Effect of Human Growth Hormone on UFA and Plasma Amino Acid Nitrogen in Man. (24748)

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Growth hormone is known to have a marked effect on fat metabolism especially on increasing lipid concentration in the liver, as has been shown by Greenbaum *et al.*(1,2,3), Weil(4), and Beaton and Curry(5). It has recently been shown by Engel *et al.*(6) that growth hormone increased plasma unesterified fatty acid (UFA) levels of hypophysectomized rats even though these animals have been fed. Raben and Hollenberg(7) (in an abstract) have reported that human growth hormone given intravenously to normal individuals will double plasma UFA in 4 hours. Human growth hormone maintained an increased UFA level for 24 hours in hypopituitary individuals and for a shorter interval in normals(7). This study was undertaken to determine the effects of intravenous growth hormone on plasma UFA and alpha amino acid levels of cancer patients some of whom had been hypophysectomized.

Methods. The 8 patients in this study all had malignant neoplasms. Four patients had undergone hypophysectomy in an effort to control the spread of their malignant disease. In all cases the hypophysectomies were functionally complete as shown by low thyroid and adrenal function and absence of urinary gonadotrophin and the patients were on maintenance compound E or F and triiodothyronine. The hypophysectomized patients used posterior pituitary powder by nasal insufflation *ad lib.*, to control symptoms of diabetes insipidus. Patient D.A. was studied prior to surgery as well as a month after a pituitary stalk section, at which time she was on maintenance Compound E and posterior pituitary powder. Three patients had normal pituitary function. At onset of study the medication schedule of patients on replacement therapy was arranged so that they received their medications at the same time each day. At 9:00 p.m. before each study, the patient was given a "milk-shake." From midnight until termination of study at about 2:00 p.m. the following day, they were allowed only water. At 8:00 a.m. on first day of study, patient's blood was drawn and 20 cc of normal saline was injected. Blood samples were obtained at 1 hr, 2 hrs, 4 hrs, and 6 hrs post-injection. Sodium ethylene diaminetetra-acetate (EDTA) was used as anticoagulant in UFA studies and heparin for alpha amino acid nitrogen determinations. The above procedure was repeated after skipping 1 day except that the patient received 10 mg human growth hormone intravenously in a 5 minute period at 8:00 a.m. The preparation was made by Dr. C. H. Li of University of California and was in an alkaline solution at pH 8.5. Unesterified fatty acids were determined by the method of Gordon(8). Alpha amino acid nitrogen was determined by the method of Frame *et al.*(9,10) and blood sugar by the method of Klendshoj(11).

Results. All but one patient given growth hormone showed a significant elevation of plasma UFA after injection of growth hormone (Fig. 1). The patient who did not respond had normal pituitary function and was the most severely ill patient in the group. Table I shows percentage change between average values of the 4-6 hour periods and 0-1 hour periods in UFA and alpha amino acid nitrogen during control test and growth hormone test. There is a significant ($p<.001$) rise in UFA after growth hormone. A downward trend is observed in alpha amino nitrogen. This held true in all cases except 2 (Table II). Blood sugar determinations showed no consistent changes and serum phosphorus determinations failed to show significant change.

Discussion. Our data indicate that growth hormone has a definite effect on mobilization of UFA in individuals with and without intact pituitary glands. Furthermore, it appears that the downward trend of alpha amino

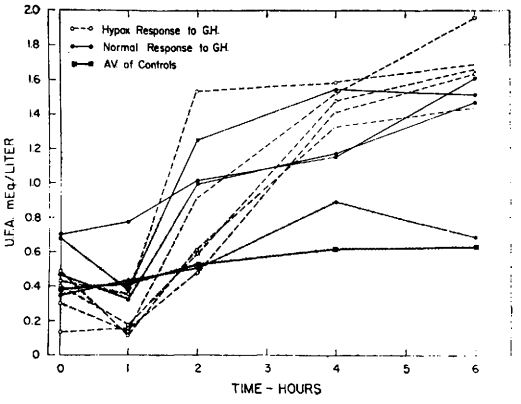

FIG. 1. Change in UFA in normal and hypophysectomized patients. Stippled area represents limits of control tests. Growth hormone was given at zero time.

acid nitrogen may indicate a sparing effect on alpha amino acids by growth hormone. This correlates well with the "protein sparing" effect of growth hormone postulated by Greenbaum(1,2) and Beaton and Curry(5). Length of time required for this action to manifest itself (4-6 hours) coincides with Greenbaum's(2) animal work in which a maximal increase in plasma and hepatic lipids occurred 6 hours after growth hormone injection.

TABLE I. Percentage Change between Average Values of 4-6 Hour Periods and 0-1 Hour Periods in the UFA and Alpha-Amino Acid Nitrogen during Control Test and Growth Hormone Test.

	UFA, % change (Avg 4, 6 hr/avg 0, 1 hr)		Alpha-amino acid, % change (Avg 4, 6 hr/avg 0, 1 hr)	
Patient	Control	HGH	Control	HGH
Normal	105	196	115	76
	106	333	103	78
	136	210	90	97
	141	280	105	90
Post-hypox	90	980	90	84
	205	812	122	56
	185	567	102	89
	120	404	100	71
	104	470	113	122
Avg	132	474	104	85
	$p < .001$		$.1 > p > .05$	

The mechanism of this delayed response of UFA to growth hormone is not readily discernible. Since Gordon(8,12) and Dole(13) have shown that insulin and epinephrine have an immediate effect on plasma UFA levels it would appear that the maximum effect of growth hormone on UFA levels requires a somewhat longer period and may require formation of some intermediate compound to activate these responses. Another possible explanation would be the slower development of insulin resistance which could produce these effects. This response to a single dose of growth hormone makes possible determination of activity of a growth hormone preparation in a patient known to respond to human growth hormone, and also to determine whether an individual will respond to a known active hormone preparation. Thus it is seen that growth hormone effects the liberation of UFA and concomitantly appears to spare alpha amino acids.

Summary. These studies reveal that intravenous injection of human growth hormone to both hypophysectomized and normal individuals cause an increase in plasma unesterified fatty acids 6 hours after injection. Also that level of plasma alpha amino acid nitrogen drops 4-6 hours after injection of growth hormone. Both fat-liberating and

TABLE II. Effect of Growth Hormone on Plasma Amino Acid Nitrogen.

	Plasma α-amino acid nitrogen, mg %					
	Control			Growth hormone		
Patient	0 hr	6 hr	Δ-6 hr pd	0 hr	6 hr	Δ-6 hr pd*
Normal	5.10	5.07	.03	6.01	4.22	−1.79
	3.97	3.63	.34	6.42	6.05	− .37
	3.27	3.22	.05	3.44	2.97	− .47
	1.69	1.30	.39	3.64	2.99	− .65
Post-hypox	5.80	5.32	.48	5.33	4.24	−1.09
	3.63	3.85	+ .22	3.97	4.90	+ .53
	3.78	5.07	+1.29	3.25	2.34	− .90
	3.10	2.80	.30	2.93	2.41	− .52
	3.34	3.65	+ .31	3.54	2.40	−1.14

* Changes in amino acid nitrogen from 0 time to 6 hr post inj. of hormone.

"protein sparing" effects of human growth hormone in man are demonstrated.

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Studies on Mammalian Tyrosinase. II. Chemical and Physical Properties Of Fractions Purified by Chromatography.* (24749)

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Our recent reports have dealt with isolation of tyrosinase from Harding-Passey mouse melanoma(1) and with its purification on diethylaminoethyl (DEAE) cellulose ion exchange columns(2). At least 3 chromatographically distinct components with tyrosinase activity were detected and designated I, II and III in the order of movement on and emergence from the column(2). It is the purpose of this paper further to describe highly purified mammalian tyrosinase in respect to some of its chemical and physical properties, and to report a rapid purification procedure using hydroxyapatite ion-exchange columns.

Methods. The highly purified tyrosinase samples used in this study were prepared by column chromatography using DEAE cellulose(2) or hydroxyapatite as the ion-exchange agent. Hydroxyapatite, prepared according to Tiselius *et al.*,(3), has been found to remove most of the impurities present in crude fractions of soluble mammalian tyrosinase(1) without adsorption of the enzyme. For these studies 1 x 11 cm, or, for preparative scale, 2.2 x 13 cm columns have been employed. Initially, development was started with pH 6.8 phosphate buffer, 0.001 M, followed by gradient elution to concentrations of 0.5 M phosphate buffer, pH 6.8. After it became clear that most of the tyrosinase activity passed through the column without absorption, simple elution with starting buffer, pH 6.8, 0.001 M, was utilized. Protein in effluent fractions was analyzed by the Folin-Lowry reaction (4). Tyrosinase activity was detected as previously described(2). After location of tyrosinase activity, fractions were combined, dialyzed from 4 to 6 hours in the cold, and lyophilized. Specific activity was determined on dried material as previously reported(2). Nitrogen for this calculation was estimated by the method of Schaffer and Sprecher(5). Ultracentrifuge studies were carried out on solutions of tyrosinase in pH 6.5, 0.05M cacodylate buffer. Aliquots containing 1.18 and 2.25 mg of protein nitrogen/ml were placed in the Spinco model E ultracentrifuge and subjected to centrifugal forces of 260,000 x g.

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