pressures of 40-60 mm Hg is a supramaximal stimulus for the adrenal medulla and the secretion of epinephrine is not markedly affected by the type of anesthetic.

Summary. Peripheral blood catecholamines were studied in dogs during hemorrhagic shock. When determined by the fluorimetric procedure, with values corrected for recovery, epinephrine increased from control levels of 0.4 ± 0.2 μg/l to 27.6 ±8.7 μg/l; norepinephrine increased from 3.4 ± 1.6 μg/l to 11.6 ± 3.4 μg/l. Thus during hemorrhagic shock in dogs the catecholamines found in the peripheral arterial blood are largely epinephrine (70%); and to a lesser extent norepinephrine (30%). Significant quantities of epinephrine-norepinephrine usually appear in the blood when the mean arterial pressure has dropped to about 80 mm Hg. As the pressure is lowered further the circulating catecholamines increase very rapidly. This increase is largely due to epinephrine and to a lesser extent to norepinephrine. Simultaneous estimation showed epinephrine concentration to be greatest in the inferior vena cava followed by the femoral artery and lowest in the femoral vein. These experiments indicate most of the catecholamines in the circulation during hemorrhagic shock in dogs are released from the adrenal medulla and are rapidly destroyed by the tissues.

1. Watts, D. T., *Am. J. Physiol.*, 1956, v184, 271.
2. Manger, W. M., Bollman, J. L., Maher, F. T., Berkson, J., *ibid.*, 1957, v190, 310.
3. Millar, R. A., Keener, E. B., Benfey, B. G., *Brit. J. Pharmacol.*, 1959, v14, 9.
4. Walton, R. P., Richardson, J. A., Walton, R. P., Jr., Thompson, W. L., *Am. J. Physiol.*, 1959, v197, 223.
5. Rosenberg, J. C., Lillehei, R. C., Longerbeam, J., Zimmermann, B., *Ann. Surg.*, 1961, v154, 611.
6. Gaddum, J. H., Lembeck, F., *Brit. J. Pharmacol.*, 1949, v4, 401.
7. Franko, B. V., Bragg, A. D., Watts, D. T., *Arch. int. Pharmacodyn.*, 1957, v111, 123.
8. Vendsalu, A., *Acta physiol. scand.*, 1960, v49, 173.
9. Goldfien, A., Zileli, S., Goodman, D., Thorn, G. W., *J. Clin. Endocrin.*, 1961, v21, 281.
10. Euler, U. S. von, Lishajko, F., *Acta physiol. scand.*, 1959, v45, 122.
11. Freeman, N. E., Freedman, H., Miller, C. C., *Am. J. Physiol.*, 1941, v131, 545.
12. Poole, T. R., Watts, D. T., *ibid.*, 1959, v196, 145.
13. Armstrong, C. G., Jr., Porter, H., Jr., Langston, J. B., *ibid.*, 1961, v201, 192.

Received September 26, 1963. P.S.E.B.M., 1964, v115.

Inhibition of Mammary Secretion in Rats by Iproniazid.*† (28982)

HIDEO MIZUNO, P. K. TALWALKER AND JOSEPH MEITES
Departments of Physiology and Pharmacology, Michigan State University, East Lansing

Monoamine oxidase (MAO) inhibitors are reported to produce alterations in the metabolism of serotonin and catecholamines in the central nervous system (CNS), and hence may influence CNS function(1). Since the CNS, particularly the hypothalamus, is involved in regulation of pituitary secretions (2), these MAO inhibitors may bring about changes in pituitary function. Thus it has been reported that iproniazid and certain other MAO inhibitors retarded sexual maturity in immature female mice(3) and rats (4), prevented development of decidual tissue in mice(5), interrupted pregnancy in mice and rabbits when administered before midpregnancy(6,7), and reduced fertility in mice and rats when given chronically throughout the periods of mating(8). Only one laboratory reported that iproniazid had no effect on implantation and litter size in mice(9). The foregoing suggests that iproniazid may inhibit gonadotropin secretion through the CNS(3-9).

Many agents, including electrical stimula-

* Published with approval of the director of Mich. Agric. Exp. Station as Journal Article No. 3246.
† This investigation was supported in part by grants from Nat. Inst. Health.

TABLE I. Inhibition of Mammary Secretory Response by Iproniazid in Estrogen-Primed Rats.

Group	Treatment*	No. of rats	Avg body wt (g) Initial	Avg body wt (g) Final	Avg secretion rating†
1	Saline, 0.1 ml	10	231 ± 6‡	238 ± 7	.05
2	Iproniazid, 100 mg/kg BW	10	229 ± 3	220 ± 3	.03
3	Electrical stimulation of uterine cervix	19	221 ± 4	227 ± 4	1.00
4	*Idem* + iproniazid, 50 mg/kg BW	10	216 ± 8	199 ± 4	.55
5	*Idem* + iproniazid, 100 mg/kg BW	17	221 ± 4	208 ± 3	.41
6	Cold exposure, 4°C	10	210 ± 2	201 ± 2	.30
7	*Idem* + iproniazid, 100 mg/kg BW	10	196 ± 3	183 ± 6	.13
8	Reserpine, 50 μg/rat	10	205 ± 4	183 ± 5	.78
9	*Idem* + iproniazid, 50 mg/kg BW	10	213 ± 6	207 ± 6	.93
10	*Idem* + iproniazid, 100 mg/kg BW	10	212 ± 3	196 ± 3	.95

* All rats were injected daily with 10 μg estradiol for 10 days, followed by above treatments for 5 days.

† Group comparisons by nonparametric Mann-Whitney U test(12). Group 3 vs 4: $P \simeq .05$; 3 vs 5: $P < .01$; 6 vs 7: $P > .1$; 8 vs 9: $P > .1$; 8 vs 10: $P > .1$.

‡ Mean ± standard error.

tion of the uterine cervix, cold stress and injections of reserpine, elicited a pseudopregnancy response in rats, indicating prolactin release. The same stimuli also induced mammary secretion in estrogen-primed rats, which was attributed to release of prolactin and ACTH(10,11). The present experiments in rats were carried out to see whether iproniazid could modify the mammary secretory response to different stimuli.

Methods. Virgin female rats of the Carworth CFN strain, 3-4 months old, were divided into groups of 10 or more each and injected subcutaneously daily with 10 μg estradiol in 0.1 ml corn oil for 10 days(10). For the following 5 days, the rats were treated as shown in Table I. Iproniazid was injected intraperitoneally in a 0.2 ml volume on alternate days beginning on the evening of the last day of estrogen pre-treatment, and reserpine was administered daily by subcutaneous injection. Electrical stimulation was conveyed to the uterine cervix once daily by platinum electrodes insulated except for the tips. A low current was supplied by an electrodyne stimulator set at a frequency of 20 cycles/second for 30 seconds. All rats were killed on the day following the last day of treatment, and the right inguinal mammary glands were removed and prepared for histological examinations by standard techniques. The ovaries, uterus, adrenals, and thymus were also removed and weighed. The mammary glands were rated for intensity of secretion from 0 to 3, in units of 0.25(10). The differences in mammary responses between groups were tested statistically by the nonparametric Mann-Whitney U test(12).

The effects of iproniazid on lactation were studied in postpartum rats. On the 4th day of lactation the litters were reduced to 8 pups each, and the mother rats were divided into 3 groups and treated on alternate days until the 18th day of lactation. Body weights of litters and mothers were recorded daily to assess the lactational performance of the mother.

Results. When saline was injected for 5 days following estrogen treatment, the mammary glands regressed from a lobulo-alveolar to almost a bare duct system in all cases (Table I). The mammary glands from rats given iproniazid alone were similar to the controls and showed no secretion. Mammary secretion in response to electrical stimulation of the uterine cervix appeared to be reduced by injection of 50 mg iproniazid/kg BW ($P \simeq 0.05$), and was decreased by 100 mg/kg BW ($P < 0.01$). Iproniazid did not significantly modify the mammary secretory responses to cold exposure or reserpine.

In rats given iproniazid alone, adrenal weights were higher ($P \simeq 0.05$) and thymus weights were lower ($P < 0.05$) than in the controls. This suggests that chronic administration of iproniazid may stimulate ACTH secretion. Iproniazid at a dose level of 50 or 100 mg/kg BW blocked the increase in

TABLE II. Effect of Iproniazid on Postpartum Lactation.

Treatment	No. of rats	Litter* wt (g) Day of lactation 4th	Litter* wt (g) Day of lactation 18th	Litter* wt (g) Avg gain	Litter* wt (g) % wt gain	Body wt of mothers (g) Day of lactation 4th	Body wt of mothers (g) Day of lactation 18th	Body wt of mothers (g) % wt gain
Control, saline	8	70 ± 2	239 ± 11	168 ± 9	239 ± 10	271 ± 9‡	284 ± 7	+5.1 ± 2.0
Iproniazid, 50 mg/kg BW	8	67 ± 3	190 ± 7‡	124 ± 6‡	186 ± 13‡	268 ± 8	269 ± 10	+ .3 ± 2.0
Iproniazid, 100 mg/kg BW	6	75 ± 2	154 ± 8‡	79 ± 5‡	106 ± 4‡	275 ± 5	257 ± 5‡	−6.5 ± 1.7‡

* 8 pups per litter. † Mean ± standard error.
‡ Significantly different from control at level of $P < .01$.

adrenal weight induced by reserpine ($P < 0.01$; $P < 0.05$), indicating inhibition of ACTH release. No significant differences were observed in ovarian and uterine weights among the various groups.

There was no significant difference in the average body weights of litters or mothers on the 4th day of lactation among the 3 groups (Table II). During 15 days of treatment the gains in body weights of the litters were significantly lower in the iproniazid injected groups than in the controls ($P < 0.01$). The per cent gain in body weight of the mothers was only slightly depressed when 100 mg iproniazid/kg BW was injected ($P < 0.01$), but was not influenced by 50 mg/kg BW.

Discussion. The mammary secretory response following electrical stimulation of the uterine cervix was significantly depressed by iproniazid treatment, suggesting inhibition of prolactin and/or ACTH secretion in response to this stimulus. The drug did not significantly modify the mammary response to cold stress or reserpine administration. As judged by adrenal and thymus weights, iproniazid decreased ACTH release in response to reserpine treatment, but not in response to cold exposure. Gaunt *et al*(13) studied the effects of iproniazid on the ACTH releasing action of reserpine and cold stress by measuring ascorbic acid in the adrenals, and observed that iproniazid completely suppressed the reserpine effect but only slightly blocked the cold effect. The present results suggest that the effects of electrical stimulation of the uterine cervix, cold exposure and reserpine may reach the pituitary by different pathways and/or the intensity of the 3 stimuli to the pituitary may have differed in degree, as suggested by Gaunt *et al*(13).

Inhibition of postpartum lactation by iproniazid cannot be attributed to the slight reduction in body weight of the mothers. A reduction of 23% in body weight of mothers was accompanied by a loss of 50% in litter weight gains(14), whereas in the present experiment a maximum reduction of 6.5% in weight of the mothers was associated with a loss of 56.6% in litter weight gains. This reduction in litter weight gains may be due to decreases in secretion of prolactin and ACTH, which are normally released following the suckling stimulus(11). There is also the possibility that the pathway of transmission of the suckling stimulus to the CNS may be blocked, since MAO inhibitors have a ganglion blocking action(15). We have recently observed that iproniazid induced central inhibition of oxytocin release whereas it did not block the milk ejection response to administration of oxytocin (unpublished). Whether inhibition of pituitary hormone secretion by iproniazid is due to inhibition of MAO and subsequent changes in the metabolism of serotonin and catecholamines in the CNS is not known.

Summary. The effects of iproniazid on the mammary secretory response to electrical stimulation of the uterine cervix, cold exposure and reserpine injection were tested in estrogen-primed rats, and on lactation in postpartum rats. At the dose levels used, iproniazid significantly depressed the mammary secretory response to electrical stimulation of the uterine cervix but did not significantly modify the mammary secretory re-

sponse to cold exposure or reserpine administration. Iproniazid significantly reduced the ability of reserpine to stimulate ACTH release while it did not block ACTH release in response to cold exposure, as judged by adrenal and thymus weights. Postpartum lactation was significantly reduced by iproniazid, without producing any significant loss of body weight in the mother rats. These results suggest that iproniazid may inhibit the release of prolactin, ACTH or oxytocin in response to electrical stimulation of the uterine cervix, reserpine injections or suckling.

The authors are indebted to Hoffmann-LaRoche, Inc. for the supply of iproniazid, and to T. E. Staley for technical assistance.

1. Shore, P. A., *Pharmacol. Rev.,* 1962, v14, 541.
2. *Advances in Neuroendocrinology,* A. V. Nalbandov, Ed., 1963, Univ. of Ill. Press, Urbana.
3. Botros, M., Robson, J. M., *J. Endocrinol.,* 1960, v20, X.
4. Setniker, I., Murmann, W., Magistretti, M. J., *Endocrinology,* 1960, v67, 511.
5. Lindsay, D., Poulson, E., Robson, J. M., *J. Endocrinol.,* 1961, v23, 209; *ibid.,* 1962, v25, 53.
6. Poulson, E., Botros, M., Robson, J. M., *Science,* 1960, v131, 1101; *J. Endocrinol.,* 1960, v20, XI.
7. Lindsay, D., Poulson, E., Robson, J. M., *J. Endocrinol.,* 1963, v26, 85.
8. Spector, W. G., *Nature,* 1960, v187, 514.
9. Fluckiger, E., Salzmann, R., *Experientia,* 1961, v17, 130.
10. Meites, J., in *Proc. of First Intern. Pharmacol. Meeting,* R. Guillemin, Ed., Pergamon Press, Oxford, 1962, vI, p151.
11. Meites, J., Nicoll, C. S., Talwalker, P. K., Chap. 8 in *Advances in Neuroendocrinology,* A. V. Nalbandov, Ed., Univ. of Ill. Press, Urbana, 1963.
12. Siegel, S., in *Non-parametric Statistics,* 1956, McGraw-Hill Book Co., Inc., New York, p116.
13. Gaunt, R., Renzi, A. A., Chart, J. J., *Endocrinology,* 1962, v71, 527.
14. Ratner, A., Meites, J., *Am. J. Physiol.,* 1963, v204, 268.
15. Gertner, S. B., *Nature,* 1959, v183, 750.

Received October 3, 1963. P.S.E.B.M., 1964, v115.

Role of Humoral Mediator in Tolerance to the Pyrogenicity of Bacterial Endotoxin. (28983)

HENRY H. FREEDMAN AND BARNET M. SULTZER
The Princeton Laboratories, Inc., Princeton, N.J.

Previous studies from this laboratory(1-3) demonstrated unequivocally that humoral factors play a role in endotoxin tolerance. The mechanisms by which tolerance develops, however, remain unknown.

Our findings suggested that the humoral mediator of tolerance, demonstrated by passive transfer, effected an altered state in the tissues rather than acting within the blood as an inhibitor of, or antibody to, the endotoxin. Recent studies by Greisman *et al*(4) have suggested that the humoral mediator of tolerance acts as an opsonin with high endotoxin specificity. The experiments reported here, based upon our earlier observation of the retention of passively acquired tolerance (2), demonstrate that rabbits given amounts of tolerant donor plasma too small to be effective against a first test dose of endotoxin display a remarkable degree of tolerance, compared to identically endotoxin-treated controls, when rechallenged with a larger dose of endotoxin the following day. The results are inconsistent with hypotheses invoking direct inhibition, detoxification, or opsonization of the endotoxin by the humoral mediator, and, most important, suggest that the events leading to the expression of tolerance by the animal are initiated by neither the endotoxin alone nor the humoral mediator alone, but by a combination of the two.

Materials and methods. The procedures used for preparing tolerant donor rabbits, collecting plasma, and performing pyrogen tests in normal recipient rabbits, and the precautions observed, have been described(2). The