Excretion of S^{35} Following Administration of S^{35}-Prochlorperazine to Rats Subjected to Experimental Stress.* (27273)

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In most pharmacological investigations every precaution is taken to ensure that the animals employed are selected from a normal population. In the majority of cases, however, the experimental animal will be used as a test subject for drugs which are intended for the individual in an abnormal physiological state. This study was undertaken to determine what effect experimental stress might have on excretion of 2-chloro-10-[3-(1-methyl-4-piperazinyl) - propyl] - phenothiazine (prochlorperazine), a compound used almost exclusively in the mentally ill, presumably stressed, patient. Fujita and Ging (1) have recently reported that 24-hour urine volumes are 20 to 25% lower in schizophrenic than in nonschizophrenic patients.

Materials and methods. Ten male Holtzman rats weighing 275 to 350 g were employed. Five served as unstressed controls while the remaining 5 were stressed for 12 hours, beginning at time of drug administration, by the bilateral hind leg ligation method of Dexter *et al.*(2). Each rat received 10 μc/175 g in a dose of 25 mg/kg of prochlorperazine (dose expressed as the base, the dimaleate salt was used in this study) as a 2% suspension in 4% acacia intraperitoneally. They were placed in stainless steel metabolism cages for 96 hours during which time urine and feces were collected separately. While in the metabolism cages the animals were allowed food and water *ad lib.* The amount of S^{35} present in the urine and feces samples obtained was determined using conventional liquid scintillation counting technics described elsewhere(3).

Results. Table I indicates the percent of the original dose appearing in the urine of each group of rats during the 4-day period. Animals subjected to stress excreted 33.4% less S^{35} in the urine during the first 24 hours, a difference significant at the 0.01 level. Amounts of S^{35} excreted in the feces of each group were not significantly different.

Discussion. The difference in urinary excretion might be the result of a number of factors, including: (a) altered drug metabolism, (b) change in pattern of drug distribution, or (c) altered renal dynamics, which may be the result of endocrine imbalance produced by stress.

As indicated by the data, the difference in amount of S^{35} excreted in the urine of stressed and control rats occurs in the first 24 hours, after this time the excretion rate is essentially the same in both groups. It has been established that exposure to stress will reduce urine formation in rats(4-5) due to an increase in antidiuretic activity of plasma(6), and in this experiment the stressed rats excreted significantly less urine during the initial 24 hours after drug administration than did the control animals. There was no significant difference in volumes of urine excreted by the 2 groups from 24 to 96 hours. Thus, the major difference in amount of S^{35} excreted occurs during the time when significantly less urine is excreted by the stressed animals. The decreased amounts of S^{35} ex-

TABLE I. Mean Cumulative Percent of Original Dose Excreted in Urine.

Time interval*	Control		Stressed	
	ml urine†	% dose†	ml urine†	% dose†
4	.74	.941	.46	.560
8	1.89	6.997	1.02	2.346
12	3.30	9.085	2.24	6.691
24	8.10	17.868	4.83	11.900
	±.9‡	±.6‡	±.1‡	±1.2‡
36	14.83	21.494	11.21	13.988
48	22.20	22.639	16.38	15.193
60	28.74	22.853	21.95	15.943
72	36.47	22.977	28.72	16.522
84	43.13	23.044	33.79	16.895
96	53.08	23.198	41.59	17.540
	±2.1‡	±.6‡	±9.8‡	±3.5‡

* Hr. † Mean of 5 rats. ‡ Stand. dev.

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creted may, therefore, be simply a result of the decreased urine formation observed in stressed rats. Further studies are being made to determine the disposition of agents in animals under varying physiological conditions.

These results indicate that information derived from certain pharmacological investigations in normal animals may be misleading when applied to agents intended for use in abnormal physiological states.

Summary. Rats subjected to experimental stress (hind leg ligation) excreted significantly smaller amounts of S^{35} in the urine following administration of S^{35}-prochlorperazine than control animals. Evidence is presented that this decreased excretion may be the result of diminished urine formation in stressed rats.

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Measurement of Streptococcal Antigen Synthesis with Fluorescent Antibody.* (27274)

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Previous reports(1,2) have described the various properties of the system in which group A streptococci regenerated the M protein after having been treated with trypsin to destroy that antigen on the surface of the cells. The present communication describes a technic for observing the rate of antigen synthesis photometrically measured by the amount of adsorbed specific fluorescein-conjugated antibody.

Procedure. Type-specific group A streptococcal antisera were prepared according to the method of Swift *et al.*(3); only the highest titer sera, giving strong precipitin reactions with antigen solutions of 1 mg/ml in 10 minutes, were employed. The type 14 streptococcus and the procedures for trypsinization and subsequent M protein synthesis in an amino acid and peptide reaction mixture were as previously described(2).

The gamma-2 fraction of type-specific antisera was isolated by the cold alcohol method of Cohn *et al.* as described by Deutsch(4) and dissolved in a volume of saline equivalent to the original volume of serum. The globulin fraction was reacted with fluorescein isothiocyanate according to the method of Riggs *et al.*, described by Pearse(5). Unreacted fluorescein isothiocyanate was separated from the conjugated globulin by passage through a column of Sephadex. This gel filtration procedure obviated the usual prolonged dialysis; the fluorescent antibody thus obtained contained no dialyzable fluorescent material. The exact procedure was as follows. To a column of Sephadex G-25 (2 × 32 cm) equilibrated with buffered saline, pH 7.0, was added 25 ml of the reacted globulin-fluorescein-isothiocyanate mixture previously centrifuged to remove traces of denatured protein or particles of dye. The solution was applied to the column and eluted with buffered saline at a rate of 2 ml per minute. The first fluorescent band to be eluted

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