Effect of Growth Hormone on Sulfate Tm, Urea Clearance and Fasting Blood Glucose.*† (22165)

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Growth hormone has a variety of actions in animals(1): it promotes nitrogen storage; it produces diabetes mellitus; and more recently it has been shown to exert a renotropic action in increasing the reduced filtration rate, renal plasma flow and Tm_{PAH} of hypophysectomized dogs(2) and increasing these functions in intact dogs after more prolonged treatment(3).

During a recent study of the effect of diet and hormones on sulfate Tm, we observed that cortisone, as well as a low-protein diet, increased this value(4). Since cortisone tends to accelerate protein catabolism, and because of the intimate relationship of sulfate to protein metabolism, we have considered the possibility that sulfate Tm is influenced by a change in direction of growth hormone, as a protein-anabolic agent, on sulfate Tm. We have measured in addition the changes induced in glomerular filtration rate, renal blood flow and fasting blood glucose.

Methods. Observations have been made on 5 trained, unanesthetized female dogs maintained on laboratory diet which supplied approximately 60 to 100 g of protein daily. They were fasted 16 to 20 hours before an experiment and to insure hydration were given 700 to 1000 cc of water by stomach tube about an hour before the experiment. Glomerular filtration rate and (effective) renal plasma flow were measured, respectively, by clearances of creatinine and PAH which were administered intravenously by means of a constant-rate infusion pump. Na_2SO_4 was incorporated in the infusion fluid to give a concentration of 1% and sulfate Tm was determined at load/T ratios of 1.5 to 2. At low plasma levels of PAH this substance does not interfere with sulfate reabsorption(5). Urea clearance (at least three 15-minute periods) was determined at constant or decreasing urine flows about 2 cc/minute. Creatinine was determined by the method of Bonsnes and Taussky(6); PAH by the method of Smith *et al.*(7); sulfate by the method of Power and Wakefield(8); urea by the method of Seligson and Seligson(9); and whole blood glucose by the Nelson-Somogyi method(10). Three growth hormone preparations were used.§ Two preparations, A and C, were made by the Raben-Westermeyer method(11), and preparation B by the Wilhelmi method(12). Preparation A was in powder form and was dissolved in distilled water acidified to pH 3 with 0.1 N HCl immediately before use. Preparation B (Armour Lots Nos. R 491131 B, R 527094 B, R 491092 B) was supplied as a powder in sterile vials and was dissolved in water before use. Preparation C (G-25-32) was in sterile solution kept frozen before use. Growth hormone was given in single intramuscular injection in the afternoon on successive days, and measurements of renal function were made in the morning after the third injection. In a few instances the hormone was injected intravenously to determine its acute effect over the following 60 minutes.

Results. 3 mg/kg/day for 3 days of preparation B increased sulfate Tm 200% and 68%, respectively, in 2 out of 3 dogs (Table I). (The third dog, No. 2, previously had shown an increase when given a low-protein diet for the first time, but not when placed on a low-protein diet a second time.) Plasma urea concentration and

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TABLE I. Effect of Growth Hormone in 4 Normal Dogs.

Dog	Wt, kg	Hct, %	Conditions	GFR,* cc/min.	RPF,* cc/min.	Sulfate Tm,* μM/min./kg	Plasma urea, mg/100 cc	Urea excretion, mg/min.	Urea clearance,* cc/min.	Blood glucose, mg/100 cc
2	16.3	38	Md†‡	61.9	238	8.3	32.0	12.9	40.5	89
	16.8	44	3 mg B†	78.2	307	8.0	15.0	7.8	50.6	137
5	12.2	44	Md	69.8	205	8.2	48.0	18.4	42.4	103
	11.7	44	3 mg B	86.0	267	24.5	11.1	6.6	59.3	102
4	12.0	37	Md	62.0	194	9.3	21.0	8.6	41.3	97
	12.5	38	3 mg B	80.7	240	15.6	6.7	4.0	60.1	146
	13.4	41	Md × 6 days	73.3	254	7.7	45.0	20.6	45.8	106
1	8.5	39	Md	32.5	92	9.1	32.0	8.6	26.7	93
	9.0	35	5 mg C†§	47.5	150	14.5	23.7	7.0	29.5	119

* Values for GFR, RPF, sulfate Tm and urea clearance are average of 3 periods.
† Md = mixed diet; B = B/kg/day × 3 days; C = C/kg/day × 3 days.
‡ This dog had just received 3 mg A/kg/day for 3 days without effect on renal function or blood sugar.
§ Lactation occurred in this dog with a dose of 3 mg/kg/day on third day, and again in a second trial with 5 mg/kg/day of preparation C after 3 days.

rate of urea excretion decreased in all 3 dogs. Plasma concentration decreased more than urea excretion in consequence of an increase in urea clearance of 25 to 47% over control values.

The filtration rate and effective renal plasma flow were increased by an average of 26% and 28%, respectively. In one dog, No. 5, blood glucose did not increase even though renal function was increased.

The two Raben preparations were less active than the Wilhelmi preparation in all respects. In dog No. 1, 3 mg/kg/day of preparation C for 3 days had very little effect on renal function or blood glucose concentration. Subsequently 5 mg/kg increased filtration rate and renal plasma flow but the effect on urea clearance and protein metabolism was slight (last experiment, Table I).

In experiments not reported here it was observed that 24-hour urine output, along with water intake, was often increased 3- or 4-fold after administration of preparation B. Moreover, during acute experiments listed in Table I, the animals treated with preparation B (in contrast to those treated with A or C) responded to a water load with a greater urine flow, resulting in greater recovery of administered water at the end of 2 hours (Table II). In view of the effect of water diuresis on urea clearance and urea excretion(13,14) we explored the possibility that diuresis in treated animals had increased urea clearance. This figure was therefore compared before and after hormone administration in the same dog at similar creatinine U/P ratios (Fig. 1). In each case the urea clearance was greater, at the same creatinine U/P ratio, after treatment, showing that diuresis was not an important factor.

In contrast to the effect of repeated administration of growth hormone intramuscularly, a single intravenous injection appeared to have no immediate effect. In 6 experiments on 4 dogs, a dose of 3 mg/kg of preparation B injected intravenously after 3 or 4 control periods produced no change in plasma urea concentration, urea excretion, filtration rate, renal plasma flow or sulfate Tm in the following 60 minutes.

TABLE II. Effect of Growth Hormone on Excretion of a Water Load in Normal Dogs.

Dog No.	Conditions	% water load excreted 1 hr	2 hr	GFR, cc/min.	Min creatinine U/P ratio
2	Md*	7	28	61.9	8.8
	3 mg B	17	62	78.2	8.2
4	Md	18	47	62.0	11.1
	3 mg B	19	70	80.7	9.8
1	Md	19	36	32.5	7.2
	5 mg C†	17	37	47.5	9.1
1	Low protein diet × 10 days	6	46	35.2	10.4
	Low protein diet and 1.3 mg B	35	99	49.7	9.0

* Md = Mixed diet; B = B/kg/day × 3 days; C = C/kg/day × 3 days.
† Dog lactating.

Discussion. Though the overall action of growth hormone is to spare protein(1), as exemplified by decrease in urea excretion in our experiments, the hormone tends to increase urea excretion at the expense of body urea in consequence of increased filtration rate. If protein metabolism remains constant, an increase in urea clearance will transiently increase excretion of urea until equilibrium is attained at a lower plasma concentration. In addition, since urea clearance increases with increasing urine flow(13), an increase in water intake and urine flow will further increase urea excretion at a constant filtration rate.

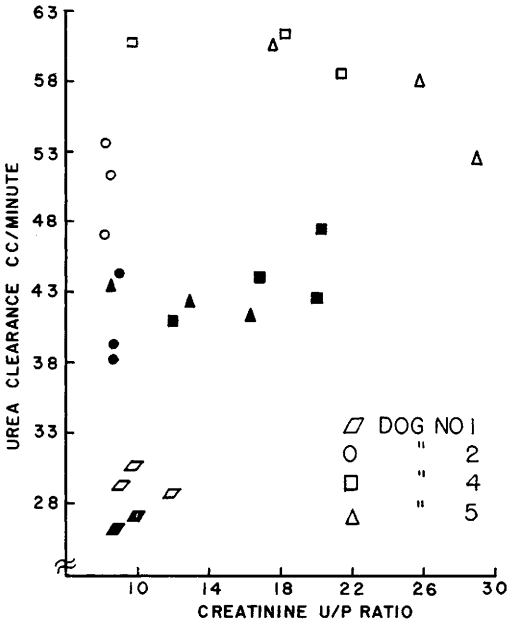

FIG. 1. Comparisons of urea clearances at similar creatinine U/P ratios before and after growth hormone in 4 dogs. Closed symbols, before treatment; open symbols, after treatment. Each symbol is a single clearance period. All observations were made at constant or decreasing urine flows.

Cortisone and growth hormone have, in general, opposite effects on protein metabolism, the first promoting protein catabolism, the second promoting anabolism; but both hormones increase sulfate Tm. Hence the effects on protein metabolism and on sulfate Tm are probably not directly related. The possibility remains, however, that some intermediate change in protein metabolism which may be induced by both hormones, such as, for example, a decrease in plasma level of one or more particular amino acids(15), induces changes in sulfate Tm.

It appears that renotropic action of growth hormone is one of its earliest observable effects. To induce diabetes mellitus, with similarly prepared extracts, either slightly larger doses have been used or injections have been continued for longer periods(16,17). Finally, it should be noted that the Wilhelmi preparation, which in our hands had greater renotropic and nitrogen-sparing effects than the Raben preparations, has been reported by Houssay and Rodriguez(17) to be more diabetogenic.

Summary. Growth hormone increased filtration rate, renal plasma flow, and urea clearance in 4 normal dogs, and sulfate Tm in 3 of these dogs, within 3 days. In 3 of the 4 dogs the fasting blood glucose concentration was elevated. Urea excretion (in short clearance periods) was decreased, but owing to the increased urea clearance, the plasma urea con-

centration decreased relatively more than urea excretion.

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Evidence for Participation of Vit. B_6 in Glutamine-α-Keto Acid Transamination-Deamidation Reaction. (22166)

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In a previous study(1) it was found that, although livers of vit. B_6 deficient rats exhibited markedly reduced glutamate-pyruvate transaminase and cysteine desulfhydrase activities, the glutamine-α-keto acid transamination-deamidation reaction proceeded at about equal rates with livers of control and experimental rats.† This finding, and our inability to resolve the glutamine enzyme by fractionation, were consistent with the concept that vit. B_6 was not involved in the glutamine-α-keto acid reaction, or alternatively, that affinity of the glutamine system for coenzyme was greater than that of the other enzymes studied. The present study supports the latter interpretation.

Patients given isonicotinic acid hydrazide (INAH) for treatment of tuberculosis developed a peripheral neuritis, ameliorated by administration of pyridoxine; such patients were also found to excrete in their urine greater than normal quantities of vit. B_6, apparently in a conjugated form involving INAH(2). These findings suggested to us that administration of INAH might represent a useful experimental procedure for depleting an animal of vit. B_6. We have therefore administered large doses of INAH to several groups of rats, and after periods ranging from 8 to 30 days, examined the livers for glutamine transaminase-deamidase activity in the presence and absence of coenzymes.

Methods. Weanling rats of Sprague-Dawley strain weighing 30 to 45 g, were used in Exp. 1 to 4, and adult Buffalo rats weighing 150 to 250 g were employed in Exp. 5 and 6. Animals were housed in individual cages and given water and diet *ad libitum*. *Exp. 1*: 26 rats were given stock Purina Chow diet; 13 of these were given 600 mg of INAH/k/day by gastric tube, and 13 received approximately same amount in drinking water. After

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† We have subsequently confirmed this observation using weanling rats of the Sprague-Dawley strain; adult Buffalo rats were used in the earlier work(1).