indicated to determine to what extent this factor may have contributed to the observed results.

Summary. Experiments were conducted on the effects of graded doses of vit. A on resistance to low environmental temperature and x-irradiation injury in the male rat. The average survival time of rats depleted of vit. A was 1.9 days at an environmental temperature of 2°C and 19.3 days under standard laboratory conditions (23°C). Adjustment to low environmental temperature (as judged by per cent surviving an experimental period of 30 days) was significantly increased in animals fed an average daily intake of 2.5 U.S.P. units of vit. A, but a minimal intake of approximately 5 U.S.P. units of vit. A daily was required for optimal adjustment to cold. A direct correlation was observed between the vit. A content of the diet and resistance to x-irradiation. All rats administered an average intake of 2.5 U.S.P. units of vit. A daily succumbed following a single exposure of 750 r x-irradiation; 90% of the rats receiving a daily intake of 50 U.S.P. units of vit. A and exposed to a similar dose of x-irradiation survived. An average daily intake of 10 U.S.P. units of vit. A was inadequate for optimal resistance to x-irradiation, although this level of vit. A was adequate under standard laboratory conditions for optimal growth.

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Reduction of Urinary Sodium and Potassium Produced by Hypophyseal Growth Hormone in Normal Female Rats.* (19454)

JOHN E. WHITNEY, LESLIE L. BENNETT, AND CHOH HAO LI.

From the Division of Physiology, Institute of Experimental Biology, and Department of Biochemistry, University of California, Berkeley.

Previous work from this laboratory(1) has shown that the administration of hypophyseal growth hormone (GH) to diabetic male rats results in the retention of both sodium and potassium even though the degree of glycosuria may be increased concurrently. It is the purpose of this paper to report that GH produces both sodium and potassium retention in normal female rats.

Methods. Three experiments are reported in this paper designated as Series I, II, and III. In each experiment 10 "plateaued" female rats of the Long-Evans strain were used. Five animals of each group were fed the regular stock diet† and 5 received the same stock diet plus an additional 600 mg of KCl

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† The stock diet consisted of ground whole wheat 67.5%, casein 15.0%, whole milk powder 10.0%, NaCl 0.75%, $CaCO_3$ 1.5%, hydrogenated vegetable oil, 5.25%. To each kg of diet were added 3.5 g Sardilene (fish oil concentrate containing 3000 USP units of vit. A and 400 chick units of vit. D per g).

TABLE I. Effect of Growth Hormone (GH) on Urinary Na and K of Normal Rats.*

Series	Diet	Urinary sodium: Pre-injection control period	Urinary sodium: GH period	Change	p§	Urinary potassium: Pre-injection control period	Urinary potassium: GH period	Change	p§
1	Control	2.2±.01† (30)‡	1.8±.07 (28)	−.4	<.01	1.19±.002 (30)	.95±.002 (28)	−.24	<.01
1	High KCl	2.5±.01 (30)	2.1±.01 (29)	−.4	"	7.3 ±.3 (19)	6.98±.18 (33)	−.33	"
2	Control	1.6±.03 (20)	1.2±.02 (20)	−.4	"	1.24±.02 (20)	.99±.01 (20)	−.25	"
2	High KCl	1.6±.01 (25)	1.4±.02 (20)	−.2	"	6.63±.04 (25)	6.12±.01 (25)	−.51	"

* Values expressed as mEq/rat/day. † Stand. dev. of the mean: $\sigma = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

‡ No. of observations in group. § Comparing the GH period with pre-injection control period. p from Fisher's(9) table of t where $t = \frac{M_1 - M_2}{\sqrt{\left[\frac{\Sigma d_1^2 + \Sigma d_2^2}{n_1+n_2-2}\right] \times \left[\frac{1}{n_1} + \frac{1}{n_2}\right]}}$

per rat per day. The animals were kept in individual metabolism cages and urines were collected daily. The food intake for each animal was constant from day to day throughout the experiment. The methods for collection of specimens, for analyses, and for maintaining the constancy of the dietary intake were the same as those previously reported (1,2). The GH was prepared by one of us according to the previously published method (3) and the same lot of hormone was used throughout. In each case the GH was given twice daily by intraperitoneal injection. The animals in Series I received 500 γ of GH per day for a 7-day injection period which followed a 6-day pre-injection control period, and was followed by a 5-day post-injection control period. The animals in Series II received 300 γ of hormone per day for a 5-day injection period following a 5-day control period. In Series III following a 7-day control period, the animals received initially 25 γ of GH per day for two days, then 50 γ per day for 2 days, then 75 γ for one day, then 150 γ per day for 2 days, and finally 1,000 γ per day for 2 days.

Results and comments. The pertinent data regarding urinary electrolytes are presented in Table I and Fig. 1. The data in Table I show that in both Series I and II there was a significant reduction of urinary Na and K during GH treatment. Significant reduction in the urinary nitrogen also occurred in all groups of animals in Series I and II, and there was no difference between the animals on the control diet and the high KCl diet in this respect. This was in contrast to two previous experiments in which there was significantly greater nitrogen retention produced by GH in the animals on the high potassium diet. Inspection of the data presented in Fig. 1 shows that the diminution of urinary Na and K produced by GH administration was present with doses of hormone as low as 75 γ per day.

That GH causes concurrent K and nitrogen retention is not surprising since both K and protein are essential intracellular components. The retention of Na is probably associated with an expansion of the extracellular fluid volume. This hypothesis is in accord with the demonstration(4) that GH administration to hypophysectomized rats is associated with an increase in the thiocyanate space but does not produce an increase in the muscle Na or any change in serum Na. That these changes in urinary sodium and potassium excretion reflect an actual positive balance for these elements is shown by the fact that fecal analy-

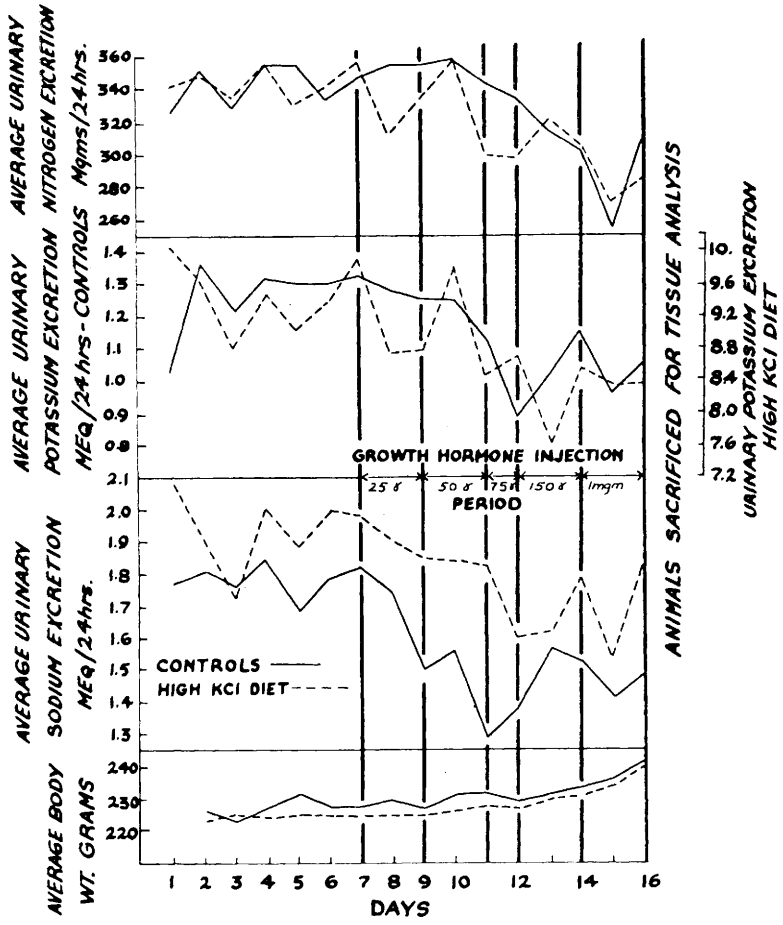

FIG. 1. Effect of growth hormone on average urinary nitrogen, potassium and sodium excretion and body wt of rats fed a high KCl diet.

ses revealed no change in fecal Na or K during the period of GH administration. This was demonstrated in an additional series of experiments which are not reported in detail here although significant reduction of urinary Na and K occurred in these experiments also. It is unlikely that unrecognized contamination of the GH could have been responsible for these results. The GH preparation used was free of antidiuretic activity as assayed by the Ham and Landis(5) procedure at a maximum dose level of 500 γ. At a dose level of 1 mg per day it was free of ACTH activity as measured by the lack of increase in the adrenal weight of hypophysectomized rats(6). A dose of 100 γ in hypophysectomized rats did not cause depletion of adrenal ascorbic acid by the procedure of Sayers *et al.*(7), again indicating that the preparation was substantially free of ACTH contamination. Even if ACTH contamination were present it is unlikely that it would have caused these electrolyte effects, since in our hands and in those of others(8) ACTH has not caused sodium retention in normal rats although it causes potassium loss, the opposite of the effect of GH on potassium balance.

Conclusion. The administration of hypophyseal GH to normal female rats produces retention of both sodium and potassium.

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Study of Ureidosuccinic Acid and Related Compounds in Pyrimidine Synthesis by *Lactobacillus bulgaricus* 09. (19455)

DANIEL S. SPICER, KATHERINE V. LIEBERT, LEMUEL D. WRIGHT, AND JESSE W. HUFF.
From the Research Division, Sharp & Dohme, Inc., West Point, Pa.

Lactobacillus bulgaricus 09 has been found to respond to orotic acid as a growth factor (1-4). Several intermediates in the chemical synthesis of orotic acid have been tested and ureidosuccinic acid was shown to have from 10-20% of the activity of orotic acid(2). In a study of certain natural products it was found that human urine gave a response equivalent to about 100 γ of orotic acid per milliliter(2). Isolation studies in these laboratories have revealed that the microbiological activity of urine is due to urea.

The utilization of urea by *L. bulgaricus* raises the question as to whether or not the organism utilizes only the urea portion of the ureidosuccinic acid molecule in the synthesis of pyrimidines. Both orotic acid and ureidosuccinic acid are utilized for the biosynthesis of pyrimidines as shown by tracer studies(5). In these studies it was demonstrated only that the ureide carbon of ureidosuccinic acid is incorporated into pyrimidines of nucleic acids. Supporting evidence is presented at this time to show that in all probability the entire molecule of ureidosuccinic acid is utilized by *L. bulgaricus* 09 in pyrimidine synthesis.

Experimental. The compounds were tested for orotic acid-like activity by the method of Wright *et al.*(2), with one exception namely that the basal medium contained no added uracil. All compounds were tested alone, but in order to obtain information as to any possible antagonistic or enhancing activity, some were tested in 2 additional ways, with orotic acid at levels of 20 γ per tube and with ureidosuccinic acid at levels of 100 γ per tube. Control tubes of orotic acid were run at levels from 0 to 100 γ per tube and ureidosuccinic acid from 100 γ to 500 γ per tube. Ureidosuccinic acid also was tested using aseptic addition to detect the possibility of structural alteration during autoclaving. No difference was found between the results obtained by the two methods. The compounds tested and the results obtained are shown in Table I. The ureides were prepared by the usual method(6) of treating the appropriate amino acid with an equivalent amount of potassium cyanate in a concentrated aqueous solution, evaporating to a syrup, or in some cases, to dryness on a steam bath, redissolving in water, acidifying with concentrated HCl with cooling and recrystallizing the product from boiling water. The succinyl thiourea was prepared by the dry fusion of succinic anhydride and thiourea (7). The 3-acetyl 2-thiohydantoin was prepared from glycine and potassium thiocyanate(8).

Results. Of the compounds tested, ureidosuccinic acid was the only compound with activity comparable to orotic acid. Urea showed a greater activity than most compounds tested. No significant enhancing effects were noted, and only thiourea showed any antagonism.

Discussion. A comparison of the various ureide structures with that of orotic acid readily shows that ureidosuccinic acid most easily meets the structural requirement as a precursor of orotic acid. That urea itself is