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THE EFFECT OF PITUITARY GROWTH HORMONE IN DWARFISM WITH OSSEOUS RETARDATION AND HYPOGLYCEMIA AND IN A CRETIN TREATED WITH THYROID¹

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The chemistry and physiology of pituitary growth hormone have recently been reviewed by Li and Evans (1). Pure samples of growth hormone have been prepared by Li and coworkers from bovine pituitary glands (2) and by Wilhelmi and coworkers from a similar source by a somewhat different method (3). This hormone has been prepared and made available to a number of workers for metabolic investigation on human subjects.²

While the recently purified growth hormone causes linear growth, weight gain and nitrogen retention in both rats and dogs (1), no such effect has been reported in humans (4). The increase in inorganic phosphate which is characteristic of growth, has been produced in rats by injection of growth hormone (1) and has been observed in humans with an increased endogenous supply of growth hormone (5), but not in human subjects treated with growth hormone (4). In fact, most individuals who have been treated with growth hormone, have shown an increased excretion of nitrogen, slight elevation of temperature and symptoms of malaise.

A dwarf with osseous retardation and hypoglycemia was selected for these studies because it was surmised that such an individual might be more sensitive to the hormone. Since it seemed possible that some of the findings in this case might be attributable to the presence of thyrotropic hormone in the preparation, an athyrotic cretin, rendered euthyroid by the administration

of thyroid, was selected for the second study in order to eliminate the possibility of a thyroid response.

METHODS

Both patients were placed on a constant diet which approximated the normal intake as closely as possible. The protein, fat, and carbohydrate composition of the diet was calculated from conventional tables and the nitrogen content checked by direct analysis. Each patient was given 1 gm. of salt daily to use in seasoning food. Because of the variable warm weather the fluid intake was not restricted.

Blood was taken for chemical analysis before breakfast. Urine was collected in 24 hour periods and preserved with acetic acid. The determinations were made on daily specimens except in the case of the steroid analyses which were made on pooled specimens.

The chemical determinations were performed by conventional methods (6) with the exception of sodium and potassium and urinary steroids. A flame photometer (7) was used to measure sodium and potassium. The 17-ketosteroid excretion was measured by colorimetric estimation with dinitrobenzene in alcoholic alkaline solution (8) and the neutral reducing lipids of the urine were measured by the phosphomolybdic acid color reaction (9) performed on extracts obtained by chloroform extraction and subjected to benzene-water partition (10) but not Girard's separation.

Urinary excretion of nitrogen was estimated colorimetrically after digestion with sulfuric acid and nesslerization, and the nitrogen content of the diet by the Kjeldahl method (6). The nitrogen content of the stools was calculated as 10% of the nitrogen content of the diet since it has been shown that when the protein content of the diet is normal this is "reasonably accurate" (11).

The growth hormone used in these experiments was prepared from beef pituitaries (2). The potency of the preparation used was equivalent to $110 \pm 6\%$ of that of the standard preparation as assayed by the manufacturers.³

CASE HISTORIES

Dwarfism with osseous retardation and hypoglycemia: The patient (H. L. H. A 67430) was a boy aged $6\frac{1}{2}$

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² Kindly furnished by Dr. John R. Mote, the Armour Laboratories, Chicago, Ill.

³ Lot. No. 6 PKR3, serial No. H 1902. Purified growth hormone assays $204 \pm 36\%$ of the standard noted above.

years. He had been stunted in growth from the age of six months. At the age of five years he started to have hypoglycemic episodes. He was well proportioned but had a height age of only 15 months and weighed only 19 pounds. He showed no evidence of hypothyroidism, being alert and energetic, and having good color and warm, moist skin. X-rays showed a bone age of less than three years. Blood chemical and urine studies are shown in Table I. Fasting blood sugar varied from 88 to 44 mg.%. The serum electrolytes were normal. He was placed on a diet of 25 gm. protein, 35 gm. fat and 110 gm. carbohydrate or 860 calories.

Cretinism: The patient (H. L. H. A 3914) was a girl aged 17 years. When first examined at the age of six years she had a height age of 15 months and a bone age of three months. There were epiphyseal dysgenesis, hypercholesterolemia, carotinemia, and other characteristic signs of hypothyroidism. From the age of six years she had received fairly continuous treatment with thyroid extract in a dose varying from 1½ to 2½ grains daily. Radioactive iodine did not localize in the thyroid region even when thyrotropic hormone was administered. At the time of these studies she was taking 1½ grains of thyroid and appeared to be euthyroid. Basal metabolism was -13% of normal. Blood chemical findings and urinary studies are shown in Table II. She was placed on a diet of 60 gm. protein, 60 gm. fat and 180 gm. carbohydrate or 1,500 calories.

RESULTS

Results of treatment with growth hormone in the case of dwarfism are summarized in Table I. More outstanding than these changes was the clinical response of the patient. He developed anorexia, listlessness and nausea when the dose of hormone was increased to 15 mg. a day after 11 days on smaller doses. When the dose was raised to 20 mg. a day he vomited and although the dose was reduced to 10 mg. a day he did not recover his well-being until a week after the hormone was discontinued.

The malaise was accompanied by a slight elevation of temperature from a previous daily maximum of 36.6° C. to a high of 37.7° C. rectal. There was no local reaction and no evidence of infection. With the anorexia there was a decrease in nitrogen intake so that although there was no rise in urinary nitrogen excretion, there was a negative nitrogen balance and a loss of weight. Five days after the hormone was discontinued, the patient reverted to the previous state of nitrogen balance and weight equilibrium.

TABLE I

Effect of growth hormone in a case of dwarfism with osseous retardation and hypoglycemia

No. days each period	Daily dose of growth hormone	Temperature maximum	Weight at end of the period (kg.) 8.6*	Average daily caloric intake †	Average daily nitrogen intake	Urine nitrogen +10% diet nitrogen	Average daily nitrogen balance	Average urine phosphate	Average urine creatinine	Average urine creatinine	Serum cholesterol level	Serum phosphate level	Serum alkaline phosphatase
days	mg.	° C.		cal.	gm.	gm.	gm.	mg.	mg.	mg.	mg. %	mg. %	units
5	0	36.6	8.6	843	4.2	3.3	+9	240	149	92	320	4.8	8.4
5	0	36.1	8.7	845	4.8	4.1	+7	305	169	155	320	4.8	7.6
5	11	36.4	8.5	869	4.8	4.3	+5	298	159	167	—	—	—
5	35	37.0	8.5	794	4.0	4.1	-.1	299	161	142	296	4.4	7.6
5	70	37.1	8.3	773	3.5	3.3	+2	255	117	141	186	4.4	8.0
5	95	37.6	8.1	738	3.2	3.5	-.3	232	157	121	156	5.4	8.2
5	60	37.7	7.8	708	3.0	3.3	-.3	250	135	98	—	—	—
5	40	37.5	7.7	639	2.4	3.1	-.7	221	152	101	146	4.2	7.8
5	0	37.0	7.7	707	2.9	3.6	-.7	174	147	83	388	4.4	6.8
5	0	36.9	7.8	753	3.0	2.9	+1	181	157	80	270	4.4	5.6
5	0†	37.3	8.2	673	2.9	2.7	+2	210	168	90	—	—	—

* Weight at the beginning of the first period.

† Methyl testosterone 25 mg. day.

‡ Includes only that portion of the diet which was ingested and retained.

TABLE II
Effect of growth hormone in a case of cretinism treated with 90 mg. thyroid extract daily

No. days each period	Daily dose of growth hormone	Temp-erature maximum	Weight at end of period (kg.) [*]	Average daily caloric intake [†]	Average daily nitrogen intake	Urine nitrogen +10% diet nitrogen	Average daily nitrogen balance	Average urine phosphate	Average creatinine	Average urine creatinine	Serum cholesterol level	Serum phosphate level	Serum alkaline phosphatase	Average daily urine volume	Average daily urine sodium	Average daily urine potassium	Average daily 17-keto-steroids	Average daily reducing lipids
days	mg.	° C.		cal.	gm.	gm.	gm.	mg.	mg.	mg.	mg. %	mg. %	units	cc.	mEq.	mEq.	mg.	mg.
5	0	37.3	41.1	1500	10.7	10.7	+ .0	721	509	432	190	3.2	4.0	736	42	68	7.7	.62
5	0	37.4	41.1	1480	10.2	11.1	- .9	806	644	470	190	3.4	4.2	1191	47	65	8.7	.38
3	30	37.6	40.7	1500	10.7	10.6	+ .1	784	615	414	144	4.0	—	815	41	66	8.5	.40
3	90	37.9	41.2	1500	10.7	11.4	- .7	742	652	567	144	3.0	3.8	926	48	61	11.1	.61
3	0	38.7	40.7	1321	9.0	10.6	-1.6	694	600	266	154	4.0	4.4	1205	58	59	10.4	.21
5	0	37.2	40.6	1500	10.7	9.8	+ .9	751	691	472	154	3.8	3.4	1100	53	73	9.5	.38

* Weight at the beginning of the first period.

† Includes only that portion of the diet which was ingested and retained.

There was no sustained increase in the serum sugar, phosphorous or alkaline phosphatase level. The most striking change was a marked fall in the serum cholesterol level from 320 mg.% to 146 mg.% and a return after the cessation of treatment to the previous higher than normal level.

The results of treatment with growth hormone in the case of cretinism are shown in Table II. Just as in the case of dwarfism, the patient developed anorexia, nausea, and vomiting complicated in this instance by severe headache. This reaction commenced on the sixth day of treatment when the dose reached a level of 100 mg. a day. The temperature rose from a previous maximum of 37.6° C. to 38.7° C. rectal. The patient returned to her normal state two days after the hormone was discontinued.

In this experiment there was no significant change in the nitrogen balance except for the last two days of hormone administration and the one day following the cessation of therapy when the lowered intake and vomiting created a large negative balance even though the urinary nitrogen was not increased. During the last period there was a return to positive nitrogen balance. The urinary phosphate excretion did not rise with the urinary nitrogen excretion and in the last period when the urinary nitrogen decreased there was a rise in the urinary phosphate. As in the previous case, there was no sustained rise in serum inorganic phosphate or in serum alkaline phosphatase. The cholesterol level fell slightly during the treatment period but did not rise again to the pretreatment level during the short observation period following treatment with the hormone.

In this case additional studies were made of the urinary excretion of 17-ketosteroid and neutral reducing lipids as well as sodium and potassium. The changes were not striking although there was a definite rise in 17-ketosteroid excretion when the dose of growth hormone was increased.

DISCUSSION

It is apparent from both of these experiments that the pituitary growth hormone preparation employed here did not cause weight gain, nitrogen retention, or a rise in serum inorganic phosphate such as has been reported to occur in species

other than man (1). It did, however, produce a state of malaise characterized by fever and anorexia which resulted in weight loss and a negative nitrogen balance as has been the experience of other investigators working with human subjects (4).

The absence of any positive nitrogen retention or elevation of serum inorganic phosphate in a case of dwarfism with osseous retardation and hypoglycemia is very significant because such a patient should be sensitive to small doses of the hormones which may be diminished or absent.

The absence of nitrogen retention in a treated cretin indicates that the failure of growth hormone to promote nitrogen anabolism cannot be ascribed to the presence of thyrotropic hormone or to any indirect effect upon thyroid activity.

It is unlikely that the presence of small amounts of adrenocorticotrophic hormone could account for the changes noted in these experiments since the adrenocorticotrophic hormone contaminant at the maximum growth hormone administration was less than 5 mg. a day as measured by the standard preparation of the Armour Laboratories. However, adrenocorticotrophic hormone usually produces an increase in nitrogen excretion (12) and the slightly increased excretion of 17-ketosteroids in the second case is compatible with an increased output of adrenal cortical hormone. It is also quite possible that an increased adrenal cortical hormone output was produced by endogenous adrenocorticotrophic hormone as a result of the toxic action of the growth hormone.

The consistent fall in the serum cholesterol level of both patients treated with growth hormone is of interest. In the athyrotic patient this decrease in cholesterol could not have been the result of thyrotropic hormone. A fall in the serum cholesterol has been reported with large doses of adrenocorticotrophic hormone (13), but less than 5 mg. a day could not be responsible for this change. It is possible that the fall in the serum cholesterol level reflects the general decrease in body fat stores which takes place when growth hormone is administered to animals (14).

The failure of growth hormone to effect the same changes in the human as have been observed in laboratory animals may be the result of improper dosage, the development of an allergic state, a species peculiarity or the presence of im-

purities in the preparation used. A similar phenomenon has been observed in dogs where a single large injection of growth hormone may cause increased nitrogen excretion with or without loss of weight (15). A further similarity between our findings and those reported by Bartlett and Gaebler in dogs is the occurrence of increased nitrogen excretion without an increase in the phosphate excretion. These authorities concluded that the negative nitrogen balance was the result of "impurities which are not chemically or biologically demonstrable." They noted further that the nitrogen loss was particularly likely to occur when single large doses of the hormone are administered and that the effect might be produced when either beef or hog pituitary growth hormone was employed.

The production of increased nitrogen excretion by the administration of growth hormone cannot be explained by the presence of contaminating thyrotropic hormone since it occurred in a cretin. It can be explained only partially by contamination with adrenocorticotrophic hormone since it is unlikely that there was enough of this substance present in the doses given to produce the observed effects, and since even large doses of adrenocorticotrophic hormone do not cause the discomfort and fever which have been observed regularly with growth hormone in the human.

SUMMARY

Pituitary growth hormone was administered to one patient with dwarfism, osseous retardation, and hypoglycemia and one patient with treated cretinism. Neither patient showed nitrogen retention or a rise in the serum inorganic phosphorous. Both patients developed fever and malaise. There was a slight increase in the urinary excretion of 17-ketosteroids in the second case. Both cases showed a fall in the serum cholesterol level which was most marked in the patient with dwarfism. Both cases showed a slight decrease in phosphate excretion.

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