarding mammary involution may be mediated by serotonin or histamine. These findings conform with those of Sawyer(5) who observed no mammary activation following injections of serotonin into the cerebral ventricles of suitably prepared rabbits. Reserpine was, however, effective in stimulating mammary growth and secretion when administered in this way.

In general, our findings are in keeping with the suggestion discussed elsewhere(27), that the effects of reserpine on the mammary gland (both lactogenic and involution-retarding effects) are due to removal of a hypothalamic inhibition of prolactin release.

Summary. 1. In rats, following weaning of litters on 4th day of lactation, administration of syrosingopine (50 μg daily) for 9 days had much less effect than the same dose of reserpine on retardation of mammary involution, but was similar in effect at a higher dose level (100 μg daily). 2. Chlorpromazine (2.5 mg and 5 mg daily) also had a lesser retarding effect on mammary involution than reserpine. 3. No noticeable effects on mammary involution were observed after administration of serotonin (250 μg 3 $\times$ daily) or histamine (1 mg 3 $\times$ daily). 4. Reserpine (100 μg daily) had no effect on mammary involution in hypophysectomized animals. 5. In adrenalectomized animals some involution-retarding effect of reserpine (100 μg daily) was still apparent and was greatly enhanced by addition of 9α fluorocortisol. 6. Reserpine, at higher dose level, depressed food consumption and body weight, and retarded return of normal cyclic function after weaning as did the higher dose of syrosingopine to a lesser degree. Marked effects on thymus and adrenal weights after reserpine administration indicated that ACTH secretion is stimulated by the drug, but neither syrosingopine nor chlorpromazine treatment resulted in any apparent stimulation of ACTH release. 7. The significance of these findings in relation to a possible hypothalamic inhibition of prolactin release is discussed.

1. Benson, G. K., Folley, S. J., *Nature,* Lond., 1956, v177, 700.

2. ———, *J. Endo.,* 1957, v16, 189.

3. Kehl, R., Audibert, A., Gage, C., Amarger, J., *C. R. Soc. Biol.* Paris, 1956, v150, 981.

4. Meites, J., PROC. SOC. EXP. BIOL. AND MED.,

1957, v96, 728.

5. Sawyer, C. H., *Anat. Rec.*, 1957, v127, 362.

6. Tuchmann-Duplessis, H., Mercier-Parot, L., *C. R. Soc. Biol.*, Paris, 1957, v151, 656.

7. Desclin, L., *ibid.*, 1957, v151, 1774.

8. Benson, G. K., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 550.

9. Sulman, F. G., Winnik, H. Z., *Lancet*, 1956, v1, 161.

10. Marshall, W. K., Lieberman, D. M., *ibid.*, 1956, v1, 162.

11. Plummer, A. J., Barrett, W. E., Maxwell, R. A., Finocchio, D., Lucas, R. A., Earl, A. E., *Arch. Inter. Pharmacol. et Therap.* (Ghent), 1959, v119, 245.

12. Shore, P. A., Silver, S. L., Brodie, B. B., *Science*, 1955, v122, 284.

13. Brodie, B. B., Pletscher, A. A., Shore, P. A., *ibid.*, 1955, v122, 968.

14. Waalkes, T. P., Weissbach, H., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 394.

15. McKinlay, H., *J. Anim. Tech. Assn.*, 1951, v2, 2.

16. Benson, G. K., *J. Endo.*, 1958, v17, xvii.

17. ———, *ibid.*, in press.

18. Sulman, F. G., Winnik, H. B., *Nature*, Lond., 1956, v178, 365.

19. Cowie, A. T., Tindal, J. S., *Endocrinology*, 1955, v56, 612.

20. Gaunt, R., Renzi, A. A., Antonchak, N., Miller, G. J., Gilman, M., *Ann. N. Y. Acad. Sci.*, 1954, v59, 22.

21. Tindal, J. S., *J. Endo.*, 1959, v18, xxxvi.

22. Harwood, C. T., Mason, J. W., *Endocrinology*, 1957, v60, 239.

23. Folley, S. J., In *Marshall's Physiology of Reproduction*, 3rd Ed. Chap. 6, p568. Ed. A. S. Parkes, London, Longmans, Green & Co.

24. Johnson, R. M., Meites, J., *Endocrinology*, 1958, v63, 290.

25. Gaunt, R., Gross, F., Renzi, A. A., Chart, J. J., in *First Hahnemann Symposium on Hypertensive Disease*, Ed. J. Moyer, 1959, p219, W. B. Saunders Co., Philadelphia.

26. Meites, J., Nicoll, C. S., Talwalker, P. K., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 563.

27. Benson, G. K., Cowie, A. T., Folley, S. J., Tindal, J. S., In *Endocrinology of Reproduction*, p457, Ed. C. W. Lloyd, Academic Press.

Received September 28, 1959. P.S.E.B.M., 1960, v103.

Indirect Evidence of Synthesis of Norepinephrine from 3-Hydroxy-Tyramine-1-C^{14} *in vivo*.* (25437)

M. GOLDSTEIN, A. J. FRIEDHOFF, C. SIMMONS AND N. N. PROCHOROFF
(Introduced by N. Nelson)
Dept. of Psychiatry and Neurology, Psychopharmacology Research Unit, N. Y. U. College of Medicine

Considerable evidence is available that 3-hydroxytyramine is, *in vitro*, a precursor of norepinephrine(1,2,3). However, little evidence is available about the formation of norepinephrine from 3-hydroxytyramine in the intact animal. The low rate at which 3-hydroxytyramine is utilized for conversion into norepinephrine, as well as the high rate of metabolism of 3-hydroxytyramine and norepinephrine *in vivo* makes it difficult to demonstrate this reaction directly in the intact animal. Since Axelrod(4) has shown that the main metabolite of norepinephrine is 3-methoxy-norepinephrine, it occurred to us that conversion of 3-hydroxytyramine to norepinephrine could be demonstrated *in vivo* indirectly by isolation of the end product of biosynthesis, namely, 3-methoxynorepinephrine. Also, since various kinds of stresses have been reported to increase excretion of norepinephrine, it seemed likely that excretion of 3-methoxynorepinephrine would be affected under these conditions.

Materials. Iproniazid phosphate was kindly supplied by Hoffmann-La Roche; 3-hydroxytyramine-1-C^{14} was obtained from New England Nuclear Corp.; 3-methoxytyramine-1-C^{14} was isolated from urine of rats treated with iproniazid; 3-methoxynorepinephrine, 3-methoxyepinephrine and 3-methoxytyramine were kindly supplied by Sterling-Winthrop

* This investigation supported by grant from Nat. Inst. Health, and presented in part to Fed. Am. Soc. for Exp. Biol., Atlantic City, April, 1959.