M. R., Exp. Eye Res., 1963, v2, 71.

4. Mueller, F. O., Casey, T. A., Trevor-Roper, P. D., Brit. Med. J., 1964, v2, 473.

5. Brown, B. L., Nagle, S. C., Jr., Science, 1965, v149, 1266.

6. Block, L. H., Drug & Cosmetic Industry, 1964, v95, 342.

7. Jacob, S. W., Bischel, M., Herschler, R. D., Curr. Therap. Res., 1964, v6, 134.

8. Fabianek, J., Herp, A., Pigman, W., Arch. Internat. Physiol. Biochim., 1963, v71, 647.

9. Fabianek, J., Herp, A., Proc. Internat. Sympos. on Nonsteroidal Anti-inflammatory Drugs, Milano, Italy, Sept. 1964, Excerpta Med. Found., Internat. Congress Series No. 82, 1965, p35.

10. Pigman, W., Bull. Soc. Chim. Biol., 1963, v45, 185.

11. Fabianek, J., Herp, A., Pigman, W., Endocrinology, 1965, v76, 408.

12. Pigman, W., Matsumura, G., Herp, A., Proc. Fourth Internat. Congress on Rheology, Providence, 1963. Symposium on Biorheology, ed. A. L. Copley, John Wiley & Sons, New York, 1965, p505.

13. Barker, S. A., Crews, S. J., Marsters, J. B., Stacey, M., Nature, 1965, v207, 1388.

14. Gibian, H., Biol. Méd., 1963, v52, 53.

15. Rosen, H., Blumenthal, A., Panasevich, R., McCallum, J., Proc. Soc. Exp. Biol. and Med., 1965, v120, 511.

Received February 10, 1966. P.S.E.B.M., 1966, v122.

Effects of d-Amphetamine on Plasma and Tissue Electrolyte Concentrations of Aggregated and of Hyperthyroid Mice.* (31116)

KENNETH E. MOORE (Introduced by R. E. Gosselin)

Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, N. H.

Many actions of amphetamine can be influenced by environmental stresses such as forced exercise(1), aggregation or crowding (2), and nonaversive electric grid shock(3). The potentiating effects of thyroid hormones on certain of the actions of amphetamine have also been described(4). The toxicity of amphetamine is markedly enhanced in both aggregated and hyperthyroid mice; similar biochemical changes precede or accompany the death of mice in both groups. For example, in aggregated and in hyperthyroid mice, but not in control mice, amphetamine produces a pronounced depletion of tissue glycogen, hypoglycemia and depletion of tissue norepinephrine stores. The importance of these chemical changes in relation to the enhanced toxicity of amphetamine has been discussed previously(5-8).

Alterations in carbohydrate metabolism are accompanied by electrolyte shifts(9). Catecholamines are also known to affect changes in electrolyte metabolism(10). Since alterations in the tissue contents of both glycogen and catecholamines accompany stress-enhanced amphetamine toxicity, it was realized that certain manifestations of this toxicity might result in part from tissue compartmental shifts of electrolytes. Accordingly, the effects of amphetamine on tissue electrolyte and water content were examined in control, aggregated and hyperthyroid mice.

Methods. Male albino mice (Charles River Mouse Farms) weighing 24-30 g were used throughout this study. Mice used in the aggregation studies were housed in groups of 24-30 until the day of the experiment. The experiments consisted of injecting mice with saline or d-amphetamine sulfate (10 mg/kg) and placing them, 4 per cage, into small wire mesh cages measuring 9 × 9 × 9 cm. Mice used in the 'hyperthyroid' study were injected with 1-triiodothyronine (0.5 mg/kg) on three consecutive days. During this time they were housed in community cages in groups of 24-30. On the fourth day hyperthyroid mice were injected with saline or d-amphetamine sulfate (10 mg/kg) and placed individually into cages similar to those described for the aggregation studies. Four hours before the start of each experiment food but not water was removed from the animal cages. The intra-

* Supported by Grant AM 06275 from Nat. Inst. of Arthritis and Metab. Dis.

peritoneal route was used for all injections. Experiments were performed in a room where the circulating air was maintained at 24° ± 0.5°C.

Mice were sacrificed by decapitation 1.5-2 hours after injection of saline or d-amphetamine. The first few drops of blood from the trunk of each animal were collected in heparinized beakers. Blood from 2-3 animals was pooled and immediately centrifuged to obtain plasma. One hind leg was quickly excised and a sample of skeletal muscle obtained. Slices of liver, renal cortex and cerebral cortex were quickly prepared, weighed in tared 10 ml Erlenmeyer flasks and dried to a constant weight at 95°C. The wet weight of tissue samples ranged from 50-125 mg. Five ml of 0.1 N nitric acid were added to the Erlenmeyer flasks containing the dried tissues and the flasks were shaken for 24 hours at room temperature. This procedure, which is similar to that described by Little(11) and by Page and Soloman(12), completely extracted sodium, potassium and calcium from the tissues since additional electrolyte could not be detected when the 'extracted' tissues were digested completely in concentrated nitric acid. Following appropriate dilutions, the sodium, potassium, and calcium contents of the acid extracts were determined with an Eppendorf flame photometer. For the calcium determination it was necessary to make a small correction for the contribution of sodium to the light emission at 623 mμ. Electrolyte concentrations were based upon the water content of the tissues as determined from the difference between wet and dry weights. Water and electrolyte concentrations were compared using Student's t test.

Results. Aggregated mice. At the time of sacrifice, those aggregated mice that had received saline lay quietly in the cages. Mice treated with amphetamine could be divided into two distinct groups. One group continued to be active (motor activity, fighting, etc.); these mice were designated as 'excited.' Animals in the other group lay quietly in their cages in an apparent exhausted condition; they exhibited labored breathing and often assumed abnormal postural positions (sitting or lying on their backs). These mice were designated as 'depressed.' Under the conditions of this experiment approximately 25% of the amphetamine-treated mice became depressed; from previous studies it is known that these animals die within 4 hours(6).

The water and electrolyte contents of the tissues from aggregated mice are summarized in Table I. The water content and electrolyte concentrations in the plasma and tissues of 'amphetamine-excited' mice were not significantly different from those of 'control' mice. Similarly, in the 'amphetamine-depressed' group there was no significant difference from controls in the water or electrolyte content of skeletal muscle or brain. However, in liver and kidney there was a significant increase in the sodium concentration and a decrease in the potassium concentration. (The reduction of the liver potassium concentration was significant at the 5% but not at 1% level.) There was also a significant decrease in the sodium and calcium concentration of the plasma of amphetamine-depressed mice; the apparent increase in plasma potassium was not significantly different from control.

Hyperthyroid mice. As reported previously (8) injection of 10 mg/kg of d-amphetamine sulfate into mice pretreated with triiodothyronine results in almost 100% mortality. At time of sacrifice all amphetamine-treated hyperthyroid mice were depressed.

The effects of amphetamine on the water and electrolyte content in the tissues of hyperthyroid mice are summarized in Table II. Triiodothyronine pretreatment caused a significant reduction in sodium concentration and an increase in potassium concentration of plasma but had no effect upon the water or electrolyte content of any tissue. Amphetamine caused no significant change in the water or electrolyte concentration in skeletal muscle or brain, but induced a significant increase in sodium concentration and a decrease in potassium concentration in both the kidney and liver. Amphetamine also caused a slight reduction in the sodium and an increase in the potassium concentration in the plasma of hyperthyroid mice; these effects, however, were not significantly different from the effects of triiodothyronine alone.

Discussion. The enhanced toxicity of am-

phetamine in aggregated and in hyperthyroid mice is accompanied by similar behavioral and biochemical events. For example, in both groups amphetamine induces excitement followed by severe depression leading to death. Certain drugs (phenoxybenzamine and chlorpromazine) block the enhanced toxicity of amphetamine in both groups(8,13). In addition, the onset of depression is accompanied by similar chemical changes in the tissues

TABLE I. Effects of d-Amphetamine on Water and Electrolyte Content of Plasma and Tissues of Aggregated Mice.

	Skeletal muscle	Liver	Kidney	Brain	Plasma
Percent water					
Control	77.7 ± .52	69.6 ± .46	76.4 ± .35	79.7 ± .31	—
d-A Ex.	76.7 ± .15	68.3 ± .02	75.1 ± .32	79.1 ± .25	—
d-A Dep.	77.0 ± .49	70.5 ± .80	75.7 ± .91	79.6 ± .40	—
Na (mEq/kg water)					
Control	32.9 ±1.03	41.5 ±1.82	68.4 ±1.08	59.0 ±1.14	134.0 ±3.49
d-A Ex.	32.7 ± .70	41.8 ± .64	66.5 ±1.98	59.6 ± .36	132.2 ±1.33
d-A Dep.	33.4 ±1.16	55.9 ±4.51*†	83.7 ±4.33*†	60.5 ± .93	124.0 ±2.02†
K (mEq/kg water)					
Control	147.0 ±1.38	141.3 ±2.32	113.4 ± .88	134.8 ± .78	7.89 ± .29
d-A Ex.	152.2 ±1.01	140.0 ±1.59	115.2 ±2.86	136.0 ± .71	9.06 ± .52
d-A Dep.	147.9 ±1.35	127.4 ±6.91	105.1 ±3.45*	135.1 ±1.04	8.48 ± .24
Ca (mEq/kg water)					
Control	4.65 ± .17	2.84 ± .07	4.03 ± .10	4.05 ± .20	4.16 ± .05
d-A Ex.	5.50 ± .33	2.98 ± .20	4.01 ± .03	4.02 ± .22	3.96 ± .15
d-A Dep.	4.68 ± .22	3.41 ± .30	4.03 ± .13	5.01 ± .60	3.53 ± .14*

Each value represents the mean ± 1 standard error obtained from 18 saline-treated mice (control), 10 d-amphetamine-treated mice that were excited (d-A Ex.) and 10 d-amphetamine-treated mice that were depressed (d-A Dep.). Plasma electrolytes are reported as mEq/l.

* Those values that are significantly different ($p < .01$) from those obtained from "control mice."

† Those values from "d-A Dep." mice that are significantly different ($p < .01$) from those obtained from "d-A Ex." mice.

TABLE II. Effects of d-Amphetamine on Water and Electrolyte Content of Plasma and Tissues of Hyperthyroid Mice.

	Skeletal muscle	Liver	Kidney	Brain	Plasma
Percent water					
Control	77.7 ± .52	69.6 ± .46	76.4 ± .35	79.7 ± .31	—
T_3	77.2 ± .44	71.3 ± .44	76.4 ± .43	79.9 ± .32	—
T_3 + d-A	77.0 ± .60	70.6 ± .65	76.1 ± .45	80.0 ± .50	—
Na (mEq/kg water)					
Control	32.9 ±1.03	41.5 ±1.82	68.5 ±1.08	59.0 ±1.14	134.0 ±3.49
T_3	35.3 ± .74	45.4 ±1.60	69.3 ±1.45	60.0 ±1.36	120.8 ±1.95*
T_3 + d-A	33.0 ± .95	60.1 ±2.88*†	106.2 ±5.97*†	61.9 ±1.47	110.1 ±3.65*
K (mEq/kg water)					
Control	147.0 ±1.38	141.3 ±2.73	113.4 ± .88	134.8 ± .78	7.89 ± .29
T_3	143.6 ±1.29	139.5 ±1.41	113.5 ±1.75	137.0 ±2.79	9.08 ± .26*
T_3 + d-A	152.0 ±3.30	124.7 ±5.02*†	79.3 ±4.61*†	136.1 ±1.34	9.72 ± .41*
Ca (mEq/kg water)					
Control	4.65 ± .17	2.84 ± .07	4.03 ± .10	4.05 ± .20	4.16 ± .05
T_3	4.73 ± .31	3.64 ± .17*	3.95 ± .21	3.70 ± .21	4.20 ± .15
T_3 + d-A	5.13 ± .43	3.72 ± .21*	4.56 ± .24	3.95 ± .17	4.53 ± .12

Each value represents the mean ± 1 standard error obtained from 18 saline-treated mice (control), 10 hyperthyroid saline-treated (T_3) and 10 hyperthyroid d-amphetamine-treated (T_3 + d-A) mice. Plasma electrolytes are reported as mEq/l.

* Those values that are significantly different ($p < .01$) from "control."

† Those values for "T_3 + d-A" mice that are significantly different ($p < .01$) from those obtained for "T_3" mice.

of both aggregated and hyperthyroid mice. These include depletion of tissue norepinephrine stores, depletion of liver glycogen and hypoglycemia(5-8). As a result of the present study a further comparison can be made with regard to tissue electrolyte changes. In both aggregated and hyperthyroid mice amphetamine caused an increase in the sodium and a decrease in the potassium concentration in kidney and liver; there were no changes in the water or electrolyte content of skeletal muscle or brain. The electrolyte concentration in the plasma changed in a direction opposite to that in kidney and liver, that is, an increase in the potassium and a decrease in the sodium concentration.

The significance of the role that the amphetamine-induced electrolyte changes might play in the death of the aggregated or hyperthyroid mice is not clear. Since previous studies have described similar electrolyte shifts occurring after various types of trauma (14), it would appear that the changes reported in the present paper are part of general chemical changes that accompany any severe stress.

Summary. The enhanced toxicity of d-amphetamine in aggregated and in hyperthyroid mice is accompanied by similar changes in tissue electrolyte concentrations. These changes include an increase in the sodium and a decrease in the potassium contents of liver and kidney and a decrease in the sodium and an increase in the potassium concentrations in the plasma.

The advice of Dr. J. T. Gatzy and the technical assistance of Mrs. L. Sawdy are gratefully acknowledged.

1. Hardinge, M. G., Peterson, D. I., J. Pharmacol. Exp. Ther., 1963, v141, 260.
2. Chance, M. R. A., ibid., 1946, v87, 214.
3. Weiss, B., Laties, V. G., Blanton, F. L., ibid., 1961, v132, 366.
4. Moore, K. E., Fed. Proc., 1965, v24, 518.
5. ———, J. Pharmacol. Exp. Ther., 1963, v142, 6.
6. Moore, K. E., Sawdy, L. C., Shaul, S. R., Biochem. Pharmacol., 1965, v14, 197.
7. Moore, K. E., ibid., 1965, v14, 197.
8. ———, ibid., 1966, v15, 353.
9. Fenn, W. O., Physiol. Rev., 1940, v20, 377.
10. Ellis, S., Pharm. Rev., 1956, v8, 485.
11. Little, J. R., Analyt. Biochem., 1964, v7, 87.
12. Page, E., Soloman, A. K., J. Gen. Physiol., 1960, v23, 327.
13. Moore, K. E., J. Pharmacol. Exp. Ther., 1964, v144, 45.
14. Selye, H., Stress: The Physiology and Pathology of Exposure to Stress, Acta, Montreal, 1950.

Received February 11, 1966. P.S.E.B.M., 1966, v122.

Precipitin and Neutralizing Antibody Response Elicited by *Crotalus atrox* Venom-Antivenom Precipitate. (31117)

A. J. LUZZIO AND G. S. TREVINO (Introduced by F. De Venuto)
U.S. Army Medical Research Laboratory, Fort Knox, Ky.

Diphtheria toxin neutralized with antitoxin was used as an immunizing agent in 1898(1). Other reports followed which indicated that such antigen-antibody complexes were unique in that antibody formation to the toxin was enhanced when compared to using the toxin alone(2,3,4). Similar results were reported for tetanus and serum albumin complexed with specific antiserum(4,5,6,7,8).

This ability of insoluble antigen-antibody complexes, in enhancing antibody formation, could have a 2-fold advantage with extremely toxic antigens. First from the added protection gained by increased antibody, and secondly, because of slower release, it is likely that more antigen could be injected, bound to antibody, than could be given alone without deleterious effects. For example, the greatest difficulty experienced with producing rapid and effective immunity to snake venoms is inherent in the extreme toxicity of the venom and its high resistance to chemical and physical detoxifying agents. These factors dictate the use of venoms in extremely low doses over