as well. The fact that this acid is the major anion of the squid axone(1) and has been suggested as a regulator of irritability in the dog heart(15) prompts study of isethionic acid levels in nervous and other human tissues.

Summary. The derivatives of orally administered ^{35}S taurine have been studied in the urine of normal and mongoloid subjects. Using paper and ion exchange column chromatography, 2-hydroxyethanesulfonic acid (isethionic acid) and sulfate were found to be the major derivatives. One major and two minor radioactive constituents remain unidentified. ^{14}C taurine administration indicated that the isethionic acid is derived directly from taurine rather than from reincorporation of sulfate. The present study also demonstrates the usefulness of anion exchange column chromatography for the separation of ^{35}S taurine derivatives.

1. Koechlin, B. A. Proc. Nat. Acad. Sci. U. S., 1954, v40, 60.

2. Braun, R., Fromageot, P., Biochim. Biophys. Acta, 1962, v62, 548.

3. Welty, J. D., Read, W. O., Shaw, E. H., Jr., J. Biol. Chem., 1962, v237, 1160.

4. Wainer, A., King, J. S., Jr., Goodman, H. O., Thomas, J. J., Proc. Soc. Exp. Biol. & Med., 1966, v121, 212.

5. Gordon, C. F., Wolfe, A. L., Anal. Chem., 1960, v32, 574.

6. Wainer, A., King, J. S., Jr., J. Chromat., 1965, v20, 143.

7. Purdie, J. W., Farant, J. P., Gravelle, R. A., ibid., 1966, v23, 242.

8. Schmidt, C. L. A., Clark, G. W., J. Biol. Chem., 1922, v53, 193.

9. King, J. S., Jr., Clin. Chim. Acta, 1964, v9, 441.

10. Salkowski, E., Chem. Ber., 1873, v6, 744.

11. Schram, E., Crokaert, R., Biochem. J., 1957, v66, 20.

12. Thoai, N. V., Roche, J., Olumucki, A., Biochim. Biophys. Acta, 1954, v14, 448.

13. Smith, I., in I. Smith ed., Chromatographic and Electrophoretic Techniques, Vol. I, Interscience Publishers, Inc., New York, 1960, 96.

14. Irreverre, F., Biochim. Biophys. Acta, 1965, v111, 551.

15. Carta, S., Frontali, N., Vivaldi, G., Rend. Ist. Super. Sanita, 1961, v24, 407.

16. Read, W. O., Welty, J. D., J. Biol. Chem., 1962, v237, 1521.

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Plasma and Adrenal Corticosterone Levels Following Exposure of the Two-Day-Old Rat to Various Stressors.* (32027)

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Previous literature on the response of the neonatal rat to stress indicate an inability of stressful stimuli to activate the pituitary adrenal axis(1-9). Jailer(2) suggested that a post-natal period was necessary for maturation of the pituitary-adrenal system and Schapiro(8) has concluded that the rat goes through a stress-non-responsive period during the first 8 days of life, which he describes as "that stage in an animal's early life when pituitary-adrenal activation is not evoked by stressor agents." Leeman(9) as well as Baca and Chiodi(10) have reported somewhat similar data. But, contrary to the above, we(11,12) and Levine(13,14) have recently demonstrated that such stress situations as heat or electric shock will induce the release of adrenal corticoids in the 2- to 3-day-old rat.

The discrepancy between our results and those of Schapiro and others has been resolved by the finding that the type of stressor employed, the time interval between stimulation and sacrifice, and the endpoint used are all critical parameters(12).

The current study is an extension of our previous findings and investigates qualitatively different stressors to determine which are capable of inducing an increased release

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of corticosterone from the adrenal gland of 2-day-old rats.

Method and materials. The subjects were Purdue-Wistar albino rats or hooded rats. All litters were reduced to 6 pups of the same sex on Day 1 of life. In all but one of the experiments described below males were used. The experimental treatments were administered on Day 2 between 6:00 A.M. and 9:30 A.M. Each experiment employed the split-litter procedure: 2 pups from each litter were randomly assigned to the control condition and 2 pups from the same litter were randomly assigned to each of the experimental treatments. Thus, it was possible to have a control condition and 2 different experimental treatments within each litter of 6 animals. Six litters were employed in each experiment described below, unless otherwise indicated.

Experiment 1: Histamine and ligation. Two control animals were decapitated as soon as they were removed from the maternity cage. Two other animals were injected (I.P.) with 2 mg of histamine dihydrochloride in 0.55 ml Krebs ringer solution. The last 2 animals within the litter had surgical silk tied tightly just above the knee of the right hind leg. The ligature was tight enough to cause the animals to squeal. Following the initial stress each pair of animals was placed in a gallon can partly filled with shavings; the can was covered with a paper towel. Eighteen minutes after stress onset the animals were decapitated.

Experiment 2: Histamine and ligation. The same experimental procedure was followed with 6 other litters except that an interval of 33 minutes elapsed between stress onset and decapitation.

Experiment 3: ACTH. Controls were killed as soon as removed from the maternity cage. Two littermates received an I.P. injection of 25 mu ACTH in .05 ml Krebs ringer solution. These pups were then placed in the gallon can as described above and killed 18 minutes after the injection.

Experiment 4: Cold stress. Two controls were killed immediately while 2 other pups from the litter were placed on crushed ice and covered with a paper towel for 3 minutes; after this they were transferred to a can containing shavings where they remained for an additional 15 minutes. These rats were killed 18 minutes after onset of the experimental treatments.

Experiment 5: Cold stress. The same experimental procedure was followed with 6 other litters except that 33 minutes elapsed between onset of the experimental treatment and decapitation.

Experiment 6: Response of females to electric shock and heat. To determine whether sex differences affected the adrenal response, previous experiments from this laboratory (11,12) were repeated except that female Purdue-Wistar rats were used. Controls were decapitated at once. Two females received 3 minutes of .8 ma. electric shock from a scrambled constant current A.C. shocker. Two other females were suspended by a nylon mesh basket in a thermo-regulated chamber maintained at 63° ± 2°C for 3 minutes. After this all animals were placed by pairs into cans partially filled with shavings and were killed 15 minutes thereafter.

Experiment 7: Response of male hooded rats to heat. To determine whether the findings could be generalized from one strain of rats to another, male hooded rats were subjected to the same heat treatment as described above and in previous experiments (11,12). Again, the controls were killed as soon as removed from the cage and the treated animals were killed 15 minutes after the exposure to heat had been terminated. Nine litters were used.

Corticosterone determinations. To obtain a sufficient quantity of material the blood from each pair of subjects was pooled; in the same fashion the adrenals from each pair were pooled. After this, levels of plasma corticosterone and adrenal corticosterone in each sample of plasma were determined by a modification of the fluorometric procedure of Silber, Busch, and Oslapas(15).

Results. The mean level of plasma and adrenal corticosterone for each experimental treatment is summarized in Table I. The analysis of variance procedure was used to evaluate the significance of the mean differences(16).

TABLE I. Mean Plasma and Adrenal Corticosterone for All Experiments. All animals were 2 days old.

Exp No.	Strain	Sex	Time lapse (min)	No. of litter-mate pairs	Treatment	Plasma (μg/100 ml)	S.E.	Adrenal (μg/100 mg)	S.E.
1	Albino	♂	18	6	Control	10.51	2.84	3.23	.56
				6	Histamine	24.15†		10.24†	
				6	Ligation	12.48		3.74	
2	"	"	33	6	Control	14.54	1.21	4.39	1.20
				6	Histamine	33.69†		23.64†	
				6	Ligation	16.72		5.80	
3	"	"	18	6	Control	12.03	1.54	5.05	1.62
				6	ACTH	30.18*		25.06†	
4	"	"	18	6	Control	13.46	1.23	2.88	.19
				6	Cold	16.30		2.67	
5	"	"	33	6	Control	13.97	2.19	3.73	.45
				6	Cold	20.82		2.83	
6	"	♀	18	6	Control	9.23	2.02	2.22	.59
				6	Shock	14.00		3.04	
				6	Heat	19.06†		6.67†	
7	Hooded	♂	18	9	Control	11.39	.98	4.32	.49
				9	Heat	15.47*		6.16*	
	Albino‡	"	18	20	Control	11.15		3.77	
				6	Shock	14.78		2.87	
				6	Heat	22.61†		7.14†	

* Significant at .05 level when compared with controls.
† Significant at .01 level when compared with controls.
‡ Data from Zarrow *et al*(12).

Effects of histamine and ligation. The histamine injection significantly increased the level of adrenal and plasma corticosterone in both experiments ($p<.01$ in all 4 instances). In none of the comparisons did the ligature have any effect upon the adrenal response.

Effects of ACTH. A significant corticosterone increase ($p<.01$) was found in both adrenal and plasma levels following ACTH injection.

Effects of cold stress. Cold stress increased the amount of plasma corticosterone found 33 minutes after onset of the experiment, and the difference approached significance *via* the analysis of variance ($p<.10$). In addition, in all 6 litters the cold stressed pair had a higher mean value than the corresponding controls. The probability of this occurring by chance alone is $(1/2)^6$ which is significant at less than the .02 level. Cold stress was not significant in any of the other 3 comparisons.

Response of females to electric shock and heat. Females subjected to heat stress differed significantly ($p< .01$) from controls on both the plasma and adrenal measures. No differences were found between shocked and control females and these findings are in complete agreement with our previous data (11,12). In Table I we have presented the mean male values from the Zarrow *et al* paper which are directly comparable to the female data in this experiment. There is good agreement between these two sets of data, indicating that sex is not a critical variable when evaluating plasma and adrenal corticosterone at this age, and that both males and females may be combined in an experiment.

Although the analysis of variance did not find any significant difference between control and shocked females, inspection of the data revealed that, for each litter comparison, the mean corticosterone value was greater for the shocked pair than for the littermate control. This was true both for the plasma corticosterone and adrenal corticosterone measures. The probability of this occurring by chance alone for either measure is less

than .02. Therefore, it may be concluded that electric shock does have a significant effect, but the effect is of a small magnitude. This small difference may be attributed to the 18 minute interval between shock onset and killing. We have previously shown that a larger, and highly significant difference will occur if the time interval is extended to 33 minutes(12).

Response of hooded rats to heat. Heat brought about a significant increase in both plasma and adrenal corticosterone ($p<.05$) in the hooded rat. Though the resting levels for the two strains are very similar, there is a quantitative difference in response to the heat stress with the albino strain responding at a much higher level than the hooded animal.

Discussion. It is apparent from these results that the pituitary-adrenal axis of the 2-day-old rat will respond to a stressful situation, that no sex difference exists at this age and that the same qualitative findings are obtained in the two strains of rats. An increase either in plasma or adrenal gland corticosterone levels was noted following such varied stimuli as ACTH, histamine, shock, and heat. To a limited extent cold also produces an increase but the results of this stimulus were not as clear-cut as the others.

The major purpose of this investigation was to determine whether the adrenal gland of a 2-day-old rat can respond to a variety of stressors. The question is answered in the affirmative except for the experiment using a ligature as the stressor. It is interesting to speculate on the failure of the ligature experiment to result in any change in plasma or adrenal corticoid levels. Fortier (17,18) has "divided stressors into two groups: 'neural' and 'systemic,' according to whether they activated ACTH release solely by the mediation of the nervous system or could act on the pituitary through the systemic circulation." The outstanding feature of the stimulus involved in the ligature experiment would appear to be its neural nature (19). These results would tend to indicate that the pitutary-adrenal-axis is operative in the 2-day-old rat but that the neural component in the system is not active as yet. Indeed Schapiro(20) points out that adequate ACTH is present in the pituitary gland to stimulate the adrenal cortex.

The present findings extend our previous conclusion(12) that activation of the adrenal gland following stress is affected by the type and severity of the stressor used and includes such parameters as dosage, length of treatment or exposure, type of response measured, and time after exposure at which the measurement was taken.

1. Jailer, J. W., Proc. Soc. Exp. Biol. & Med., 1949, v72, 638.

2. ———, Endocrinology, 1950, v46, 420.

3. Irwin, E., Buchanan, A. R., Longwell, B. B., Holtkamp, D. E., Hill, R. M., ibid., 1950, v46, 526.

4. Eskine, I. A., Ann. D'Endocrinol., 1957, v18, 343.

5. Milkovic, K., Milkovic, S., Endokrinology, 1959, v37, 301.

6. Levine, S., Alpert, M., Lewis, G. W., J. Comp. Physiol. Psychol., 1958, v51, 774.

7. Schapiro, S., Fed. Proc., 1960, v19, 154.

8. Schapiro, S., Geller, E., Eiduson, S., Proc. Soc. Exp. Biol. & Med., 1962, v109, 937.

9. Leeman, S. E., Fed. Proc., 1963, v22, 165.

10. Baca, Z. V., Chiodi, H., Endocrinology, 1965, v76, 1208.

11. Haltmeyer, G. C., Denenberg, V. H., Thatcher, J., Zarrow, M. X., Nature, 1966, v212, 1371.

12. Zarrow, M. X., Haltmeyer, G. C., Denenberg, V. H., Thatcher, J., Endocrinology, 1966, v79, 631.

13. Levine, S., VIth Pan American Congress of Endocrinology Excerpta Med. Intern. Congress Series No. 99, 1965.

14. Levine, S., Mullins, R. F., Jr., Science, 1966, v152, 1585.

15. Silber, R. H., Busch, R. D., Oslapas, R., Clin. Chem., 1958, v4, 278.

16. Winer, B. J., Statistical Principles in Experimental Design, McGraw-Hill, New York, 1962, p191.

17. Fortier, C., Endocrinology, 1951, v49, 782.

18. ———, in The Pituitary Gland, G. W. Harris, B. T. Donovan, ed., Univ. of Calif. Press, Berkeley, 1966, v2.

19. Gordon, M. L., Endocrinology, 1950, v47, 347.

20. Schapiro, S., ibid., 1962, v71, 986.

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