6. Bronner, F., *Trans. N. Y. Acad. Sci.*, 1962, v24, 265.

7. Johnston, C. C., Jr., Deiss, W. P., Holmes, L. B., *Endocrinology*, 1961, v68, 484.

8. Dische, Z., *J. Biol. Chem.*, 1950, v183, 489.

9. Tashjian, A. H., Jr., Munson, P. L., *J. Chron. Dis.*, 1963, v16, 269.

10. Bernstein, D. S., LeBoeuf, B., Cahill, G. F., Jr., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 458.

11. Dziewiatkowski, D. D., *J. Cell. Biol.*, 1962, v13, 359.

12. DeLuca, H. F., Engstrom, G. W., Rasmussen, H., *Proc. Nat. Acad. Sci.*, 1962, v48, 1604.

Received March 24, 1964. P.S.E.B.M., 1964, v116.

Drug Effects on Epineprine Release from Rat Adrenal Medulla Homogenates and Granule Preparations.* (29350)

R. GREENBERG AND H. C. SABELLI†

Department of Physiology, University of Illinois College of Medicine and Department of Pharmacology and Therapeutics, Chicago Medical School

We previously(1) characterized the *in vitro* release of the catecholamines from the chromaffin granules of the adrenal medulla of the rat. The initial release and the absolute release of the catecholamines were increased in electrolyte media as compared with equiosmolar sucrose and at 20°C and 37°C as compared with the release at 0°C.

We will report here the presence or absence of a releasing effect by 35 different drugs upon homogenates of the adrenal medulla of the rat and upon the partially isolated chromaffin granules separated from the homogenates. Although there are many *in vivo* studies of the effects of drugs on the catecholamine content of the adrenal medulla of the rat, we are not aware of any previously reported *in vitro* studies. It seemed pertinent, therefore, to evaluate a given drug in *in vitro* preparations from the same species in which the *in vivo* studies were made. Many of these agents were ineffective or quantitatively less effective on rat medullary granules as compared with the reported data on bovine adrenal medullary and adrenergic nerve granules. Their effects on rat medullary granules are more comparable with those recently reported on rabbit medullary granules(2) except that: a) d-lysergic acid diethylamide (LSD) and mescaline were effective on rat granules and ineffective on rabbit granules; b) reserpine, at high concentrations, was effective (as a releaser) on rabbit granules and ineffective on rat granules; c) at low concentrations (10^{-5}-10^{-7} M) reserpine and chlorpromazine inhibited release of adrenaline from rabbit granules while they were ineffective at similar concentrations on the rat granules.

Methods. The preparations of the homogenates and the fractionated suspensions of the chromaffin granules of the adrenal glands of rats were previously described(1). Briefly, the medullae were mechanically expressed, gently homogenized (0°C) either in sucrose (0.3 M), NaCl (0.155 M) in Tris buffer (0.02 M) adjusted to pH 7.45, or phosphate buffer adjusted to pH 6.5 (0.125 M KCl, .005 M KH_2PO_4, .006 M $MgCl_2$), in the proportion of 3 medullae/0.5 cc, and incubated together with equal volumes of the various drugs made up in the same suspending medium. After various incubations (50 minutes, 0°C; 15 minutes, 20°C; 15 minutes, 37°C) the mixture was layered over 0.5 M sucrose and centrifuged at 20,000 *g* (Servall-ss-34 head) for 20 minutes at 0°C to bring down the granules. The catecholamine content of the supernatant represented the portion released while the catecholamine content of the pellet was presumably contributed almost entirely by the granules.

Alternatively the chromaffin granules were in part fractionated from the homogenate by

* This investigation was supported by research grants from Nat. Inst. Health, Bethesda, Md.

† Dept. of Pharmacology, Chicago Med. School, Chicago, Ill. Fellow of Consejo Nacional de Investigaciones, Argentina.

a 2-layered centrifugation procedure in which the original homogenate (in 0.3 M sucrose) was layered over 0.5 M sucrose before centrifugation in the cold at 3000 *g* for 10 minutes. About 50% of the original adrenaline content remained in the supernatant layer. Under control conditions, about 86% of the adrenaline content of the supernatant was sedimentable and presumably in medullary granules. Fourteen percent of the adrenaline content was unsedimentable; presumably this represented the adrenaline originally present in the cell sap together with whatever was released from granules due to mechanical damage. In this procedure, in contrast to most previously described procedures, the granules were not sedimented and resuspended and perhaps damaged prior to incubation; the granules remained suspended in their original cell sap together with the original microsomes and many mitochondria.

The supernatant layer together with equal volumes of the various drugs made up in the same suspending medium were now layered over dense sucrose (0.5 M) and incubated for 15 minutes at 0°C prior to final contrifugation at 20,000 *g* for 20 minutes to bring down the granules.

The data are calculated in terms of percent of adrenaline released into the supernatant of the total present in the supernatant and pellet combined. The increased or decreased release of adrenaline in each experiment was interpreted in terms of its own control value in the absence of the drug.

Adrenaline and noradrenaline determinations, performed in duplicate, were made by Wiegand and Perry's(3) modification of the Shore and Olin(4) fluorimetric method. Due to the large amounts of adrenaline present in relation to the noradrenaline content, we do not have confidence in the noradrenaline determinations. Therefore we are reporting only the adrenaline determinations.

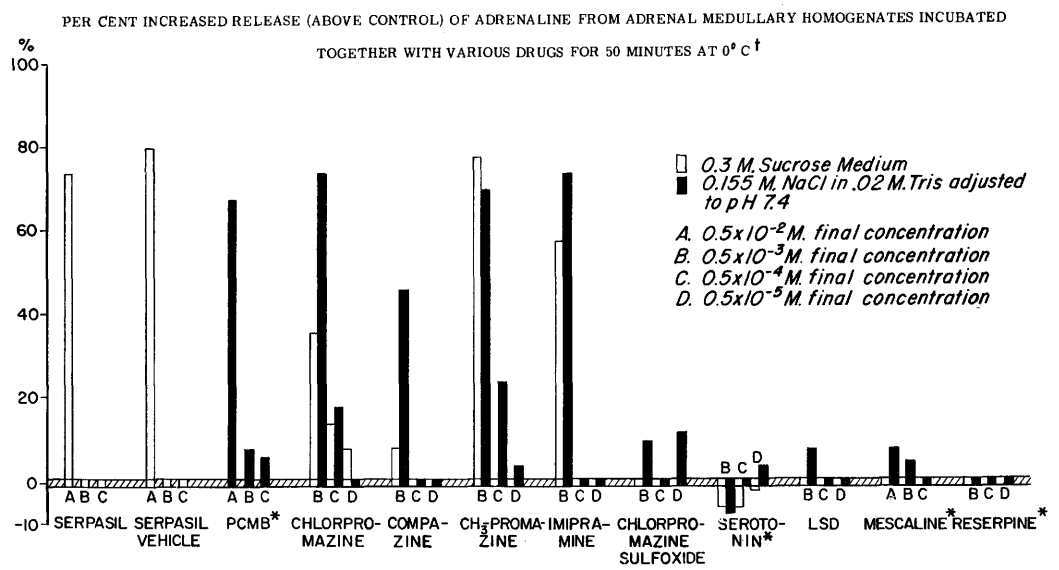
Interference controls were run in each drug experiment in which there was an experimentally observed change by adding the several doses of each drug to standard solutions of adrenaline prior to extraction and development. The influence of the drug on the final adrenaline fluorescence was zero or negligible in every case.

Results. The average adrenaline content of the homogenates (55 determinations) was 38.0 ± 17.3 μg in 0.5 ml. Each 0.5 ml aliquot was equivalent to 3 adrenal medullae. The average release of adrenaline from the various control preparations under standard conditions was: a) 12.1% ± 4.1 (20 determinations) from the homogenates in 0.3 M sucrose for 50 minutes at 0°C. b) 27.7% ± 8.2 (20 determinations) from the homogenates in NaCl-Tris for 50 minutes at 0°C. c) 14.0% ± 3.9 (18 determinations) from the fractionated granules in 0.3 M sucrose for 15 minutes at 0°C. The increased release of adrenaline from the homogenates in NaCl-Tris (b above) as compared with sucrose (a above) was significant at the 1% confidence level (Student t Test).‡ In no instance was there an increased release of adrenaline (above control) by a given drug from the homogenate preparation in sucrose (a above) as compared with the fractionated suspension in sucrose (c above). However, in a number of instances a given drug effected an increased release in the NaCl-Tris (b above) as compared with either of the sucrose preparations.

In Fig. 1 are reported all of the drugs which modified the release of adrenaline from the homogenates under the standard conditions (0°C, 50 minutes). Data for 12 of 35 drugs examined are given. All of these increased the release of adrenaline (above control) except serotonin which slowed release and reserpine which was without effect. Chlorpromazine, prochlorperazine (Compazine), CH_3-promazine, imipramine and p-chloromercurobenzoate (PCMB) markedly increased the release of adrenaline from the chromaffin granules. (Except for PCMB, all of these agents have somewhat similar structure and may be described pharmacologically as centrally-acting drugs with weak atropinic and antihistaminic properties.)

Chlorpromazine sulfoxide, LSD and mescaline slightly increased the release of adren-

‡ Student t Test, in Fisher, R. A., Design of Experiments. Oliver & Boyd, London, 1942.



PCMB* Similar increased release noted at same concentration in NaCl-Tris media at 37°C. for 10 min.

Serotonin* Similar blocking of release noted at same concentrations in sucrose and NaCl-Tris media at 37°C. for 10 min.

Mescaline* Similar increased release noted at same concentrations in NaCl-Tris media at 37° C. for 10 min.

Reserpine* No increased release at same concentrations in phosphate media at 0°, 20°, 25°C. for 50 min. 5-10% increased release at 2 x 10^{-3} M. concentration of reserpine in phosphate and NaCl-Tris media at 37°C. for 15 min.

† Determinations were made in duplicate; differences were with ± 5%.

aline. Reserpine was without effect (see below); however, commercial Serpasil and Serpasil Suspending Vehicle§ (without reserpine) markedly increased the release of adrenaline. We have not yet identified the substance responsible for this effect.

Under special conditions (NaCl-Tris, phosphate, 37°C, 15 minutes) reserpine (2×10^{-3} M final concentration) slightly increased (5-10%) the release of adrenaline; however the reserpine is in part in suspension at this concentration and most probably this is a non-specific effect not related to the concentration of reserpine in the saturated solution.

In Table I are listed the remaining 23 of 35 drugs. These did not modify the release of adrenaline above control whether from the homogenates (a and c above) or from the fractionated granule preparations (b above).

In selected cases as noted in the Figure and Table the individual agents were incubated under still different experimental conditions in order to approximate more nearly the conditions under which other investigators reported *in vitro* releases of adrenaline from the chromaffin granules of other species.

The spontaneous release of adrenaline was even higher from the homogenates made up in the phosphate media than from the NaCl-Tris media(1). The average release of adrenaline from the control preparations was: 28% at 0°C for 50 minutes, 45% at 20°C for 50 minutes, 65% at 37°C for 15 minutes. In no instance was there a markedly increased effect in phosphate media at 20°C (50 minutes) or at 37°C (15 minutes) as compared with the % release of adrenaline in sucrose and NaCl-Tris media at 0°C (50 minutes). Histamine, acetylcholine, 48-80, sodium cyanide, and sodium fluoride were

§ Serpasil Suspending Vehicle. Ciba Pharmaceutical Co., Summit, N. J. Adipic acid: 30 g; N,N-dimethyl acetamide: 300 g; Versene FD-3: 0.3 g; benzyl alcohol: 30 ml; polyethylene glycol 300: 150 ml; ascorbic acid: 1.5 g; sodium sulfite: 0.3 g; distilled water q.s. to 3000 ml.

equally ineffective at 0°C and in the phosphate media at increased temperatures.

Under special conditions (phosphate medium, 37°C, 15 minutes) the sympathomimetic amines, methamphetamine and ephedrine (0.5×10^{-3} M final concentration) increased slightly (6%) the release of adrenaline. However tyramine and cocaine were still without effect.

Under special conditions (phosphate memium, 37°C, 15 minutes) and at low concentrations (10^{-4}-10^{-7} M) reserpine and chlorpromazine did not inhibit the release of adrenaline from the rat medullary granules.

TABLE I. Experimental Conditions Under Which 23 of 35 Drugs Did Not Modify Release of Adrenaline from Chromaffin Granules of Rats: Methamphetamine and Ephedrine Were Slightly Effective Under Special Conditions.*

Insulin	A†, B†
Histamine	A, B, D
Acetylcholine	B‡, C‡
RO-1-9569	C
Physostigmine	C
Tremorine	C
Dibenzyline	C
TM-10	C
Cocaine	C, D
Tyramine	C, D, E
Methamphetamine	C, D§
Ephedrine	C, D§
Bretylium	A, B
Guanethidine	A, B
Librium	A, B
Nialamide	A, B
Iproniazid	C
JB-329	C
Bufotenin	C
DOPA	C
48-80	C, D, E
Sodium cyanide	A, B, D
Sodium fluoride	A, B, D

A. Adrenal medullary homogenate in 0.3 M sucrose at 0°C for 50 min. Final concentrations of drugs in incubation medium: $0.5 \times (10^{-2}, 10^{-3}, 10^{-4}$ M).

B. Adrenal medullary homogenate in NaCl-Tris at 0°C for 50 min. Final concentrations of drugs in incubation medium: $0.5 \times (10^{-2}, 10^{-3}, 10^{-4}$ M).

C. Fractionated granule preparation in 0.3 M sucrose at 0°C for 15 min. Final concentrations of drugs in incubation medium: $0.5 \times (10^{-3}, 10^{-4}, 10^{-5}$ g/ml).

D. Adrenal medullary homogenate in phosphate medium at 37°C for 15 min. Final concentrations of drugs in incubation medium: $0.5 \times (10^{-3}, 10^{-4}, 10^{-5}$ g/ml).

E. Adrenal medullary homogenate in phosphate medium at 20°C for 50 min. Final concentrations of drugs in incubation medium: $0.5 \times (10^{-3}, 10^{-4}, 10^{-5}$ g/ml).

* Determinations were made in duplicate; differences were within ± 5%.

† Final concentrations of insulin in incubation media: 0.5, .05, .005 units/ml.

‡ No increased release if physostigmine (10^{-3} M) is added.

§ 6% increased release at concentration of 0.5×10^{-3} g/ml.

Discussion. Thirty-five different drugs were incubated either with adrenal medullary homogenates or with fractionated suspensions of rat chromaffin granules. The compounds were selected for a variety of reasons: a) Reserpine(5), insulin(6), histamine(7), acetylcholine(8), Ro-1-9569 (tetrabenazine)(9), physostigmine(10), TM-10 (choline-2, 6-xylylether bromide)(11), tremorine (1,4 dipyrrolidino-2-butyne), and dibenzyline(12) are depleters of the catecholamines of the adrenal medulla *in vivo*. b) Guanethidine (13), bretylium(14), tyramine(15), cocaine (16), methamphetamine(17) and ephedrine (18) are depleters of adrenergic nerve endings *in vivo*. c) The psychopharmacologic agents because they might possibly accelerate or slow the release of biogenic amines in the central nervous system. d) Several metabolic inhibitors because they might possibly modify the accumulation or the release of catecholamines from the granules if these are energy dependent reactions.

None of the catecholamine depleters (a and b above) accelerated the release from the rat chromaffin granules except ephedrine and metamphetamine which were only slightly effective (Table 1). The remarkable lack of parallelism between these *in vitro* effects and the well known *in vivo* effects of these same drugs suggests that they must act indirectly upon some structural feature of the chromaffin cell as a whole.

Reserpine and Serpasil. Von Euler and Lishajko(19) observed a marked release of catecholamines from bovine adrenergic nerve granules by reserpine and Serpasil at high concentrations and blocking of release by reserpine at low concentrations(20,21,22). Weil-Malherbe and Posner(2) reported similar findings with reserpine acting upon rabbit chromaffin granules. In the present report: a) Reserpine was essentially ineffective as a releaser of adrenaline from the rat medullary

granules at high concentrations (0.5×10^{-3}-0.5×10^{-5} M) and under comparable experimental conditions (phosphate buffer, 20°-25°C, 50 minutes). There was a 5-10% increased release above control at extremely high concentrations (2×10^{-3} M) (37°C, 15 minutes) under which the drug was frankly in suspension. b) Reserpine did not inhibit the spontaneous release of adrenaline from the rat medullary granules at low concentrations (10^{-4}-10^{-7} M), under experimental conditions comparable to those used on bovine adrenergic nerve granules(21), and on rabbit medullary granules(2) (phosphate buffer, 20°-25°C for 50 minutes, 37°C for 15 minutes). c) Both Serpasil and the Serpasil Suspending Vehicle§ caused a marked release above control at high concentrations; therefore, the Serpasil Suspending Vehicle contains some other agent, not reserpine, which causes the observed release.

In agreement with our findings, Stjarne and Schapiro(23) did not observe any effect by reserpine on isolated bovine medullary granules; their concentrations (approx. 10^{-5}-10^{-7} M) were within the range at which the inhibition of release of adrenaline should have been observed. In contrast to their earlier report, von Euler and Lishajko(21) failed to observe a release by reserpine at high concentration (0.2 mg/ml) upon an original suspension of bovine adrenergic nerve granules. In summary, the published reports on the action of reserpine on the granules of the various species are contradictory.

Our *in vitro* data in the rat support the thesis that reserpine does not act directly upon the adrenal medullary granules. There are equally contradictory *in vivo* data in the rat with respect to whether reserpine acts directly upon the adrenal chromaffin cells (24,25,26) or indirectly via the innervation (27,28,29). Our *in vivo* data would support the thesis that reserpine does not act directly upon the adrenal chromaffin cell(29).

Sympathomimetic agents. Previously tyramine was observed to increase the release of catecholamines *in vitro* from bovine chromaffin granules(30) and adrenergic nerve granules(31). However, it was ineffective upon rabbit granules(2). Previously cocaine was reported to increase the release from bovine adrenergic nerve granules(20) while failing to increase the release from bovine (30) or rabbit chromaffin granules(2). Previously ephedrine and methamphetamine increased the release from bovine chromaffin granules(32). However they were ineffective upon rabbit chromaffin granules(2).

In this report: a) Ephedrine and methamphetamine were slightly effective (6% increased release of adrenaline above control) upon the rat chromaffin granules under special conditions of increased temperature and concentration (Table I). b) Tyramine and cocaine were ineffective even under special conditions of increased temperature and concentration.

The relative ineffectiveness of sympathomimetic amines on rat and rabbit chromaffin granules as compared with bovine medullary and nerve granules would support the hypothesis that species specificity may be an important variable to be considered when these agents use used *in vivo*.

Bretylium, guanethidine, TM-10. Bretylium, guanethidine and TM-10 were ineffective (Table I) on the rat granules. Previously, they had been reported ineffective on rabbit chromaffin granules(2) and on bovine nerve granules(22) except that TM-10 was a releaser at high concentrations when incubated with the nerve granules. Their ineffectiveness as releasers of noradrenaline at nerve endings *in vivo* had been previously considered(33,34,35,36). Alternatively it was suggested that they might: a) interfere with conduction at fine nerve terminals by as yet unknown mechanisms(35,36), b) interfere with an acetylcholine induced release of adrenaline(37,38), c) interfere with noradrenaline synthesis at adrenergic endings (34).

Psychopharmacologic agents. a) Tranquilizers. Reserpine (discussed above) and librium were ineffective *in vitro* on the rat granules; however, all of the phenothiazines examined (chlorpromazine, compazine, CH_3-promazine; Fig. 1) were markedly effective. Interestingly, chlorpromazine sulfoxide, an inactive phenothiazine metabolite, was only

slightly effective. The observed *in vitro* releasing effect of chlorpromazine on rabbit (2,21) and bovine(39) medullary granules were previously reported. However, the phenothiazines are generally considered to be ineffective as releasers *in vivo*. One might speculate that chlorpromazine altered the permeability of the isolated granule membrane as has been reported for other cellular membranes(40,41,42), or interfered with catecholamine binding possibly by forming a complex with a metal present in tracer amounts(43).

b) Anti-depressives. Iproniazid and nialamide were ineffective on the rat granules. Imipramine, which is an analogue of chlorpromazine was markedly effective (Fig. 1), and had been so reported on rabbit medullary granules(2).

c) Hallucinogens. Bufotenin and JB-329 (Ditran) were ineffective, while mescaline and LSD (lysergic acid diethylamide) caused a slight release (8% above control, Fig. 1) in the electrolyte medium and not in the sucrose media.

The small *in vitro* effects noted with mescaline and LSD if paralleled *in vivo* would require evaluation in the several hypotheses involving catecholamines in higher mental functions. Both of these agents were reported ineffective on rabbit medullary granules(2).

Metabolic inhibitors. Previously D'Iorio (44) reported an accelerated release by PCMB from ox granules at 37°C. In this report PCMB caused a markedly increased release of adrenaline from the rat granules both at 0°C and at 37°C (Fig. 1), while sodium cyanide and sodium fluoride were ineffective both in the cold and at 37°C (Table I). The lack of a differential effect at the 2 temperatures would suggest that the metabolic processes which these agents block are not involved in maintaining the granule stores under the present experimental conditions. In some way, however, the *in vitro* process destroys some mechanism of the intact cell which helps to maintain the granule integrity, which leads to markedly increased adrenaline release from the isolated granules at physiologic temperatures. If either the accumulation or release of adrenaline by the granules were linked to metabolic processes in the granules *per se,* or in the accompanying mitochondria, then provided the proper inhibitor was administered, we should observe a quantitative difference at increased temperature which would be eliminated in the cold.

Other agents. The endogenous agents DOPA (dihydroxyphenylanine) and serotonin (5-hydroxytryptamine) were of obvious interest in this system. DOPA was ineffective; however, serotonin slowed adrenaline release from the rat granules at 0°C and 37°C (Fig. 1). These data may afford a partial explanation of the reported antagonism by serotonin of physiologically released noradrenaline as reported by Gordon *et al* (45).

48-80 did not deplete the rat granules under our standard conditions (Table I), nor was it effective in phosphate media (20°C, 50 minutes; 37°C, 15 minutes); previously(46) it has been reported effective on bovine granules in Ringer's phosphate solution under somewhat similar conditions (33°C, 30 minutes).

Our negative findings with histamine, acetylcholine, and dibenzyline (Table I) are in agreement with previously reported *in vitro* observations on bovine chromaffin granules (47,48), on bovine nerve granules(49), and on rabbit chromaffin granules(2) respectively. Dibenzyline has also been reported to slow the release from bovine nerve granules while accelerating the release from the adrenal medullary glands of the same species (22).

Summary. 1. Adrenal homogenates and fractionated medullary granule suspensions from rats were incubated in various media at several temperatures with and without addition of various concentrations of drugs, after which adrenaline concentration was measured in the centrifuged particulates and the supernatant. 2. Both homogenates and granule suspensions showed minimal spontaneous release of adrenaline at 0°C, markedly increased with temperature. All drug incubations were carried out routinely at 0°C. In many instances determinations were repeated at increased temperature and drug

concentration under experimental conditions which approximated those of other investigators who studied the release of catecholamines from the chromaffin granules of other species. 3. Thirteen of the 35 drugs examined modified the release of adrenaline from the rat chromaffin granules. a) Ten of these were previously reported to increase the release *in vitro* in other species. They were chlorpromazine, compazine, CH_3-promazine, chlorpromazine sulfoxide, imipramine, PCMB, Serpasil, Serpasil Suspending Vehicle, ephedrine and methamphetamine. b) LSD and mescaline increased the release from the rat granules; they had been previously reported ineffective in other species. c) Serotonin slowed the release; this had not been previously reported.

4. Twenty-two of the 35 drugs examined were ineffective as releasers when incubated with the rat chromaffin granules. a) At least 6 of these, which we found ineffective, were previously reported effective in other species. They were reserpine, TM-10, tyramine, cocaine, dibenzyline and 48-80. b) Reserpine deserves special comment because, in other species, it has been reported to be effective as a releaser at high concentrations and as an inhibitor of spontaneous release at lower concentrations. Neither of these effects was observed in comparable experiments with rat medullary granules. c) The 16 remaining drugs which we found ineffective were: histamine, acetylcholine, guanethidine, bretylium, insulin, RO-1-9569, physostigmine, tremorine, librium, nialamide, iproniazid, JB-329, bufotenin, DOPA, sodium fluoride, and sodium cyanide. Many of these had been previously reported ineffective in other species. The metabolic inhibitors, sodium cyanide and sodium fluoride, deserve special comment. They were equally ineffective both in the cold and at 37°C.

5. Many of these agents were ineffective or quantitatively less effective on rat medullary granules as compared with the reported data on bovine adrenal medullary and adrenergic nerve granules. There are greater similarities between the rat and rabbit medullary granules as compared with bovine granules, except that LSD and mescaline were effective on rat granules and ineffective on rabbit granules while reserpine was effective on rabbit granules and ineffective on rat granules. The very high concentrations of drugs required for any effect on the rat granules, in most instances, suggest that great care be taken when attempting to transfer these mechanisms into *in vivo* systems.

We are grateful to A. Bragner, G. Falk and C. Wegrzyn for excellent and conscientious technical assistance. We are grateful to Drs. H. Tedeschi, J. E. P. Toman and R. G. Wiegand for valuable advice. Dr. L. G. Abood kindly supplied 2-methylpromazine, chlorpromazine sulfoxide, and Ditran (JB 329). We wish to thank the following for donation of drugs: Abbott Laboratories: methamphetamine HCl (Desoxyn HCl), 1,4-dipyrrolidino-2-butyne (Tremorine). Burroughs Wellcome & Co.: bretylium toslylate (Darenthin Brand), 48-80. Ciba Pharmaceutical Co.: guanethidine (Ismelin), reserpine, Serpasil and Serpasil Vehicle. Chas. Pfizer & Co.: nialamide (Niamid). Geigy Pharmaceuticals: impramine (Tofranil). Hoffmann-LaRoche, Inc.: chlordiazepoxide (Librium HCl), tetrabenazine (RO 1-9569). Sandoz Pharmaceuticals: d-lysergic acid diethylamide (LSD). Smith, Kline and French Laboratories: chlorpromazine (Thorazine), choline -2,6 xylyletherbromide (TM-10), prochlorperazine (Compazine).

1. Greenberg, R., Falk, G. S., Toman, J. E. P., submitted to *Biochemica et Biophysica Acta.*

2. Weil-Malherbe, H., Posner, H. S., *J. Pharm. Exp. Ther.,* 1963, v140, 93.

3. Weigand, R. G., Perry, J. E., *Biochem. Pharmacol.,* 1961, v7, 181.

4. Shore, P. A., Olin, J. S., *J. Pharm. Exp. Ther.,* 1958, v122, 295.

5. Holzbauer, M., Vogt, M., *J. Neurochem.,* 1956, v1, 8.

6. Udenfriend, S., Cooper, J. R., Clark, C. T., Baer, J. E., *Science,* 1953, v117, 663.

7. Burn, J. H., Dale, H. H., *J. Physiol.,* 1926, v71, 185.

8. Douglas, W. W., Rubin, R. P., *ibid.,* 1961, v159, 40.

9. Quinn, G. P., Shore, P. A., Brodie, B. B., *J. Pharm. Exp. Ther.,* 1959, v127, 103.

10. Edmunds, C. W., Smith, R. G., *J. Lab. and Clin. Med.,* 1932, v17, 399.

11. Coupland, R. E., Exley, K. A., *Brit. J. Pharmacol. and Chemother.,* 1957, v12, 306.

12. Shapiro, S., *Acta Physiol. Scand.,* 1958, v42, 371.

13. Maxwell, R. A., Plummer, A. J., Schneider, F.,

Povalski, H., Daniel, A. I., *J. Pharm. Exp. Ther.*, 1960, v128, 267.

14. Siegel, J. H., Gilmore, J. P., *Fed. Proc.*, 1961, v20, 126.

15. Weiner, N., Draskoczy, P. R., Burach, W. R., *J. Pharm. Exp. Ther.*, 1962, v137, 47.

16. Muscholl, E., Vogt, M., *J. Physiol.*, 1958, v141, 132.

17. McLean, J. R., McCartney, M., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 77.

18. Chidsey, C. A., Harrison, D. C., Braunwald, E., *ibid.*, 1962, v109, 488.

19. Euler, U. S. v., Lishajko, F., *Science*, 1960, v132, 351.

20. ———, *Biochem. Pharmacol.*, 1961., v8, 62.

21. ———, *Acta Physiol. Scand.*, 1961, v52, 137.

22. ———, *Proc. 1st Internat. Pharmacol. Meeting*, Lowry, O. H., Lindgren, P., Eds., 1963, v5, 77.

23. Stjarne, L., Schapiro, S., *Nature, Lond.*, 1959, v184, 2023.

24. Kroneberg, G., Schumann, H. J., *Arch. Exp. Path. Pharmak*, 1957, v231, 349.

25. Callingham, B. A., Mann, M., *Nature*, 1958, v182, 1020.

26. Mirkin, B. L., *ibid.*, 1958, v182, 113.

27. Coupland, R. E., *J. Endocrin.*, 1958, v17, 191.

28. Eranko, O., Hopsu, V., *Endocrinology*, 1958, v62, 15.

29. Greenberg, R., Jeffay, A. I., Toman, J. E. P., *J. Pharm. Exp. Ther.*, 1960, v130, 119.

30. Schumann, H. J., Weigmann, E., *Arch. Exp. Path. Pharmak*, 1960, v240, 275.

31. Euler, U. S., v., Lishajko, F., *Experientia* (Basel), 1960, v16, 376.

32. Schumann, H. J., Philippu, A., *Nature*, 1962, v193, 890.

33. Bain, W. A., *Adrenergic Mechanisms*, Ciba Foundation Symposium, Little, Brown & Co., Boston, 1960, p131.

34. Bein, H. J., *ibid.*, 1960, p162.

35. Exley, K. A., *ibid.*, 1960, p158.

36. Green, A. F., *ibid.*, 1960, p148.

37. Burn, J. H., *ibid.*, 1960, p502.

38. ———, *Brit. Med. J.*, 1961, vii, 1625.

39. Carlsson, A., Hillarp, N. A., *Med. Exp.*, 1961, v5, 122.

40. Mann, T., *Proc. Study Fertility*, 1954, No. 6, 41.

41. Nathan, H. A., Friedman, W., *Science*, 1962, v135, 793.

42. Miller, Z. B., Hancock, R., *J. Cell. Biol.*, 1963, v19, 50A.

43. Borg, A. C., Cotzias, G. C., *Fed. Proc.*, 1960, v19, 248.

44. D'Iorio, A., *Canada, J. Biochem.*, 1957, v35, 395.

45. Gordon, P., Haddy, F. J., Lipton, M. A., *Science*, 1958, v128, 531.

46. Hogberg, B., Hillarp, N. A., *Kungl. Fysiografiska Saliskapetsi Lund Forhandl.*, 1956, v26, 117.

47. Blaschko, H., Hagen, P., Welch, A. D., *J. Physiol.*, 1955, v129, 27.

48. Eade, N. R., *Brit. J. Pharmacol. and Chemother.*, 1957, v12, 61.

49. Euler, U. S. v., Lishajko, F., *Acta Physiol. Scand.*, 1961, v51, 193.

Received March 25, 1964. P.S.E.B.M., 1964, v116.

Effects of CE^{144} Administered to Pregnant Rats.* (29351)

A. F. MCFEE (Introduced by F. R. Mraz)

University of Tennessee-Atomic Energy Commission, Agricultural Research Laboratory, Oak Ridge, Tenn.

Although numerous reports concerning the responses of embryos to external irradiation have been published(1), the consequences of administering radioisotopes to pregnant females have received only occasional attention. The LD_{50} dose for the rat embryo of maternally administered P^{32} increases from 0.46 mc per gram of embryonic tissue on day 6 of gestation to 1.29 mc on the tenth day(2). Injections of 0.2, 0.4, or 0.6 mc of P^{32} on day 14 or 17 of gestation have caused pronounced interference with spermatogenesis in the resulting progeny(3). Body size and growth rates were reduced in litters of females injected with 5 to 17 μc of P^{32} per

* This manuscript is published with the permission of the director of Univ. of Tennessee Agric. Exp. Station, Knoxville. The work was completed under Contract between Univ. of Tennessee College of Agriculture and Atomic Energy Commission.