served changes could not be ascribed to either technical errors or to disturbances of rats by extraneous laboratory noises. Potential errors in analytic technique were eliminated by routinely running calibration checks on freshly prepared standards. Noise was eliminated as a factor on the basis of other experiments in our laboratory where no differences were found between ion levels of rats that were exposed to noises approximating jet-engine intensity levels and ion levels of nonexposed controls. In the latter studies, significant day-to-day fluctuations in serum sodium and potassium occurred although the means, standard deviations, and coefficients of variation were the same in noise-exposed and control rats bled at corresponding times on any one day.

The net effect of these investigations points to the need for considering diurnal and day-to-day variations in serum ions when dealing with electrolyte changes in animals. Rigid standardization of the time of sampling is mandatory in experiments when small numbers of animals are used to establish "normal" ion levels and when the interpretation of electrolyte shifts is predicated on the assumption that such levels represent a stable base line.

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Preparation of Growth Hormone from Pituitaries of Man and Monkey

Despite occasional suggestions of effectiveness (*1*), growth hormone prepared from the pituitaries of slaughterhouse animals has thus far been generally ineffective in man. Preliminary tests by Beck and Venning (*2*) have shown pronounced anabolic effects in man with growth hormone extracted from human and from simian pituitaries, and we wish to record the method of preparation of the materials used in these tests (*3*). The preparation from monkey was also found by Knobil, Wolf, and Greep to be effective when it was tested in monkeys, although bovine and porcine preparations were inert in that species (*4*).

Table 1. Weight yield of various fractions during preparation of growth hormone from pituitaries of three species. Human and monkey pituitaries were used as obtained, without removal of posterior lobe; the pig preparation was made from anterior lobes only. The values cited were obtained from human pituitary batches of 11.25 g and 24 g of acetone-dried powder, two batches of monkey pituitaries (20.8 and 13 g of powder) and many batches of pig pituitaries.

	Man		Monkey	Pig
	Batch 1 (%)	Batch 2 (%)	(%)	(%)
Acetone-dried pituitary powder	100	100	100	100
Acetone precipitate from glacial acetic extract	4.5	5.4	7.2	7.0
Ether precipitate—"crude extract"	9.4	13.2	20.6	12.0
Adsorbed by oxycel—"corticotropin fraction"	0.16	0.26*	0.14	0.24
Second oxycel adsorption	0.12	0.4*	0.26	0.20
Precipitate at *p*H 8.5	3.12	4.6	7.2	~7.0
Alcohol precipitate—"growth hormone"	3.1	4.6	3.0	1.5

* Double amounts of oxycel were used with batch 2.

The human pituitaries were collected post-mortem and stored in acetone after the amount necessary for microscopic study had been removed (*5*). The activity of the growth hormone survived the delay between death and autopsy, in agreement with observations on the survival of activity in animal pituitaries (*6*). The pituitaries from monkeys were collected in the laboratories of several pharmaceutical companies from rhesus monkeys used in the preparation of poliomyelitis vaccine and were stored frozen (*7*).

Acetone-dried pituitary powder was prepared by homogenization in acetone with a Virtis 45 or Waring Blendor, further washing with acetone on a sintered-glass filter, and desiccation in a vacuum. Growth hormone was extracted and purified by the method originally devised for porcine glands (*8*). The procedure involved extraction with glacial acetic acid at 70°C, removal of an acetone precipitate, precipitation of a crude fraction with ether, removal of corticotropin and intermedin from a weak acetic acid solution with 11 percent COOH oxycellulose (*9*), removal of a *p*H 8.5 precipitate, and precipitation of growth hormone with ethyl alcohol. The weight yield from pituitaries of man and monkey was greater than the yield from pituitaries of pig (Table 1). The activity per unit weight as assayed in the hypophysectomized rat was approximately the same for the three species, with the human material perhaps slightly more active, and the monkey slightly less active, than the pig. It is assumed that the product is chemically impure, in view of the recent finding that animal growth hormone prepared by this and other methods can be further fractionated chromatographically (*10*).

Certain features of this method of preparation of growth hormone made it particularly suitable for its present use. Storage of the human pituitaries in acetone simplified the collection of glands, treatment of the pituitary powder with acetone, ether, and hot glacial acetic acid provided strong bactericidal and viricidal action in the extraction of human pituitaries of indeterminate origin, and the virtual absence of thyrotropin, as well as the low degree of contamination with other pituitary hormones, made the final product well suited for clinical use.

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Metabolic Effects of Human and Monkey Growth Hormone in Man

The growth-promoting activity of crude anterior pituitary extracts was first demonstrated by Evans and Long in 1921 (*1*). Li and his associates (*2*) isolated and characterized the properties of the hormone responsible for this effect in 1944 and summarized the diverse physiological effects observed in animals in 1951 (*3*). Wilhelmi, Fishman, and Russell in 1948 (*4*) and Raben and Westermeyer in 1951 (*5*) developed procedures for crystallizing growth hormone in good yield, permitting wider exploration of its metabolic effects in animals and man. While previous work in animals was confirmed and elaborated using these preparations, the data on the effects in man, particularly with regard to its protein anabolic action and its influence on carbohydrate metabolism, are at best inconclusive (*6*).

Shorr and his associates (7), using growth hormone prepared according to the method of Wilhelmi *et al.*, in two female subjects of short stature, showed some enhancement of nitrogen, calcium, and phosphorous storage as well as impairment of glucose tolerance and increased insulin resistance. The same report included data on three males of short stature who failed to exhibit any metabolic response to growth hormone. In addition, Knobil (*8*), in a study of these preparations in the normal and in the partially pancreatectomized rhesus monkey, failed to elicit any physiological effect. Wilhelmi (*9*) and Knobil (*10*) suggested that the failure to demonstrate significant and consistent metabolic effects in the rhesus monkey and in man with preparations of bovine growth hormone might be the result of species differences in the growth hormone. Support for this concept was provided by the further work of Knobil (*11*), who found monkey growth hormone to be metabolically active in monkeys. This preliminary report deals with some of the metabolic effects observed in a human pituitary dwarf during the administration of human and monkey growth hormone prepared by Raben (*12*).

A 13-year-old male with well-documented hypopituitarism secondary to a craniophyaryngioma was studied on the metabolic ward of the Royal Victoria Hospital in Montreal, Quebec. Replacement therapy from May 1952 to April 1956 had consisted of methyl testosterone administered intermittently with resultant growth in height and maturation of skeletal age. This therapy also resulted in moderate genital maturation and the development of sparse axillary and pubic hair. For the immediate four months prior to this study, daily replacement therapy consisted of thyroid extract, 60 mg; cortisone acetate, 15 mg; and methyl testosterone, 10 mg. The latter was stopped 16 days prior to the beginning of this study, while the thyroid and cortisone administration was continued. There was no alteration in weight associated with the cessation of androgen therapy.

During the period of study, a known dietary intake was given consisting of 80 g of protein, 60 g of fat, and 178 g of carbohydrate, a total of 1700 kcal per 24-hour period. The sodium and potassium contents of the diet were 86 and 88 milliequivalents per 24 hours, respectively, the calcium and phosphorus contents 1358 and 1390 mg per 24 hours, respectively. Corrections were made for dietary returns when they occurred. The fluid intake was kept constant at 2500 ml per 24 hours.

A wide variety of determinations were performed on daily 24-hour urine collections, 72-hour stool collections, and frequent blood samples. The preliminary data concern the effects of growth hormone on the nitrogen, potassium, phosphorus, calcium, and sodium balance, as well as on the urinary excretion of aldosterone.

The human growth hormone was prepared from pituitaries obtained at autopsy (*13*), and the monkey growth hormone from pituitaries made available as a consequence of the poliomyelitis vaccine program. The extraction procedure was carried out by M. S. Raben (*12*). The growth hormone in a slightly acid *p*H was administered intramuscularly every 6 hours. There were no local reactions to its injection, but certain personality changes occurred (*14*). In addition, the patient was oliguric on the fifth day and anuric on the sixth day of administration of monkey growth hormone.

The data on metabolic balance are summarized in Table 1. Human and monkey growth hormone resulted in a significant enhancement of nitrogen storage, which was evident on the second day of its administration. During the ad-

Table 1. Data on the metabolic balance. Day 1 was 26 Aug. 1956. HGH, human growth hormone; MGH, monkey growth hormone; meq, milliequivalents.

Day of study	Therapy (mg/ 24 hr)	Wt.	N balance (g/ 24 hr)	P balance (mg/ 24 hr)	Ca balance (mg/ 24 hr)	K balance (meq/ 24 hr)	Na balance (meq/ 24 hr)	Urinary aldosterone (mg/ 24 hr)
1		73¾						
2		73¾	+ 0.36	− 8.3	+ 50.6	− 9.0	− 5.0	
3		74½						
4		73½						
5		73½	+ 0.8	− 4.6	+ 77.3	− 2.8	+ 32.5	2.8
6		73¼						
7		73						
8		74½	+ 2.5	+ 78.3	+ 15.0	+ 4.5	+ 15.0	2.8
9	HGH	73¼						
10	10	73						
11	10	73	+ 4.2	+ 464.0	+ 340.0	+ 28.1	+ 58.5	6.1
12	10	74¼						
13	20	74½						
14	20	75	+ 5.2	+ 578.3	+ 434.0	+ 15.1	+ 69.3	11.6
15	20	74¾						
16	20	76						
17	20	75	+ 5.9	+ 392.0	+ 266.6	+ 11.3	+ 54.8	14.2
18	20	75						
19	20	75½						
20	20	76	+ 5.8	+ 475.0	+ 355.5	+ 42.0	+ 57.6	20.2
21		75½						
22		76	+ 5.0	+ 180.0	+ 81.0	+ 8.4	+ 23.9	14.2
23		76½						
24		75½						
25		74½	+ 3.1	+ 279.0	+ 171.0	+ 10.0	+ 4.1	5.5
26		74½						
27		74½						
28		73	+ 3.7	+ 321.0	+ 213.0	+ 15.7	− 11.3	3.2
29	MGH	73						
30	40	73						
31	40	76	+ 5.6	+ 546.0	+ 525.0	+ 36.9	+ 28.2	7.7
32	40	76						
33	80	76						
34	80	75½	+ 7.6	+ 528.0	+ 287.3	+ 39.1	+ 65.9	12.0
35	80	74						
36		73						
37		73¾	+ 4.5	+ 407.0	+ 433.3	+ 18.6	+ 75.7	7.5
38		73¾						
39		73¾						
40		74	+ 1.2	+ 128.3	− 188.3	− 3.1	− 22.1	9.3