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Received July 29, 1952. P.S.E.B.M., 1952, v81.

Effects of Single Intravenous Injections of Pituitary Growth Hormone to Normal Adult Men.* (19765)

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Highly purified preparations of pituitary growth hormone† are capable of inducing consistent effects in laboratory animals. Thus growth hormone accelerates the growth rate of both hypophysectomized and normal animals(1); induces a permanent state of diabetes mellitus in certain species(2),‡ increases the ketosis of the fasting state(3); decreases total body fat(4); and diminishes the urinary excretion of sodium and potassium(5). In contrast this hormone has, in general, been ineffective in man. Recently, however, positive nitrogen and phosphorus balance has been reported with growth hormone in a case of hypopituitarism(7), and hyperglycemia of short duration has been observed in a patient with pancreatic islet cell tumor(8).

It is the purpose of this paper to report briefly experiments which demonstrate consistent, rapidly occurring effects of a highly purified growth hormone preparation§ in man.

Methods. Eight subjects were studied, 7 normal men (ages 20-29) who were ambulatory except during the experiment, and a 42-year-old patient with cancer of the stomach and malnutrition. Five of the normal subjects were admitted to the Metabolic Ward of the Royal Victoria Hospital for 4 days. Dietary intakes were not controlled before or during the course of the experiments. On day 1 no food was taken after 6 p.m. The bladder was emptied at midnight and 500 cc of water administered orally. On day 2 urines were collected at 6 a.m. and hourly thereafter until 1 or 2 p.m. Amino acid nitrogen(11), glucose, phosphorus and creatinine (Evelyn modification of Folin-Wu) determinations were done on these specimens. 250 cc of water were given at 6, 7, 10 and 11 a.m. and 12 noon to insure adequate urine volumes but the fast was continued until the last urine was collected. Beginning at 8:15 a.m. on day 2, 320 cc of an amino acid solution (1.24% amino N) was administered intravenously at a constant rate over a period of 26 minutes. Venous blood samples were drawn without stasis and the following determinations done: amino acid

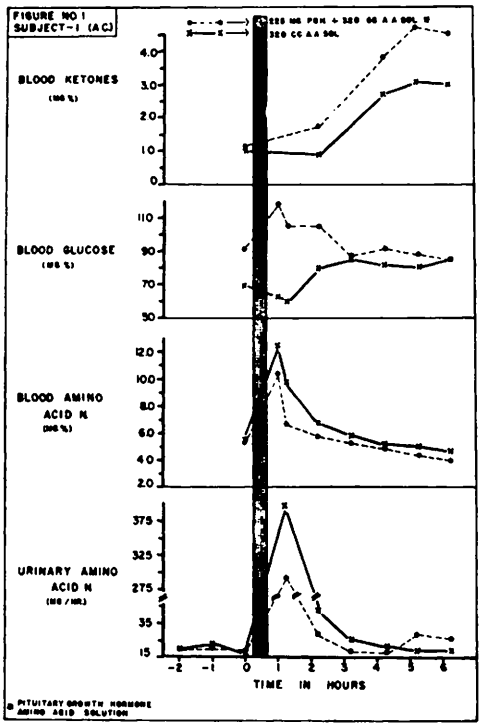
* The expense of these studies was partially defrayed by the National Research Council, Ottawa, the Damon Runyon Memorial Fund, and Frank W. Horner Ltd., Montreal.

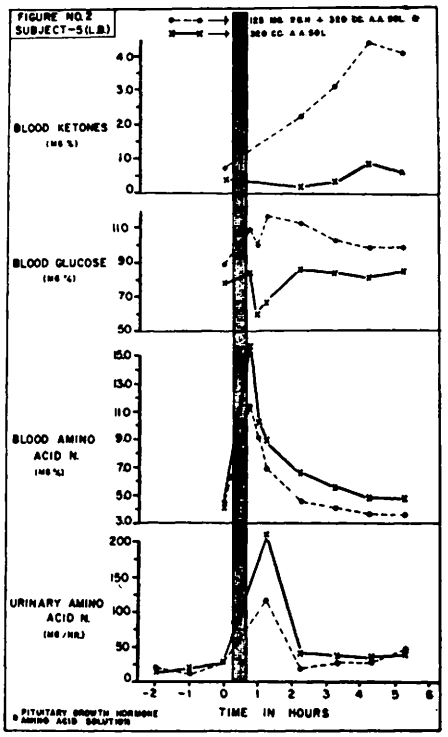
† Hereafter referred to as growth hormone.

‡ Raben and Westermeyer(6) reported that they had obtained a purified growth hormone preparation which did not exhibit diabetogenic properties in 3 dogs.

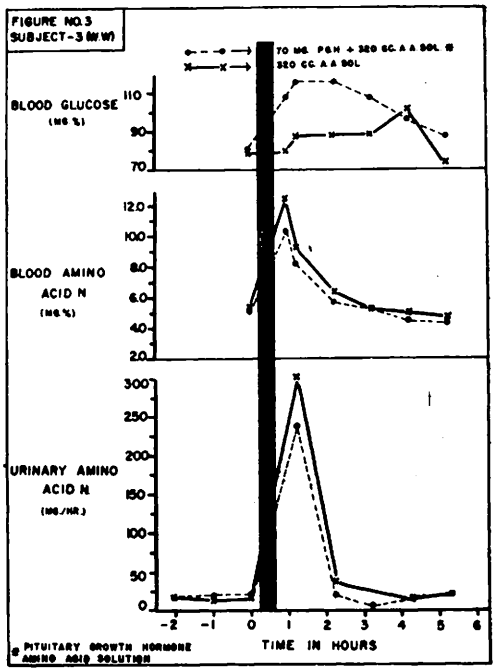
§ Generously supplied by Dr. Leonard Mitchell of Frank W. Horner Ltd., Montreal. This preparation was isolated from beef pituitaries by the Wilhelmi method(9). Its biological activity is equal to that of the electrophoretically homogeneous preparation of Li(10). It is very low in ACTH, TSH, gonadotropin and pitressin activity by biologic assay.

‖ Kindly supplied by Dr. J. H. Laurie of Merck Co. Ltd., Montreal. This solution contains 12 amino acids; 10 are the l-form, 1 the d-l (tryptophane).





nitrogen(12), glucose (Folin-Wu), non-protein nitrogen (Evelyn modification of Folin-Wu), ketones(13), eosinophiles, phosphorus (Fiske-Subbarow) and alkaline phosphatase (King-Armstrong). Amino acid nitrogen, glucose, ketones, eosinophiles were determined on heparinized blood. Day 3 was a rest period. On day 4 the injection of amino acids was repeated with the addition of growth hormone (through the rubber tubing of the I.V. set) at a constant rate over the entire injection period. Three dose levels of growth hormone were used: 1, 2 and 3 mg/kg body weight. In two normal subjects the amino acid solution was administered the second time without growth hormone. In the patient with cancer the order of the experiment was reversed, that is the hormone given on day 2 and 250 cc of amino acid solution administered over a period of 30 minutes instead of 320 cc over a period of 26 minutes.



Results. Fig. 1, 2 and 3 illustrate the changes observed in blood and urinary amino acid nitrogen levels, blood glucose and blood ketones in 3 cases at the various dose levels. The principal changes in blood and urinary amino acid nitrogen in seven experiments are presented in Tables I and II. In the control experiments the blood amino acid nitrogen

TABLE I. Blood and Urinary Amino Acid Nitrogen Data.

Subject	Dose of PGH	Blood amino acid nitrogen (mg %) — Fasting — Control	Fasting — PGH	Maximum* — Control	Maximum* — PGH	Diff.	% diff.	Total urinary amino acid nitrogen (mg) — Control	PGH	Diff.	% diff.
1–A.C.	225 mg (3 mg/kg)	5.44	5.30	12.52	10.40	1.98	29	517.1	406.2	110.9	21
2–R.B.	200 mg (3 mg/kg)	5	4.80	11.54	9.24	2.10	32	340.3	252	88.3	25
3–W.W.	70 mg (1 mg/kg)	5.28	5.92	12.52	10.50	2.66	36	377.9	292.3	85.6	23
4–D.K.	125 mg (2 mg/kg)	4.80	4.88	16.16	13	3.24	28	332.2	254.2	78	23
5–L.B.	125 mg (2 mg/kg)	4.48	4.72	15.38	11.52	4.10	37	332.1	232.1	100	30
7–M.I.†	92 mg (2 mg/kg)	4.88	4.72	18.04	13.88	4.16	31	372.5	198.7	173.8	47
8–V.S.‡	0	5.20 5.26		12.04 11.50		.60	8	245.8 279		33.2	11

* In subjects 1, 2 and 3 these samples were taken 15 min after the intrav. infusion was ended, in the others 5 min. after the end of the infusion.
† Subject with cancer of the stomach and malnutrition. ‡ Subject who received amino acid solution alone twice.

TABLE II. Time Required for Blood Amino Acid Nitrogen Levels to Return to Fasting Level after Start of the Amino Acid Infusion (in Hr).

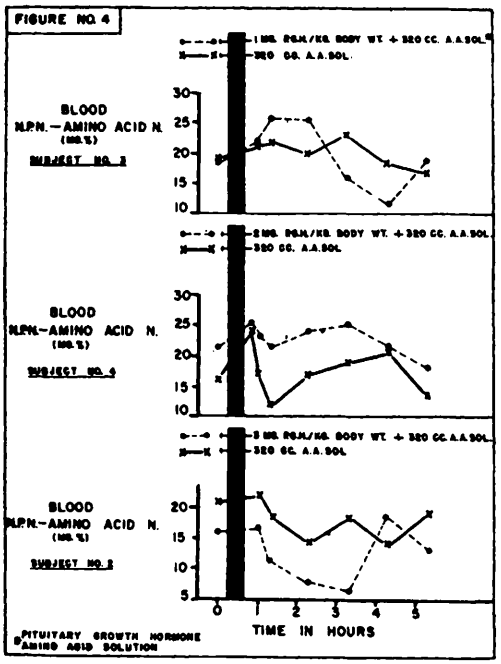
Subject	Control	Growth hormone
1	4	3
2	3	2
3	3	2
4	3	1
5	5	2
7	5	3
8	3	—
(2 controls)	3	

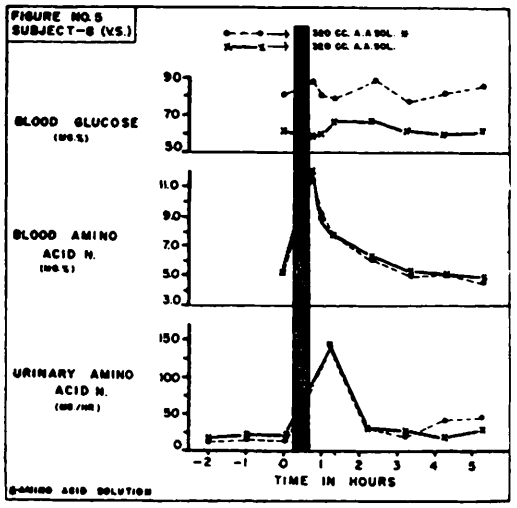
values rose to a maximum immediately after the cessation of the amino acid infusion, returned to fasting levels within 3 to 5 hours and remained approximately at this level, the terminal values falling slightly in two cases. With growth hormone the maximum values were reached at the same time but were consistently lower than the controls by 1.98 to 4.16 mg %, which represents an average of 32% (range 28-37%) less than the control values, and they remained below control figures throughout. The values returned to fasting levels within 1 to 3 hours as compared with 3-5 hours in the controls, and subsequently fell below the fasting values, remaining so for the duration of the experiment. Blood non-protein nitrogen minus amino acid nitrogen values were not increased in the growth hormone experiments (Fig. 4).

The total excretion of amino acid nitrogen from 8 a.m. to the end of the experiment was 68-111 mg less under the influence of growth hormone. This represents an average reduction of 24% (range 21-30%) in relation to control values.

The blood glucose levels during the first part of the experiment were consistently elevated under the influence of growth hormone, whereas in the controls a depression of the fasting level usually resulted at this time. Glycosuria was not observed under the influence of growth hormone.

In the 3 cases in which blood ketones were studied, the ketonemia of fasting was accentuated by growth hormone: in subject 1 the increase in the maximum blood ketone value was from 3.18 to 4.80 mg %, in subject 4 from 2.81 to 3.75 mg %, and in subject 5





from 0.87 to 4.39 mg % in the last 2 hours of the experiment. Serum and urinary phosphorus and serum alkaline phosphatase showed no consistent variation from control values. In all but 2 cases (those receiving 3 mg/kg) blood eosinophiles remained the same or showed the usual diurnal variation. With the 3 mg/kg dose of growth hormone there was a depression below control curves. Blood hematocrit values in 2 cases so studied were not significantly altered.

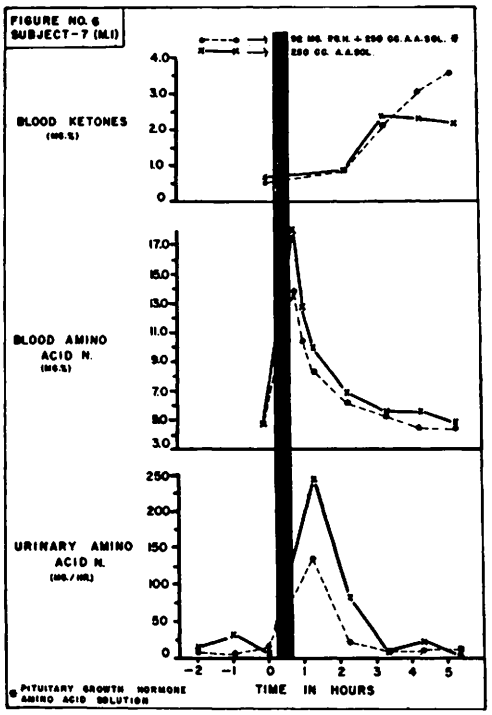
Of the 3 subjects tested, No. 3 and 4 showed a marked increase in the urinary sodium retaining activity (method devised by Miss Bertha Singer, based on the retention of radioactive sodium in adrenalectomized rats); in the third subject (No. 2) the assay was unsatisfactory.

In the patient with malnutrition and cancer, reversing the order of the experiment did not qualitatively alter the amino acid nitrogen results (Fig. 6). With growth hormone the maximum blood amino acid nitrogen value was 30% less and the total amino acid nitrogen excretion in the urine 47% less than the control values. The maximum blood ketone value with growth hormone was 1.33 mg % higher than the corresponding control figure. The glucose curves could not be interpreted because the patient had a diabetic glucose tolerance curve.

In order to determine whether the prior administration of the amino acid solution influenced the effect of the subsequent infusion, 2 amino acid infusions alone were administered to two normal subjects on days 2 and 4. In one the maximum blood amino acid nitrogen value was 8% less in the second experiment but subsequent values were either slightly above or below the corresponding ones in the first (Fig. 5). The total quantity of amino acid nitrogen excreted in the urine during the second experiment was 11% higher than during the first. The curves were similar in the second control.

The 2 subjects receiving the largest dose (3 mg/kg body weight) had nausea and vomited a small amount. The amount of amino acid nitrogen in the vomitus was negligible. Two subjects receiving 2 mg/kg body weight complained of a mild, transient headache. The remaining 2 subjects had no symptoms. None of the subjects developed pyrexia during the experiments. No significant changes in blood pressure or pulse rate were observed.

Discussion. The data reveal that growth hormone given intravenously has a rapid effect (within 5 minutes after the end of the infusion) on blood amino acid nitrogen and glucose levels. The effect on the former lasted



at least 6 hours, whereas the glucose effect disappeared in one to 2 hours. These effects were observed with doses of growth hormone ranging from 70 to 225 mg per subject. The amino acid nitrogen changes in blood and urine without increase in non-protein nitrogen probably means a significant retention of the administered amino acids somewhere in the body—presumably the intracellular and/or extracellular space. The results might also be explained by an increase in blood volume, an increased catabolism of the administered amino acids, or an expansion of the extracellular space. The fact that blood hematocrit values were unchanged by growth hormone (two cases studied) and the non-protein nitrogen not increased (3 cases studied) is against the first two possibilities. It is planned to do measurements of extracellular space to rule out the third explanation. Finally, the possibility remains that an increased rate of anabolism took place under the influence of growth hormone.

In the rat growth hormone depresses the fasting level of amino acid nitrogen(14), increases the rate of removal of amino acids from the plasma when protein hydrolysate is administered(15), and diminishes the formation of urea from administered protein hydrolysate(16). In the rat, hypoglycemia is observed following growth hormone administration(17), whether or not protein hydrolysate is given(15). However, in these experiments the blood glucose determinations were done on samples taken 1½ to 2 hours after growth hormone was administered intraperitoneally. In our experiments the hyperglycemia occurred during the first 1½ hours after the end of the intravenous infusion of growth hormone. Failure to obtain the serum phosphorus(18) and alkaline phosphatase(19) changes which occur in the rat in more prolonged experiments is not surprising owing to the short duration of the experiments.

The failure of others to demonstrate positive effects in man with large doses of growth hormone may be due to the fact that it was given subcutaneously or intramuscularly. It may be that the hormone is rapidly inactivated by subcutaneous and muscle tissue. Another possible explanation is the rapid formation of antibodies with repeated doses, although no such antibody formation has been demonstrated in man(20) or in the rat(21). Studies on antibody formation after intravenous growth hormone administration are being done.

Summary. A highly purified preparation of pituitary growth hormone given intravenously, together with an infusion of amino acids, to 5 normal young adult men and a patient with cancer caused a consistent, rapid, sustained lowering of blood amino acid nitrogen levels and of amino acid nitrogen excretion in the urine; a rapidly occurring but transient hyperglycemia; an increase in the ketonemia of the fasting state in three subjects so studied and an increased urinary excretion of sodium-retaining material in the 2 subjects so tested. No consistent effects were noted on blood and urine phosphorus, serum alkaline phosphatase or blood eosinophiles.

We wish to thank Dr. Norman Kalant for permission to use his ketone determinations and Miss Bertha Singer for permitting us to use the sodium retention assay data. Thanks are due to Miss Lois

Boyd for technical assistance and Miss Zina Malevitch and Miss Barbara Emmett for technical advice.

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Received July 29, 1952. P.S.E.B.M., 1952, v81.

Effect of Nucleic Acid Derivatives in the Reversal of Aminopterin Inhibition in the Chick Embryo.* (19766)

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Aminopterin (4-amino-pteroylglutamic acid) is a powerful inhibitor of embryonic development in the chick(1,2). The injection of 10 μg or more into eggs prior to incubation completely inhibits embryonic development. This inhibitory effect is partially alleviated by thymidine and more completely by thymidine plus hypoxanthine desoxyriboside(2). The inhibitory effect is also alleviated by high levels of "leucovorin," but not by folacin itself(3). These results were interpreted to mean that aminopterin acts in the chick embryo, as in other organisms, as an antagonist of folacin and its derivatives; that these substances are directly or indirectly essential for synthesis of thymidine and one or more purine derivatives; and that synthesis of these essential components of nucleic acid is the first process to become limiting in the aminopterin inhibited chick embryo. When thymidine, which is highly specific, and a suitable purine derivative are supplied preformed, these inhibited reactions are by-passed; nucleic acid synthesis, and hence initiation of embryonic development, can then occur in their absence.

The effectiveness of various purine bases and their nucleosides in enhancing the effects of thymidine is compared below. If the viewpoint cited above is correct, each of the effective purine derivatives must be able to serve as a precursor for synthesis of nucleic acid by the chick embryo.

Experimental. The procedures followed in

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Presented at the 121 National Meeting of the American Chemical Society, Milwaukee, Wisc., April 1952.

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