not inhibited by 50 mg % of acetoacetate. 3. A rapid hydrolysis of the glycogen of liver slices was unaffected by 125 mg % of acetoacetate in the buffer in the absence of glucose, but glycogenolysis was markedly decreased if both were present. 4. Acetoacetate in these concentrations does not appear to inactivate insulin, to inhibit glycogen storage in the muscle or liver tissue, or to affect appreciably the utilization of carbohydrate as measured by the glucose tolerance.

The assistance of Anne M. Miller, W. W. Burr, Jr., and James C. McPherson is gratefully acknowledged.

1. Nath, M. C., and Brahmachari, H. D., *Nature*, 1944, v154, 487.

2. Nath, M. C., and Chakrabarti, C. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 326.

3. Nath, M. C., and Brahmachari, H. D., *Nature*, 1946, v157, 336.

4. Nath, M. C., and Chakrabarti, C. H., *Indian J. Physiol. and Allied Sc.*, 1951, v5, 43.

5. Nath, M. C., Chakrabarti, C. H., and Hatwalne, V. G., *Sc. and Culture*, 1948, v14, 211.

6. Tidwell, H. C., and Axelrod, H. E., *J. Biol. Chem.*, 1948, v172, 179.

7. Tidwell, H. C., and Nagler, M. E., *J. Biol. Chem.*, in press.

8. MacKay, E. M., *J. Clin. Endocrinol.*, 1943, v3, 101.

9. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

10. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

11. Villee, C. A., and Hartings, S. B., *J. Biol. Chem.*, 1949, v179, 673.

12. Stadie, W. C., and Zapp, J. A., Jr., *J. Biol. Chem.*, 1947, v170, 55.

13. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism, Burgess Publishing Co., Minneapolis, 1949, 119.

14. Deutsch, W., *J. Physiol.*, 1936, v87, 56P.

15. Fisher, R. A., Statistical Methods for Research Workers, Edinburgh, 7th edition, 1938.

16. Gemmill, C. L., *Bull. Johns Hopkins Hosp.*, 1940, v66, 232.

17. Wick, A. N., and Drury, D. R., FED. PROC., 1952, v11, 310; *J. Biol. Chem.*, 1952, v196, 129.

18. Chari, A., and Wertheimer, E., *Nature*, 1953, v171, 44.

Received March 12, 1953. P.S.E.B.M., 1953, v82.

Epinephrine Hypertensive Effects Before and After Cocaine. (20208)

ROBERT V. BROWN AND GALE C. BOXILL.* (Introduced by J. P. Quigley.)
From the Division of Pharmacology, University of Tennessee, Memphis.

Numerous reports have indicated that cocaine increases the pressor potency of epinephrine(1-11). The questionable experimental methods employed leave this claim unsettled. The following work investigates the situation in the dog.

Procedures. Mongrel dogs were anesthetized by the intravenous injection of 30 mg of pentobarbital sodium/kg of body weight. This agent was chosen because the control blood pressure remains within physiological limits during anesthesia. Atropine sulfate, 1.0 mg/kg, was given intravenously; when necessary, additional atropine sulfate, 0.2 mg/kg/dose, was given until tetanic stimulation of the distal end of the cut left vagus failed to alter blood pressure. Blood pressure was recorded by a mercury manometer from the left common carotid artery. After securing a control record, the first dose of epinephrine was injected into the right external jugular vein. The next dose of epinephrine was injected only after the blood pressure had stabilized at approximately the preinjection level; all doses were rapidly injected, *i.e.*, within 15 seconds. Each of the 10 dogs in series 1 received repeated identical doses of epinephrine;† among the dogs, the range covered was 0.125-12.5 μg/kg. The results provide estimates of the variability of responses to such repeated doses. In series 2 each dog received the progressively increasing

* Present address: College of Pharmacy, University of Georgia, Athens.

† Eastman Kodak Co., epinephrine, containing some arterenol.

TABLE I. Summary of Data on Variability of Consecutive Responses to Identical Doses of Epinephrine in 3 Dogs.

Dose in μg of base/kg	No. of doses	Preinj. blood pressure	Rise in blood pressure	Max pressure attained	Coef. of variation* (1)	(2)	(3)	(4)
.125	11	143.3 ± 2.93	7.1 ± 2.85	150.4 ± 3.75	.020	.401	.025	—
1.25	9	158.3 ± 4.20	84.1 ± 4.20	242.4 ± 7.05	.027	.050	.029	.58
12.5	7	142.6 ± 2.83	124.7 ± 2.42	267.3 ± 3.76	.158	.127	.057	.45

* (1) For preinj. blood pressure; (2) for rise in blood pressure; (3) for max b. p. attained; (4) ratio of (3) to (2).

TABLE II. Blood Pressure Responses before and after Cocaine HCl: Mean (Stand. Dev.); P>0.1 Except as Noted. *s* has 9 degrees of freedom in every case.

Epinephrine, μg/kg	Cocaine HCl, 2 mg/kg Before	Cocaine HCl, 2 mg/kg After	Cocaine HCl, 5 kg/kg Before	Cocaine HCl, 5 kg/kg After
	Max blood pressure attained in mm Hg			
Control	156.8 (14.2)	165.1 (13.9)	147.1 (14.8)	148.0 (16.7)
.01	—	—	150.5 (13.3)	148.4 (16.5)
.1	174.4 (13.9)†	187.9 (9.5)†	164.4 (13.7)	165.2 (13.7)
.5	190.6 (12.8)‡	209.2 (18.7)‡	180.7 (16.1)	195.2 (19.4)
1.0	191.6 (23.5)*	210.2 (20.0)*	184.8 (18.6)	203.1 (19.3)
5.0	—	—	232.6 (26.1)	249.8 (21.1)
10.0	250.2 (17.7)	251.0 (21.8)	280.4 (21.1)	283.9 (29.1)
	Rise in blood pressure (peak minus original control): mm Hg			
.01	—	—	3.4 (5.7)	.4 (.8)
.1	17.6 (9.0)	22.8 (14.7)	17.3 (8.1)	17.2 (11.4)
.5	33.8 (7.5)	44.1 (16.3)	33.6 (13.1)	47.2 (20.4)
1.0	45.3 (12.6)	56.5 (19.6)	44.5 (23.3)	62.2 (24.2)
5.0	—	—	85.5 (27.3)	101.8 (26.6)
10.0	123.6 (18.1)	118.8 (25.4)	103.1 (20.8)	103.0 (27.1)

* These values differ with P<.01.
† " " " " .02<P<.05.
‡ " " " " " " .
For homogeneity of variance of the controls, $\chi^2 = .123$ with P>.5.

doses of epinephrine‡ shown in Table II; after the intravenous injection of 2 mg of cocaine HCl/kg, the doses of the hormone were repeated. The 10 dogs in series 3 received the graded doses of epinephrine‡ shown in Table II; after 5 mg of cocaine HCl, these graded doses were repeated. Epinephrine HCl was injected. Doses of the hormone are reported in μg of base/kg. The two doses of cocaine HCl did not affect blood pressure *per se*, *cf.*, Table II.

Analyses of the data follow Snedecor(12).

Results. A summary of some of the responses to repeated identical doses of epinephrine is given in Table I, including the coefficient of variation, C; results in the other seven dogs in this series were similar. The last column of this table indicates that the criterion of maximum blood pressure attained (MBPA) is more precise than is rise of pressure. The results in series I show no evidence that the first response is the largest; variance analysis, comparing the first response with the second in all 10 dogs, leads to the conclusion that such differences as exist are randomly distributed, with a probability exceeding 50%.

Table II lists the mean values of the control blood pressures, MBPA, and rises in blood pressure for the graded doses of epinephrine, both before and after the respective cocaine injections; the standard deviations are included parenthetically. Results of significance tests are given.

A dose-response curve was fitted to the data from each dog in series 2 and 3, before and again after cocaine, by the method of Brown (13). Table III contains a summary of the estimates of the parameters of the 40 dose-response curves, in so far as the estimates with probably homogeneous variances are

‡ Courtesy of Parke, Davis and Co.; this epinephrine contained less than 0.1% arterenol.

TABLE III. Summary of Parameter Estimates of the Individually Fitted Dose-Response Curves Before and After Cocaine HCl: Means and stand. dev.

Parameter estimated	Before cocaine, 2 mg/kg	After cocaine, 2 mg/kg	Before cocaine, 5 mg/kg	After cocaine, 5 mg/kg
$\bar{k}$	506.7 (539.4)	220.4 (166.6)	440.3 (547.6)	112.1 (84.5)
$\bar{b}$	3.1 (2.17)	1.8 (1.33)	3.2 (3.13)	0.9 (0.87)
$\bar{a}$	158.5 (39.0)	136.5 (28.9)	132.2 (21.9)	117.9 (27.2)

There are 9 degrees of freedom in every case. P exceeds 5% in all comparisons which are valid.

concerned(13). The administration of cocaine did not alter the estimates to any significant extent; the probability exceeds 5% for all valid comparisons.

Discussion. These results are contrary to those of most other investigators(1-13), although Melville(6) reported no marked potentiation of epinephrine effect by cocaine. All workers(6-11) who have used dogs report sensitization. Considering the variability shown in Table I, significance tests are indicated. For example, even accepting the validity of the *percentage rise* method of stating results, Tainter's data(1) yield a probability between 10-20%; this probability level is not usually considered significant. With one exception, in Tainter's data the first and smallest dose of epinephrine produced the largest response. Probably the control blood pressure in his animals, which were either pithed or urethanized, was excessively low, but the first epinephrine injection produced a more normal blood pressure, which was then maintained.

Rosenblueth(5) noted that cocaine did not alter the type of the dose-response curve for the pressor response to epinephrine except by alteration of the parameter estimates, specifically the asymptote parallel to the dosage axis. The analyses of $\bar{a}$, this asymptote, in Table III do not support this finding, as the estimates do not differ significantly from their previous values after either dose of cocaine. Neither do the analyses of the MBPA, in Table III, before and after cocaine, bear out Rosenblueth's report(5) that the MBPA are increased by cocaine, except at the single epinephrine dosage level of 1.0 μg/kg, where the probability is less than 1%.

Conclusions. The responses to repeated identical doses of epinephrine in the same dog show marked variability. In general, cocaine does not sensitize the dog to the hypertensive effects of epinephrine injections. Neither do the doses of cocaine used, 2 and 5 mg/kg, alter the level of the control blood pressure.

Support. This study was partially supported by the Memphis Heart Assn.

1. Tainter, M. L., *J. Pharmacol. and Exp. Therap.*, 1929, v36, 569; 1930, v40, 43; 1932, v46, 27.

2. MacGregor, D. F., *J. Pharmacol. and Exp. Therap.*, 1939, v66, 393.

3. Rosenblueth, A., *Am. J. Physiol.*, 1932, v101, 149.

4. Melville, K. I., *J. Pharmacol. and Exp. Therap.*, 1937, v59, 317.

5. Hazard, R., Beauvallet, M., and Guidicelli, R., *Compt. rend. Soc. biol.*, 1948, v142, 185.

6. Hermann, H., Morin, G., and Vial, J., *Comp. rend. Soc. biol.*, 1936, v121, 998.

7. Lands, A. M., Luduena, F. P., Ananenko, E., and Grant, J. I., *Arch. internat. pharmacodyn.*, 1950, v83, 602.

8. Lands, A. M., *Am. J. Physiol.*, 1952, v169, 11.

9. Luduena, F. P., Ananenko, E., Siegmund, O. H., and Miller, L. C., *J. Pharmacol. and Exp. Therap.*, 1949, v95, 155.

10. Raymond-Hamet, *Compt. rend Soc. biol.*, 1937, v124, 1293.

11. Tainter, M. L., and Chang, D. K., *J. Pharmacol. and Exp. Therap.*, 1927, v30, 193.

12. Snedecor, G. W., *Statistical Methods*, 1946, Iowa State College Press, Ames, Iowa.

13. Brown, R. V., *J. Pharmacol. and Exp. Therap.*, 1952, v105, 139.

Received March 12, 1953. P.S.E.B.M., 1953, v82.