these amino acids are added in very low concentration. Rate of uptake of cystine by either normal or "cystinuric" leukocytes is not affected by presence of high concentrations of arginine and lysine. The mechanism of renal tubular concentration may therefore be different in kind from that of leukocytes.

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Effects of Handling on the Adrenalectomized Rat.* (24467)

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Previous studies using normal young albino rats have shown that handling or gentling results in significant increases in weight gain and skeletal length(1,2), superior learning ability(3), and increased resistance to certain forms of stress(4,5). It has recently been shown that hypophysectomy alters some of the response to handling(6). The purpose of this paper is to report the influence of adrenalectomy on certain effects induced by handling.

Methods. Three consecutive experiments were done using young male rats of Sprague-Dawley strain.† Bilateral adrenalectomy was performed in a single operation on 23rd day of life in Exp. I and on 21st day in Exp. II and III. The animals were weighed and then divided into 2 equal groups: 1) Beginning on day following adrenalectomy each animal of experimental group was handled individually for 10 minutes every day at same time of day for 2 months. In each experiment a different investigator handled the animals. Handling consisted of holding the animal in one hand and stroking it repeatedly with the other hand from head to tail with animal facing the handler. 2) Control animals were never touched after their original placement in cages following adrenalectomy. A weighed amount of food in dish (Exp. I and II) or special feeding cup‡ to minimize spillage (Exp. III) was placed in each cage and leftover food weighed to determine individual daily food consumption. Food used in Exp. No. I and II was as follows: 480 g canned dog food (Pard®), 2 slices of crumbled whole wheat bread, and 2.5 ml Vi-Daylin® (liquid multivitamin preparation marketed by Abbott Labs.). These ingredients were thoroughly mixed and formed a soft, adherent mass such that food loss from spillage was minimal. In Exp. III powdered Purina Rat Chow was used. In all experiments 5% glucose in normal saline was supplied as drinking water. Animals were housed

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† Obtained from Hormone Assay Labs., Chicago.
‡ Obtained from A. S. Aloe and Co.

TABLE I. Effect of Handling on Adrenalectomized Rats.

Exp. No.	I			II			III		
Diet	Pard			Pard			Purina		
Exp. group	Not handled	Handled	p†	Not handled	Handled	p†	Not handled	Handled	p†
No. of rats—Initial No.	14	13		11	11		14	14	
Final "	14	13		8	9		6	8	
Initial wt (g)*	49.2 ± 7.1	47.9 ± 6.9	.7	47.0 ± 6.1	48.5 ± 7.5	.6	45.8 ± 2.6	45.7 ± 3.2	.9
Wt gain (g)*	164.5 ± 35.4	166.0 ± 25.4	.9	135.0 ± 40.7	140.0 ± 51.5	.9	187.0 ± 42.7	174.0 ± 45.9	.6
Total food consumed (g)*	2173.0 ±179.5	1999.0 ±277.0	.1	1453.0 ±162.0	1512.0 ±182.0	.5	565.0 ± 59.0	521.0 ±110.0	.4
Food consumed (g)/g wt gained*	13.7 ± 3.2	12.2 ± 1.9	.2	11.3 ± 2.6	11.6 ± 2.8	.9	3.0 ± .3	3.0 ± .3	.9
Maze learning—No. animals tested	14	13					5	6	
Trials to criterion*	103.0 ± 30.0	120.0 ± 38.0	.2				130.0 ± 30.0	118.0 ± 24.8	.5
No. of errors in first 100 trials*	30.0 ± 7.3	33.0 ± 10.3	.4				33.0 ± 9.9	28.0 ± 5.6	.4
Thiourea toxicity—No. animals tested	14	13					5	6	
Mortality	5	5	.9				1	2	.9

* Mean ± stand. error.

† Significance of difference between handled and unhandled groups.

individually in wire cages arranged in rack so that handled and unhandled animals alternated. The animals were weighed weekly in their cages and weight of cages subtracted to avoid handling. The temperature of animal room was maintained at 80 ± 2° F. After 6 weeks animals of each group were tested on a black-white discrimination problem. The apparatus was a T-shaped discrimination box, described elsewhere(3). Either arm of the T could be lighted, and the training was light-positive. The sequence of turns for the lighted alley was L (left) R (right) RLLRLRRL for odd tens of trials and RLLRRLRLLR for even tens. Animals were allowed 5 runs a day for the first 2 days and 10 runs a day thereafter, until each animal reached the criterion of learning (2 successive errorless days of 10 trials each), except that all animals were given at least 100 trials. Record was also kept of number of errors made by each animal. An error was considered to have been made if the animal's body, exclusive of tail, entered the unlighted T-arm. Following completion of testing, animals in Exp. I and III were tested for resistance to a single intraperitoneal dose (50 mg/kg) of thiourea as previously described(7). At end of experiment, the animals were sacrificed and examined for residual adrenal tissue.

Results. Table I shows the data from the 3 experiments. There was no significant difference in food consumption, weight gain, maze learning ability, or resistance to thiourea toxicity between handled and unhandled animals.

Small amounts of residual or regenerating adrenocortical (but no medullary) tissue were found at autopsy in animals from each experiment. Since the autopsies were done about one month after experiments were completed, it is assumed that all animals were deficient in adrenal hormones during the study. A comparison of animals with and without residual adrenal tissue with respect to weight gain, food consumption, maze learning ability and resistance to thiourea toxicity revealed no significant difference.

The results of our experiments indicate that a certain minimum level of adrenal hormones is necessary for the enhancing action of hand-

ling on weight gain, efficiency of food utilization, maze learning ability and resistance to thiourea toxicity previously reported in the normal young albino rat. Whether the effects of handling in question depend on presence of adrenal medullary and/or adrenocortical hormones remains to be determined. The fact that significant effects of handling on weight gain and food utilization occur in the hypophysectomized rat(6) does not resolve this problem. It could be argued that because these changes occur in the absence of ACTH, adrenal medullary hormones (not known to be influenced by hypophysectomy) are the crucial factors. On the other hand, it could be postulated that the diminished effects observed in the hypophysectomized rat (as compared to normal rat) implicate the adrenocortical hormones known to be diminished by hypophysectomy.

Summary. Experiments indicate that the adrenal gland is necessary for the enhancing effects on weight gain, efficiency of food utilization, maze learning ability and resistance to thiourea toxicity previously shown to result from handling, in the intact young albino rat.

ADDENDUM: Experiments completed since this manuscript was submitted indicate that handling does not regularly cause an increased weight gain in normal rats (Sprague-Dawley and Wistar strains) whereas it does consistently increase learning ability and resistance to stress. These findings indicate that the lack of effect of handling on weight gain in the experiments reported in this paper may be due to factors other than the absence of the adrenal glands.

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Susceptibility of Garter Snakes (*Thamnophis* Spp.) to Western Equine Encephalomyelitis Virus. (24468)

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In temperate zones, the importance of a mosquito-bird-mosquito cycle for disseminating western equine encephalomyelitis (WEE) virus in summer is well established(1). A major problem, however, in a study of the ecology of WEE virus is the overwintering mechanism. One such mechanism suggested has been a continuing bird-mosquito-bird cycle in warmer climates with seasonal invasion of colder climates through migrating birds. Reeves *et al.*(2) reported that chronic latent infection of 1 to 10 months' duration occasionally occurs in wild birds experimentally infected with WEE virus and suggested that this may account for maintenance of virus during winter months. However, Kissling *et al.*(1) did not isolate WEE virus from blood of 764 birds wintering in southern U. S. or from 739 birds entering the same area on their spring migration. The relative paucity of evidence that birds served as a winter reservoir of virus or reintroduced it into northern areas each year suggested a consideration of hibernating animals. With regard to mosquitoes themselves, evidence is mounting that they are relatively unimportant as a winter reservoir of infection(3,4,5,6). Hibernating vertebrates were considered, since on theory of latent infection(2), such species, if susceptible, might serve to maintain virus during winter months and to disperse it when vectors become active. Field studies showed that *Culex tarsalis,* the mosquito vector, overwinters in rock piles(5) and that many snakes