shortened either by a combination of PTA and HF deficiency plasmas or by each purified activity combined with its deficiency plasma (Table II). In contrast, addition of the combined purified activities did not shorten clotting time. These data suggest that plasma contains an additional activity which is missing from EP.

Summary. 1) Hageman factor and plasma thromboplastin antecedent have been separated from human plasma and each other by ion exchange chromatography. 2) The defect in "exhausted plasma" is not corrected by purified PTA, purified HF, or a combination of the two.

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Early Metabolic Effects of Human Growth Hormone.* (26139)

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The diabetogenic action of chronic administration of growth hormone is well established, both in experimental animals and in man. Insulin-like effects of the hormone have also been described, especially as a result of its acute administration(1) or of its presence *in vitro*(2,3). These effects have been related either to an insulin-like activity of the hormone itself or, *in vivo,* to a tropic effect of growth hormone upon the islets of Langerhans(4,5). In recent years, the physiologic importance of species differences in structures and function of growth hormone have become apparent(6,7,8), suggesting that previously observed effects should be reevaluated using growth hormone obtained from the same species. Although the metabolic effects of chronic administration of human growth hormone to human subjects with and without diabetes mellitus have been extensively described(9,10), the early metabolic effects have not been outlined as clearly.

In this report, an acute hypoglycemic effect of human growth in man will be described and related to changes in plasma free fatty acids described by Raben(11) and to measurements of serum insulin-like activity.

Materials and methods. Human growth hormone was prepared from pituitaries collected at autopsy according to the technic outlined by Raben(9). The potency of the preparation was tested against a sample obtained from Dr. Raben and exhibited approximately the same activity, mg per mg, using its effect upon plasma free fatty acids in man as activity index. When tested for contamination with ACTH, using the bioassay procedure of Nelson and Hume(12), 100 μg of the preparation contained less than 40 $\mu\mu$g purified ACTH.§

Growth hormone administration was carried out in 6 male human volunteers in apparent good health, ranging in age from 25 to 37 years. They were kept fasting over-

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night and at 8:00 a.m. a control blood sample was obtained. Human growth hormone dissolved in 3 ml of normal saline was then rapidly injected intravenously, subsequent blood samples being obtained 10, 20, 30, 60, 90 and 240 minutes after injection. No food intake was allowed during that time. The first subject received 4 mg of human growth hormone without side effects and 9 days later, 8 mg. Again, side effects were absent, but changes in the selected metabolic indices were greater. The 5 subsequent volunteers received therefore only a single dose of 8 mg of the hormone.

Blood glucose was measured according to Somogyi's modification of the Nelson procedure(13) and plasma free fatty acids according to the method of Gordon(14). Insulin-like activity (ILA) was determined according to Martin, Renold and Dagenais(15) using oxidation of glucose-1-C^{14} to $C^{14}O_2$ by rat epididymal adipose tissue as index of ILA. In this procedure 6 segments of epididymal adipose tissue are obtained from each rat, of which 2 are needed for standards, thus leaving 4 segments for determination of unknowns. We have previously shown that the inter-assay error is greater than the intra-assay error(16). Accordingly, only 4 samples for determination of ILA were obtained in each subject at 0, 30, 60 and 240 minutes. All 4 samples were in each instance tested within the same assay on the same day. Assays were carried out on undiluted serum.

Before applying this assay for ILA to human serum after the intravenous injection of human growth hormone, it was necessary to establish whether the injected growth hormone preparation itself would interfere with the procedure. According to results reported by Read(17) with an immunoassay procedure for human growth hormone, normal plasma levels in man do not exceed 0.2 to 0.6 μg/ml. Furthermore, if the injected 8 mg of human growth hormone were entirely retained in plasma, the expected maximal level of injected growth hormone would be 3 μg/ml. Unpublished observations from this laboratory have established that concentrations of 0.5 and 5.0 μg/ml human growth hormone do not exhibit significant insulin-like or anti-

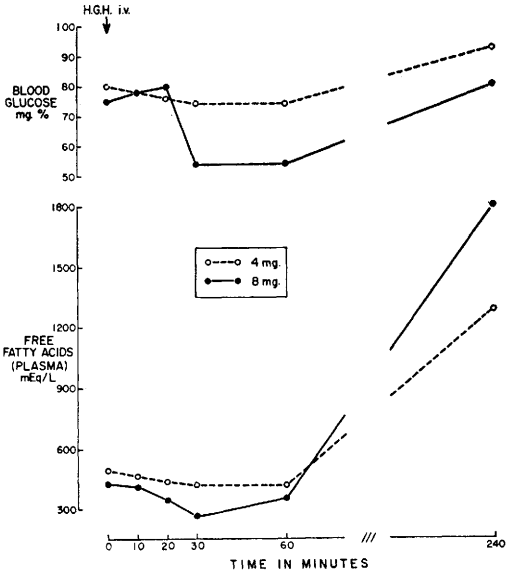

FIG. 1. Acute effects of human growth hormone given in a single inj. on blood glucose and free fatty acids (plasma) of a normal subject.

insulin activity in the assay procedure used, either in buffer or in human serum. This is in contrast with the marked effects of larger concentrations of growth hormone which have been reported(18).

Results. The results obtained are shown in Fig. 1 and Table I. Fig. 1 demonstrates the relative effects of the smaller (4 mg) and larger (8 mg) dose of human growth hormone given at 9 day intervals to the same subject, while Table I summarizes all the results obtained with the higher (8 mg) dose. It is apparent from the data that intravenous administration of human growth hormone produced a very small initial rise in blood glucose, of doubtful significance ($P < 0.2$), then a marked and highly significant ($P < 0.001$) hypoglycemia with a mean blood glucose fall of 25 mg per 100 ml 30 minutes after administration of hormone. Free fatty acids first decreased ($P = 0.02$) then rose significantly ($P < 0.01$) to a mean level 3 times greater than control value 240 minutes after administration of the hormone. With continued fasting alone, for this relatively short period of 4 hours, plasma free fatty acids would not be expected to rise by more than 50%(11) and significant changes in blood glucose are unlikely. Furthermore, in

TABLE I. Acute Changes in Levels of Glucose, Free Fatty Acids and Insulin-Like Activity in Blood of 6 Normal Subjects after a Single Injection of Human Growth Hormone (8 mg i.v. at Time 0).

Subject	Time in min. 0	10	20	30	60	90	240
				Blood glucose (mg %)			
J.F.	75	78	80	54	54		80
J.C.	85	85	66	59	85		114
R.Z.	74	114	66	61	70		85
J.K.	73	70	58	47	43	62	77
A.F.	80	87	68	49	39	81	83
L.P.	85	94	80	54	44	78	85
Mean ± S.E.	79 ± 2	88 ± 7	70 ± 4	54 ± 2	56 ± 8	74	87 ± 3
				FFA-plasma (meq/l)			
J.F.	420	408	348	276	360		1800
J.C.	340	320	300	340	400		1240
R.Z.	635	830	528	540	595		1025
J.K.	360	340	260	200	340	500	800
A.F.	460	540	380	320	460	1100	1630
L.P.	600	320	320	340	360	780	2360
Mean ± S.E.	469 ± 55	460 ± 89	356 ± 42	336 ± 51	419 ± 43	793	1476 ± 255
				ILA-serum (μU/ml)			
J.F.	390			190	300		400*
J.C.	170			200	260		430
R.Z.	340			600	220		620
J.K.	170			120	170		220
A.F.	240			220	270		300
L.P.	140			130	120		210
Mean ± S.E.	242 ± 42			243 ± 73	223 ± 64		363 ± 63

* Indices of precision (lambda) of the individual assay runs follow: J.F., .18; J.C., .18; R.Z., .24; J.K., .20; A.F., .16; L.P., .22.

the subject who received 2 doses of growth hormone, both blood glucose and the plasma free fatty acid changes were considerably greater with the larger than with the smaller dose.

Values for ILA measurements are recorded together with the index of precision (lambda) for each assay day. Even with relatively satisfactory values for lambda, the 95% confidence limits for each single value range from approximately one-half the estimate to double the estimate. However, standard statistics may be correctly applied to each group of 6 values obtained at each of the times indicated in the 6 subjects, since no meaningless values were present as indicated by analysis of the assay characteristics of each day. ILA did not significantly vary from the control value in this group at either 30 or 60 minutes but showed a probably significant increase ($P = 0.05$) at 240 minutes. By way of comparison it may be pointed out that administration of glucose results in a 3- to 4-fold increase in serum ILA with the method used(16).

Three of the 6 subjects complained of a moderately severe headache between 3 and 5 hours after injection, without change in pulse rate or blood pressure. One of the subjects, furthermore, fainted when he suddenly assumed the vertical position at the end of experimental period. He reported that he had previously fainted with sudden changes in position. Ikkos, Luft and Gemzell(10) reported headaches and dizziness in 3 out of 4 patients given human growth hormone intramuscularly.

Discussion. These studies confirm the occurrence of early insulin-like effects of growth hormone preparations, even in the absence of species differences between preparations and test system and, more specifically, even for preparations of human growth hormone in *man*. The effects are insulin-like both in terms of hypoglycemia and in terms of an early decrease in circulating levels of

free fatty acids. Failure to observe increased serum ILA at the time of maximum hypoglycemia and decrease in plasma free fatty acids suggests that the insulin-like effect of growth hormone preparations is neither secondary to a stimulatory effect on the pancreatic beta cells, nor associated with occurrence in serum of substances capable of potentiating the effect of serum insulin upon rat adipose tissue. It would seem more likely, therefore, that the early insulin-like action of growth hormone preparations is the result of intrinsic *in vivo* insulin-like activity of these preparations. Whether this insulin-like activity is directly related to growth hormone itself, or rather to presence of possible unrelated insulin-like components in the preparation is not clear. Huggins and Ottaway(19) and Bornstein and Hyde(20) have recently reported that peptides with insulin-like activity *in vitro* can be isolated from preparations of both ox and human growth hormone. Thus, presently available growth hormone preparations might be contaminated with an insulin-like peptide even as most crystalline insulin preparations are contaminated with glucagon. As in the case of glucagon, it will be necessary to establish whether the activity is an artefact of preparation or a substance of physiologic significance.‖ It is, of course, impossible to rule out that an increase in serum ILA, too small to be detected yet sufficient to account for the hypoglycemia, did in fact occur.

The late (240 minutes) mild hyperglycemic effect of growth hormone, associated with the marked increase in free fatty acid levels, is consistent with the chronic "diabetogenic" action of human growth hormone in man (10). Increased ILA at that time could be considered as the secondary consequence of the rising level of blood glucose, although growth hormone induced stimulation of insulin-producing cells cannot be excluded. It may be of interest to consider the possibility that the early hypoglycemic effect of growth hormone contributes to some extent to the increased free fatty acid and blood glucose levels noted after 4 hours.

Summary. Human growth hormone, 8 mg, has been administered intravenously to 6 normal volunteers, and blood samples have been obtained before and from 10 to 240 minutes after injection of the hormone. The growth hormone preparation used exhibited a definite early insulin-like effect both in terms of blood glucose and plasma free fatty acid levels. This was not associated with a change in serum insulin-like activity, as measured with rat epididymal adipose tissue. Four hours after growth hormone had been given, there was a mild hyperglycemic effect, and a marked increase in plasma free fatty acids, together with a small but significant elevation in insulin-like activity of serum. These observations are interpreted as compatible with an intrinsic *in vivo* insulin-like activity of the growth hormone preparation used, which could be either a property of the growth hormone molecule itself, or represent the activity of unrelated substances present in the preparation.

‖ During preparation of this manuscript the authors have become aware of the studies of Domingues, Greenberg, Pazianos, Ray and Pearson, *Acta Endocrinol.* (Copenhagen), 1960, Suppl. 51, p. 241. These authors observed an early decrease in blood glucose and plasma free fatty acid levels following intravenous administration of 5 mg human growth hormone to *both diabetic and non-diabetic subjects.* They concluded that "the hypoglycemic effect of growth hormone does not appear to be due to insulin release, since it occurs in the juvenile-type diabetic patient." This observation further substantiates the interpretation given to our results reported here. An early decrease in plasma free fatty acids has also been described by Beck and his collaborators, *Ciba Found. Colloquia on Endocrinology,* 1960, v13, 164.

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Passive Transfer of Delayed Sensitivity to the Newborn Rabbit.* (26140)

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Recent studies of the immune capacity of the human newborn infant have demonstrated that the newborn infant is unable to accept or to manifest a passive transfer of delayed allergy(1). A similar unresponsiveness to passively transferred delayed allergy in the newborn guinea pig was demonstrated by Waksman and Matoltsy(2). They were unable to transfer delayed sensitivity to tuberculin to newborn guinea pigs with peritoneal exudate leukocytes, but were uniformly successful with such transfers to mature guinea pigs. Parallel limitations in capacity of the newborn human and newborn rabbit have been demonstrated by several workers(3,4,5, 6). Data presented here show that like newborn infants newborn rabbits are limited in this capacity to express or to accept passively transferred delayed allergy.

Methods and materials. The animals used in the experiment were New Zealand white rabbits, newborn and mature. The newborn rabbits were 7, 14, 21, and 28 days of age, and the mature rabbits weighed 5 to 6 lb.

Delayed sensitivity was induced by infecting adult animals with Dematco strain of streptococcus. The Dematco streptococcus is group A, type 12 strain of beta-hemolytic streptococcus which was originally obtained from a patient with acute glomerulonephritis. This strain produces large quantities of streptokinase and streptodornase B. One-tenth cc of an 18-hour broth culture of the Dematco strain streptococcus was injected into each hind foot pad of the 5-6 lb rabbits.

These rabbits were skin tested 7 to 10 days after infection to determine their degree of sensitivity. Skin testing was done with 0.01 cc of physiologic (8.5%) saline containing 100 units of streptokinase and 3.3 units of streptodornase‖ injected intradermally in the inner surface of the rabbit's ear. As a control, 0.01 cc of physiologic saline was injected in the opposite ear. The skin testing sites in the ears were examined 18-24 hours later for induration and erythema. The skin reactions were read on the basis of square millimeters of erythema and induration, as follows: 0-9 mm^2 – no transfer, 10-19 mm^2 – 1+, 20-39 mm^2 – 2+, 40-59 mm^2 – 3+, 60-79 mm^2 – 4+, over 80 mm^2 – 5+. The same rating system was used for grading the passive transfers.

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‖ Varidase (Lederle).