factors which enable the hypophysectomized animal to maintain adequate electrolyte metabolism. Perhaps the hypophysectomized dog, in spite of the lack of adrenocorticotrophic hormone, produces a sufficient amount of adrenocortical steroids to conserve sodium and excrete potassium but not enough to handle water properly. A further possibility would be that the adrenals of the hypophysectomized dog can produce a "salt regulating" but not a "water regulating" hormone. An alternative hypothesis might be that a complete lack of adrenocortical steroids in the hypophysectomized dog is counterbalanced by the absence of one or more pituitary hormones.

The effect of the anterior pituitary growth hormone upon water metabolism in the hypophysectomized dog is the subject of one of the papers of this series.

Summary. Study of hypophysectomized dogs that manifest (a) atrophic changes in all three cortical layers of the adrenals, (b) severe disturbances in carbohydrate and protein metabolism and (c) reduced renal functions (glomerular filtration rate, renal plasma flow and tubular transfer maxima) reveals that: (1) their ability to excrete an orally administered water load is impaired, (2) their ability to withstand sodium restriction is normal and (3) their renal tubules retain the ability to excrete potassium and the animals are able to handle potassium loads almost as well as the normals.

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Effect of Growth Hormone on Water Metabolism in Hypophysectomized Dogs.* (18576)

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During the course of a study of the effects of growth hormone on carbohydrate metabolism in hypophysectomized dogs(1-3) it was observed that the animals developed a severe polyuria. Since there was no glycosuria and the specific gravity of the urine was very low, the increased water exchange appeared comparable to that seen in experimentally induced diabetes insipidus. The polyuria disappeared after cessation of growth hormone administration. These observations suggested that the hypophysectomized dog's impaired ability to excrete a water load(4,5) might, at least in part, be due to lack of growth hormone.

The present investigation deals with the study of the effects of purified anterior pituitary growth hormone on the response to water load and the daily water exchange in hypophysectomized dogs. Glomerular filtration rate and renal plasma flow were concomitantly determined in an effort to evaluate the role of these functions in the alterations in water

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‡ Dazian Foundation Fellow.

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metabolism produced by growth hormone.

Methods. Seven female, hypophysectomized dogs were used. The technic of hypophysectomy§,‖ and the after-care have been described in a previous publication(6). All animals used in this study exhibited maximal sensitivity to the hypoglycemic action of insulin thereby indicating that the hypophyses had been successfully removed(6,7). Thus far 2 of the animals (K-3 and K-8) used in this investigation have come to autopsy. Study of serial sections of a block consisting of a portion of the sphenoid bone, the tissue occupying the region of the sella turcica and the hypothalamus has revealed that neither adenohypophyseal cells (pars distalis, pars tuberalis and pars intermedia) nor cells of the pars nervosa were present in either of the animals(8). The remaining animals will be sacrificed and studied in a similar fashion at a later date. The technics employed for the measurement of renal function (glomerular filtration rate and renal plasma flow), urinary sodium concentration and the response to water load have been described in an earlier paper(5). The daily water exchange was determined by measurement of the 24 hour water intake and urine output. The animals were placed on a constant diet which was mixed with water(3,9). In addition water was provided *ad libitum*. Occasionally, there were periods (several days to 2 and 3 weeks) when the dogs consumed only a part or none of their basic food allotments and at such times, a diet of meat, fish, milk or eggs was substituted. The administration of growth hormone, however, was started only after the animals had been returned to and maintained on their standard diet for at least one week. The food allotment was not increased during the growth hormone administration. Growth hormone (Armour¶—Lot No. 22KR1) was given daily, intramuscularly, 1 mg/kg body weight for 14 to 33 days. In 2 experiments the dose of growth hormone was reduced after 7 days to 0.5 mg/kg. These doses of the hormone (same lot number) produced a marked anti-insulin and diabetogenic action in all hypophysectomized dogs studied(2,3).

Results. The effect of growth hormone was observed in 7 hypophysectomized dogs. Protocols of 2 representative dogs S-4 and E-4 are recorded in Tables I and II respectively.

Response to oral water loads. Before hypophysectomy the dogs excreted the orally administered water load (40 ml/kg) quantitatively in three hours(5,9). Following hypophysectomy the ability of all 7 dogs to excrete the standard water dose was considerably reduced. In dogs S-4 and E-4 the average excretions of the given water loads were 15 and 34%, respectively (Tables I and II). Gastric drainage revealed that no water remained in the stomach 40 minutes after the administration of the water load(5). During the growth hormone regimen the response to the water load progressively increased in every dog, reaching an excretion of at least 50 to 80% of the administered water. On one occasion dog S-4 excreted 109% of the load. After discontinuing growth hormone administration the excretion of the water load gradually declined to the range observed before the hormone regimen.

Daily water exchange. Within 24 hours following hypophysectomy the animals developed the usual transient diabetes insipidus which lasted from several days to 3 weeks. The severe polyuria gradually diminished and

§ Topical Thrombin, which was very helpful in controlling hemorrhage during the operation, was kindly supplied by Dr. A. C. Bratton, Jr. of Parke Davis and Co.

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¶ We are greatly indebted to Drs. E. E. Hays and I. M. Bunding of Armour and Co. for their generous supply of growth hormone.

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TABLE I. Effect of Growth Hormone on Water Exchange, Response to Water Load and Renal Functions in a Hypophysectomized Dog Having Mild Polyuria. Dog S-4.

Date	Status	Body wt, kg	Water exchange,* ml/24 hr: Water intake	Urine output	Urine sp. gr.	% water load excreted, 3 hr	GFR, ml min.	RPF, ml min.
6/13/50	Normal	15.8	700	450	1.028		66.8	320
16						113		
22		14.1	750	480	1.025		75.3	318
6/30/50	Hy-ectomy	14.2						
7/ 1-2			5135	4340				
20-27		15.8	2638	2054				
28			2560	1900			17.1	58
8/ 3-10			1457	1200†				
11			1800	1100†		14		
15			1240	1200†			15.7	61
9/ 7-13		15.2	1019	1020†				
14			1080	950†	1.010	16		
9/26 } 10/ 3 }		16.0	2234	1885	1.005			
10/ 4	Growth H.‡	16.0	1810	1875				
5	"		1980	2075	1.005			
7	"		3270	2625	1.003			
9	"	17.7	3250	2800	1.001	57		
10	"		4158	3815	1.002		26.3	102
12	"		5450	4820	1.001			
13	"		4770	4250	1.002	72		
15	"	18.0	6350	5130	1.002			
16	"		5400	4500	1.002		30.3	104
17	"	18.8	5810	4675	1.001	109		
26	"	17.9	4394	4480	1.002	81		
27	"	17.9	4990	4800	1.002		28.7	98
10/29			4370	3940	1.002			
30		17.8	4190	4270	1.002	68		
11/ 2		18.0	3240	3380	1.004	59		
4		17.3	2820	2830	1.004	57		
8		17.4	2350	2335	1.005	47		
9			2035	1920	1.004		16.3	63
11		17.6	2010	2560	1.004	53		
12-18		17.5	1835	1652	1.007			
12/29 } 1/ 5/51 }		18.4	2300	2060	1.006			

* When the time period is >1 day the water exchange figure represents a 24-hr average for the given period. In no case did the spread between successive days in the averaged periods exceed ±400 ml. GFR: Glomerular filtration rate (creatinine clearance). RPF: Renal plasma flow (p-aminohippurate clearance). Hy-ectomy: Hypophysectomy.

† Dog ate poorly.

‡Growth H.: Growth hormone, 1 mg/kg body wt daily.

the water exchange returned to within normal levels in 2 animals (*e.g.* the urine output of dog E-4 averaged 560 ml between 10/8/50 and 10/13/50), while in the others it remained at a level 2 to 3 times that found normally (*e.g.* the urine output of dog S-4 averaged 1885 ml between 9/26/50 and 10/3/50). The difference between these 2 groups was not due to variations in dietary intake since all animals had consumed their full basic diet for at least one week prior to growth hormone administration. Nevertheless dietary intake influences water exchange as evidenced by dog S-4 (Table I). Between 8/3/50 and 9/14/50 this dog ate poorly and consequently her urine output was approximately one-half that observed during her periods of full diet consumption (7/20-27 and 9/26-10/3/50).

Growth hormone administration resulted in a progressively increasing excretion of urine of low specific gravity containing neither sugar nor increased amounts of sodium chloride. The increased excretion of urine persisted

TABLE II. Effect of Growth Hormone on Water Exchange, Response to Water Load and Renal Functions in a Hypophysectomized Dog Having Normal Water Exchange. Dog E-4.

Date	Status	Body wt, kg	Water exchange,* ml/24 hr: Water intake	Urine output	Urine sp. gr.	% water load excreted, 3 hr	GFR, ml/min.	RPF, ml/min.
2/21/50	Hy-ectomy	15.0						
23-24			6305	5280				
27 } 3/ 5 }		15.3	2144	1794				
3/18-25		15.5	1371	895				
31 } 4/ 6 }		15.7	819	649				
5/22-28		16.4	750	616				
7/25		17.4				33		
10/8-20		18.9	835	560	1.015			
21			800	540			15.7	58
23			845	575		36		
10/24/50	Growth H.†	18.9	1236	900	1.009			
25	"	18.9	750	300	1.016			
26	"		900	450	1.015			
27	"		940	550	1.016			
28	"	19.3	1300	870	1.005	36		
29	"	19.8	1992	1540	1.004			
30	"	18.6	2060	1760	1.002		22.2	77
31	"	19.7	2440	2120	1.003	69		
11/ 1	"	19.8	2845	2155	1.002	79		
2	"	20.0	1990	1500	1.004			63
3	"	20.1	2080	1820	1.004		23.2	73
4	"	19.8	1770	1471	1.006	53		
5	"	19.6	2132	1955	1.005			
6	"	19.6	1370	1260	1.008		23.5	84
7	"	19.9	1530	1091	1.007			
11/ 8/50		19.7	957	725	1.008	50		
10		19.4	750	700	1.011		17.0	65
11		19.6	1080	979	1.010	49		
13-18			892	699	1.012			
12/29/50 } 1/ 5/51 }			905	532				
1/ 6/51		18.5	900	480	1.014	25		

* When the time period is >1 day the water exchange figure represents a 24-hr average for the given period. In no case did the spread between successive days in the averaged periods exceed ±400 ml. GFR: Glomerular filtration rate (creatinine clearance). RPF: Renal plasma flow (p-aminohippurate clearance). Hy-ectomy: Hypophysectomy.

† Growth H.: Growth hormone, 1 mg/kg body wt daily.

throughout the period of hormone treatment. In some animals the polyuria declined from the peak values while still on growth hormone, even though the animals were consuming their full basic diet (dog E-4). Several animals ate poorly during growth hormone administration and although their water exchange was reduced, it still remained well above the control levels. When the hormone injections were discontinued the urine excretion returned to the pre-hormone levels within one to 3 weeks.

Renal functions. In all animals after hypophysectomy there was a considerable decrease in the glomerular filtration rate and renal plasma flow. The filtration fractions did not change appreciably(4,5). During growth hormone treatment both functions increased, approximately in the same proportion. Full restoration of these functions to the pre-operative status was not achieved in any of the hypophysectomized dogs. The improvement of these renal functions prevailed throughout the growth hormone treatment. In 2 dogs some decline from the maximum levels was noted. White, Heinbecker and Rolf(10) observed full restoration of the renal dynamics during growth hormone ad-

ministration with approximately the same dosage of hormone. The discrepancy in results is perhaps due to the fact that there was a greater reduction of glomerular filtration and renal plasma flow rates in our dogs.

Discussion. The data just presented indicate that growth hormone administration to the hypophysectomized dog significantly improves its ability to excrete a water load and increases the level of water exchange. The growth hormone preparation used contained small amounts of adrenocorticotrophic and thyrotrophic hormones(3). However, the administration of ACTH in amounts 50 times that contained in the growth hormone preparation did not significantly alter the water exchange of hypophysectomized dogs. Very large doses of thyroxin did produce a severe diabetes insipidus in hypophysectomized dogs (11) along with signs of thyrotoxicity (*i.e.* persistent tachycardia and marked loss of weight). None of the growth hormone treated dogs exhibited any such signs. The observed effects on water metabolism, therefore, cannot be attributed to these two contaminants.

The mechanism by which growth hormone improves the ability to excrete a water load and increases the level of water exchange in the hypophysectomized dog is not yet clear. The diabetes insipidus produced by interruption of the hypothalamico-hypophyseal tract represents a hormonal deficit in that antidiuretic hormone (and possibly other posterior pituitary hormones) is absent while the anterior pituitary hormones are present and are indeed indispensable for the development of a severe permanent diabetes insipidus. According to Handley and Keller(12) the filtration rate, renal plasma flow and $Tm_{Glucose}$ are reduced in diabetes insipidus. However, White, Heinbecker and Rolf(13) indicate that the reduction in filtration rate, renal plasma flow and $Tm_{Diodrast}$ is only slight and transient when diabetes insipidus is produced without injury to the anterior hypophysis at the time of operation. In any case, the filtration rate in the diabetes insipidus dog is less affected than in the hypophysectomized animal. Further, the action of pitressin on water reabsorption by the tubules is independent of the filtration rate(14). In contrast, the hypophysectomized animal is deficient in all pituitary hormones and as a consequence develops atrophy of all the target glands. These animals manifest great reduction in filtration rate and renal plasma flow, severe impairment of all tubular functions, and inability to excrete a water load. The decreased filtration rate of the hypophysectomized dog appears to contribute to its impaired excretion of a water load and is in all probability a factor in limiting the diabetes insipidus expected in these animals on the basis of their deficiency of antidiuretic hormone.

The administration of growth hormone to hypophysectomized animals results in an increase in the filtration rate and renal plasma flow, improvement of tubular functions, return toward normal of the ability to excrete water loads and the production of a severe polyuria. The increased filtration rate induced by growth hormone appears to be responsible, at least in part, for the improved excretion of a water load. It may also be postulated that, in the absence of effective amounts of antidiuretic hormone, the increased filtration rate results then in severe polyuria. However, the changes in filtration rate and the ability to excrete a water load are not strictly parallel during growth hormone administration (*e.g.* dog E-4, Table II) and a direct hormonal effect on the renal tubular reabsorption of sodium and water cannot be excluded at present. The growth hormone induced polyuria in the hypophysectomized dog suggests that this hormone is one of the anterior pituitary principles the presence of which will permit the development of diabetes insipidus following interruption of the hypothalamico-hypophyseal tract.

The lack of growth hormone is shown to

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11. Unpublished observations.

12. Handley, A. C., and Keller, A. D., *Am. J. Physiol.*, 1950, v160, 321.

13. White, H. L., Heinbecker, P., and Rolf, D., *Am. J. Physiol.*, 1942, v136, 584.

14. Shannon, J. A., *J. Exp. Med.*, 1942, v75, 387.

be responsible for some of the disturbances in water metabolism found in the hypophysectomized dog. It may be a factor in the maintenance of normal water metabolism but probably not in the sense of a direct regulator as is the role given to antidiuretic hormone.

Summary and conclusions. Continued administration of purified growth hormone significantly improves the hypophysectomized dog's impaired response to water load. Concomitantly it increases the daily water exchange to levels seen in surgically induced diabetes insipidus. Growth hormone increases the glomerular filtration rate and renal plasma flow of hypophysectomized dogs but not to the pre-operative levels. It is suggested that growth hormone is one of the factors concerned with the maintenance of normal water metabolism.

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