Groeihormoon vetstofwisseling en energievoorziening

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De overwegingen, die ertoe geleid hebben een onderzoek te verrichten naar de relatie tussen groeihormoon en het gedrag van de vrije vetzuren tijdens vasten, worden in de inleiding besproken.

In hoofdstuk I wordt een overzicht gegeven van de stofwisseling van vrije vetzuren. Bij de energievoorziening van het lichaam spelen vrije vetzuren een belangrijke rol. De F.F.A.-concentratie in serum is sterk afhankelijk van de beschikbaarheid van voedsel, in het bijzonder van koolhydraten. Vele hormonale factoren beïnvloeden de F.F.A.-spiegel. Het effect van de toediening van een hormoon op het gedrag van de vrije vetzuren is echter moeilijk te beoordelen, omdat dit naast de invloed op de F.F.A.-spiegel, ook aanleiding is tot een verandering in de endogene produktie van andere hormonen, die eveneens de F.F.A.-concentratie beïnvloeden.

De methoden, die voor de bepaling van vrije vetzuren en bloedsuiker werden gebruikt, worden in hoofdstuk II besproken. De vrije vetzuren werden volgens Dole in serum bepaald. Het bleek, dat wisselingen van de serumconcentratie van melkzuur, β-hydroxyboterzuur, pyrodruivenzuur en fosfolipoïden in de gebruikte proefopstelling van geen belang waren voor de resultaten, die met de methode van Dole werden verkregen. Het bloedsuikergehalte werd bepaald volgens Somogyi-Nelson.

Het verloop van de serumconcentratie van F.F.A. en het bloedsuikergehalte bij normale individuen en patiënten met panhypopituïtarisme op substitutietherapie met cortisol en thyreoid wordt in hoofdstuk III behandeld. De nuchtere F.F.A.-spiegel van de normale individuen en de patiënten met hypofyse-insufficiëntie toonde geen duidelijke verschillen. De stijging van de serumconcentratie van vrije vetzuren na 24 uur vasten was echter bij de normalen veel groter dan

bij de patiënten. De geringere F.F.A.-stijging tijdens vasten bij patiënten met panhypopituïtarisme berust op een afwijking in de vetmobilisatie. Uit onderzoek bij patiënten met gesubstitueerd myxoedeem en met bijnierinsufficiëntie op substitutietherapie bleek, dat deze patiënten wel in staat zijn tot F.F.A.-mobilisatie tijdens vasten. Hiermee was aannemelijk gemaakt, dat de gestoorde vetmobilisatie tijdens vasten bij de patiënten met hypofyse-insufficiëntie niet berustte op het onvermogen de schildklier- of bijnierfunctie te variëren, maar dat de oorzaak gelegen was in het ontbreken van een vetmobiliserende factor in de hypofyse. Na de toediening van groeihormoon verbetert deze stoornis in de vetmobilisatie (hoofdstuk IV).

Er is echter geen directe relatie tussen de aan- of afwezigheid van groeihormoon en de F.F.A.-mobilisatie (hoofdstuk V). Dit maakt het onderzoek naar de F.F.A.-mobilisatie tijdens vasten ongeschikt als hypofysetest.

Het verband tussen F.F.A., bloedsuiker en groeihormoon werd daarom verder onderzocht na de ontwikkeling van een methode voor de bepaling van groeihormoon in serum (hoofdstuk VI). Hiertoe werd gebruik gemaakt van de methode van Read, waarin twee belangrijke wijzigingen werden aangebracht.

1e. in plaats van schapenerytrocyten werden mensenerytrocyten gebruikt, bloedgroep O, Rh. neg.

2e. het principe van een bloktitratie werd toegepast.

Met deze methode kunnen geen absolute groeihormoonconcentraties worden bepaald; wel is het mogelijk om verschillen in groeihormoonconcentratie aan te tonen, mits sera afkomstig van één persoon in één reeks ten opzichte van dezelfde standaard worden bepaald.

In hoofdstuk VII ten slotte worden de resultaten van een onderzoek naar de invloed van vasten en het gebruik van voedsel op de groeihormoonspiegel, de F.F.A.-concentratie en het bloedsuikergehalte besproken. Het blijkt, dat bij normale personen de groeihormoonconcentratie in serum tijdens een periode van 24 uur vasten niet parallel loopt aan de F.F.A.-spiegel. Tijdens de optredende vetmobilisatie is er geen duidelijke verandering van de groeihormoonspiegel. De groeihormoonconcentratie daalt echter na het gebruik van voedsel, met name van glucose. Het schijnt dus, dat de groeihormoonspiegel aan wisselingen onderhevig is, die worden beïnvloed door de

beschikbaarheid van snel utiliseerbare calorieën afkomstig van koolhydraten. Dit kan erop wijzen, dat het volwassen menselijk lichaam groeihormoon nodig heeft, om in staat te zijn depotvet te gebruiken voor de energievoorziening, en dat de endogene groeihormoonproduktie afhankelijk is van de mate, waarin hieraan behoefte bestaat.

SUMMARY

In recent years it has been found, that free fatty acids (F.F.A.) are of major importance in the overall metabolism and energy supply of the human body. Normally occurring in amounts of 400-800 micro-eq./l. serum, F.F.A. levels rise markedly in situations, where energy supply is largely derived from fat as during fasting and in decompensated diabetes mellitus.

It is known, that patients with panhypopituitarism are less prone to ketosis during fasting; in hypophysectomised patients with diabetes mellitus metabolic acidosis rarely occurs.

On the other hand growth hormone is the most potent hypophysial fat mobilising hormone in man, as judged by the rise of F.F.A. levels in serum, that occurs after its administration.

Following the considerations described above, it was thought interesting to study the effect of fasting on F.F.A. levels in patients with panhypopituitarism, in whom absence of growth hormone can reasonably be expected. The effect of fasting and ingestion of food on growth hormone levels was also studied in normal people.

The literature concerning the metabolism of free fatty acids in man is reviewed in chapter I. F.F.A. are probably mainly derived from lipid tissue and are extracted from the blood by muscles, the heart and the liver. The turnover rate of F.F.A. is very high, the biologic half life time of radioactive palmitate is 1 to 4 minutes. Many factors influence the F.F.A. level, among them fasting, ingestion of food, especially carbohydrates. Hormonal effects on F.F.A. metabolism are difficult to evaluate, because administration of a hormone, apart from its effect on F.F.A., also brings contraregulatory hormonal mechanisms into action.

In chapter II the methods used for the determination of F.F.A. and bloodsugar levels are discussed. F.F.A. were determined with Dole's method. Whether plasma or serum is used makes no difference to the determination of F.F.A. Storing of both serum and plasma at —6° C. for a week had no effect on the result of the procedure. Keeping the samples at 37° C. for 24 hours gave appreciably higher F.F.A. values in serum and especially in plasma. Addition to 1 ml. serum of lactic acid (to obtain an estimated lactic acid value of about 10000 microeq./l. serum) did not give a consistent elevation of the titratable acidity in 10 experiments. Addition of β hydroxybutyric acid (to obtain an estimated β hydroxybutyric acid level of 27000 microeq./l.) did have an effect on the outcome of the titration procedure. However only 1% of the added β hydroxybutyric-acid was recovered as titratable acidity. The presence of varying amounts of lactic- and β hydroxybutyric acid, under the experimental conditions, was of no consequence for the outcome of Dole's titration procedure. The same holds true for pyruvic acid and the phospholipids.

Bloodsugar levels were determined with the Somogyi Nelson method.

The effects of 24 hours fasting on F.F.A. and bloodsugar levels in 16 normal individuals and in 16 patients with panhypopituitarism adequately treated with cortisol and thyroid are described in chapter III.

The fasting F.F.A. level of the normal individuals and the patients with panhypopituitarism were similar. After a 24 hours' fast F.F.A. rose much more in normal individuals than in patients with panhypopituitarism. The results are shown in fig. 26(A). The rise of F.F.A. after the 24 hours' fast is plotted against the initial value of each individual. The difference of the F.F.A. rises between both groups is statistically significant (rank sum test, $P < 0.01\%$). This is in agreement with the results of Perry and Gemmell. The bloodsugar values did not show a difference between the two groups.

The difference in F.F.A. behaviour in patients and in normal individuals might possibly be explained by differences in physical activity and emotional stress during the experiment. Therefore F.F.A. levels were also determined in ten patients without endocrine disease before and after a 24 hours' fast (fig. 26(B)). They were in the same environmental circumstances as the patients with panhypopituitarism. It was found that the patients without endocrine disorders

showed higher F.F.A. rises than the patients with panhypopituitarism (rank sum test, $P < 1\%$).

Since the turnover rate of F.F.A. is not accelerated during a prolonged fast (Brown), it was concluded that in patients with panhypopituitarism fat-mobilisation, not fat-utilisation is impaired. The inability of these patients to vary their thyroid- and adrenal function during starvation might be the cause of this disturbance.

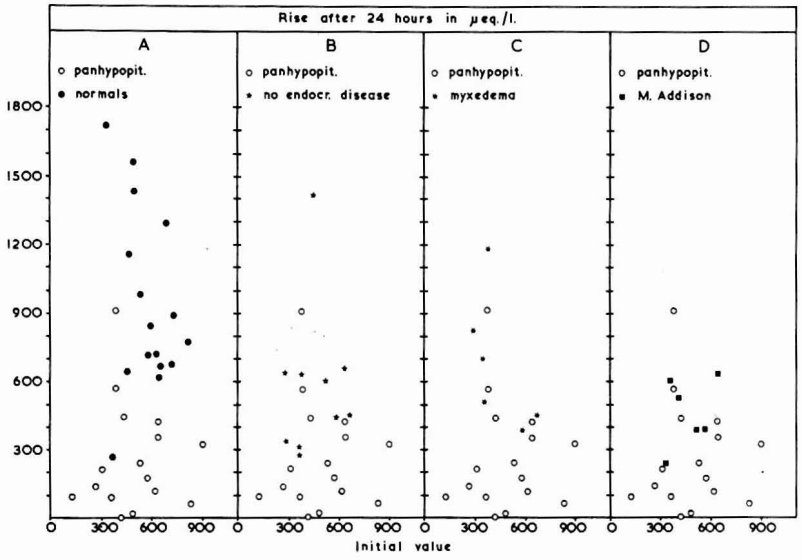

FIG. 26. The rise of F.F.A. after a 24 hours' fast, plotted against the initial value, in patients with panhypopituitarism compared with the rise of F.F.A. in normal individuals (A), in patients without endocrine disease (B), in cases of primary myxedema (C) and in cases of Addison's disease (D).

However patients suffering from myxedema and Addison's disease on substitution therapy show F.F.A. rises during a 24 hours' fast of the same magnitude as the patients without endocrine disorder. Six patients with treated myxedema had a higher rise of F.F.A. during a 24 hours' fasting period than the sixteen patients with panhypopituitarism (rank sum test, $P < 1\%$, fig. $26^{(C)}$). Similar findings were obtained, when six patients with treated adrenal insufficiency, studied under the same conditions, were compared with the hypophyseal patient group (rank sum test, $P < 5\%$, fig. $26^{(D)}$). It was concluded,

that the disturbance of fat mobilisation in patients with panhypopituitarism should be sought in the hypophysis.

As it is known that growth hormone injection gives a sharp rise of F.F.A. in man, the effect of administration of a human growth hormone preparation was investigated in nine out of the sixteen patients with panhypopituitarism (chapter IV). F.F.A.-level rose following the growth hormone injection after a four hours' fast to higher levels in all patients than without growth hormone after a four hours' and a twenty-four hours' fast. The effect of growth hormone in patients with panhypopituitarism can be interpreted as an amelioration of the disturbed fat mobilisation during starvation.

However there is no direct relationship between F.F.A. mobilisation and the presence or absence of endogenous growth hormone (chapter V). Administration of epinephrine gave a rise of F.F.A. levels in patients with panhypopituitarism (7), primary myxedema (9) and Addison's disease (4) of similar magnitude as found in normal individuals (10). This makes it probable, that extra-pituitary factors are also of importance in fat-mobilisation in these patients. In patients with active acromegaly the rise in F.F.A. level during a 24 hours' fast was not more pronounced than in normal people. Moreover in some extremely obese patients without demonstrable endocrine abnormality an inability to mobilise fat during fasting was also found. Thus no conclusions concerning pituitary functional integrity can be drawn from the ability to mobilise fat during starvation, since this is only partially determined by pituitary factors.

The relationship between free fatty acids, blood sugar and growth hormone was therefore further investigated after the introduction of a method for measuring serum growth hormone levels (chapter VI).

Human growth hormone (H.G.H.) was measured in serum by the passive haemagglutination inhibition method of Read and Bryan, applied to human erythrocytes, bloodgroup O, Rh.—. A 'chessboard titration' was introduced to increase accuracy. First blocking doses for antiserum dilutions are determined. The same antiserum dilutions are then analysed with small step dilutions of a human serum. The tests are read according to the haemagglutination pattern by the same person, who is unaware of the outcome of concomitant or previous series of tests. A positive (+) haemagglutination pattern means, that less H.G.H. is present in the human serum dilution than

in the standard dilution of H.G.H., that blocks the corresponding H.G.H. antiserum dilution. Serum dilutions giving negative (—) reactions contain the same amount or more H.G.H. than the blocking dose for the antiserum dilution. The results of a chessboard titration have to agree with each other. The method of calculation is demonstrated in fig. 27. With this method it is not possible to estimate absolute amounts of growth hormone in serum, owing to an inhibiting factor in serum, that influences the passive haemagglutination inhibition reaction. Indeed 'growth hormone' has been found in sera of patients with panhypopituitarism, though generally in lower concentration than in normals. This was evident in our series and in the results of other authors using this assay. Utiger using a radioimmunoassay also had difficulty to distinguish normal people and patients with panhypopituitarism. Nowadays it is therefore not yet possible to estimate absolute amounts of growth hormone in serum.

Anti H.G.H. dilutions	Blocked by H.G.H. (mμg. per ml.)	Serum dilutions 1/4	1/5	1/6	1/7	1/8	mμg. per ml.
1/2400	45	+	+	+	+	+	$< 180 = <(4 \times 45)$
1/3200	30	-	+	+	+	+	$135 = \frac{(4 \times 30)+(5 \times 30)}{2}$
1/4800	23	-	-	±	+	+	$138 = \frac{(5 \times 23)+(7 \times 23)}{2}$
1/6400	15	-	-	-	-	-	$\geqslant 120 = \geqslant(8 \times 15)$

FIG. 27. Example of chessboard titration for determination of H.G.H. in serum.

However we can determine differences of growth hormone content of sera from the same patient when analysed at the same time against the same standard. The in vitro recovery of growth hormone added to serum of normal individuals is shown in tables VI end VII. Following intravenous injection of 6 mgr. H.G.H. in four patients with panhypopituitarism, there was a constant rise in serum H.G.H. level after one and four hours. Twenty-four hours later the values had returned to the preinjection level in three of the four cases (fig. 28).

Measured H.G.H.-values			Recovery	
normal serum	normal serum + 125 mμg/ml.	normal serum + 250 mμg/ml.	125 mμg	250 mμg
90	180	320	72%	81%
115	220	380	91%	102%
210	320	480	92%	120%

Table VI. The in vitro recovery of H.G.H. added to the sera of three normal individuals.

Measured H.G.H.-values		Recovery
normal serum	82	—
normal serum + 200 mμg/ml.	360	278 = 139%
normal serum + 100 mμg/ml.	218	136 = 136%
normal serum + 50 mμg/ml.	128	46 = 92%
normal serum + 25 mμg/ml.	108	26 = 104%

Table VII. The in vitro recovery of H.G.H. added to the serum of a normal individual.

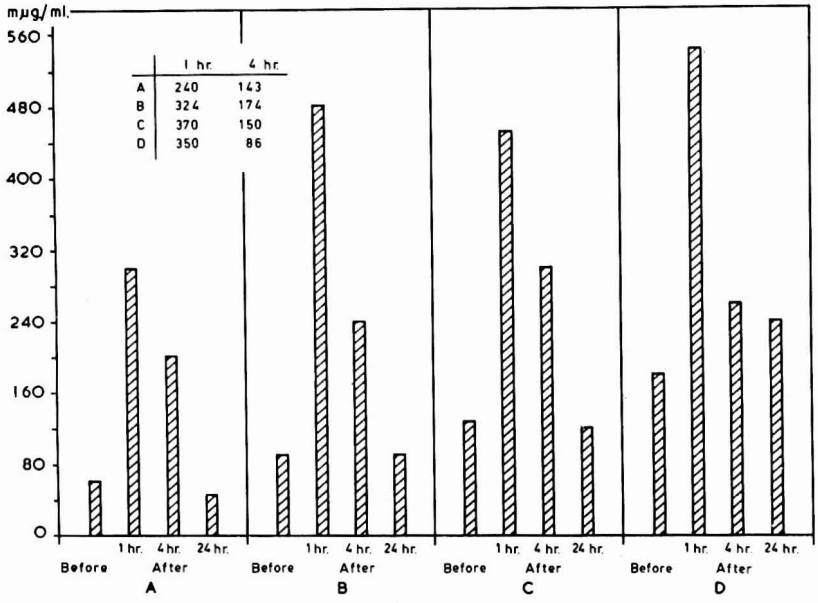

FIG. 28. The measured growth hormone level in sera of 4 patients with panhypopituitarism before and 1 hour, 4 hours and 24 hours following the intravenous injection of 6 mg. H.G.H.

Departures from the initial value after 1 and 4 hours are also noted in figures for each patient.

Growth hormone levels were investigated in the fed and the fasting state in normal persons. At the same time F.F.A. and bloodsugar were determined. Growth hormone level was found to be higher in the fasting than in the fed state in a group of nine persons. After a 24 hours' fast however there was no further rise of the growth hormone level. During this period there was a sharp rise of F.F.A.

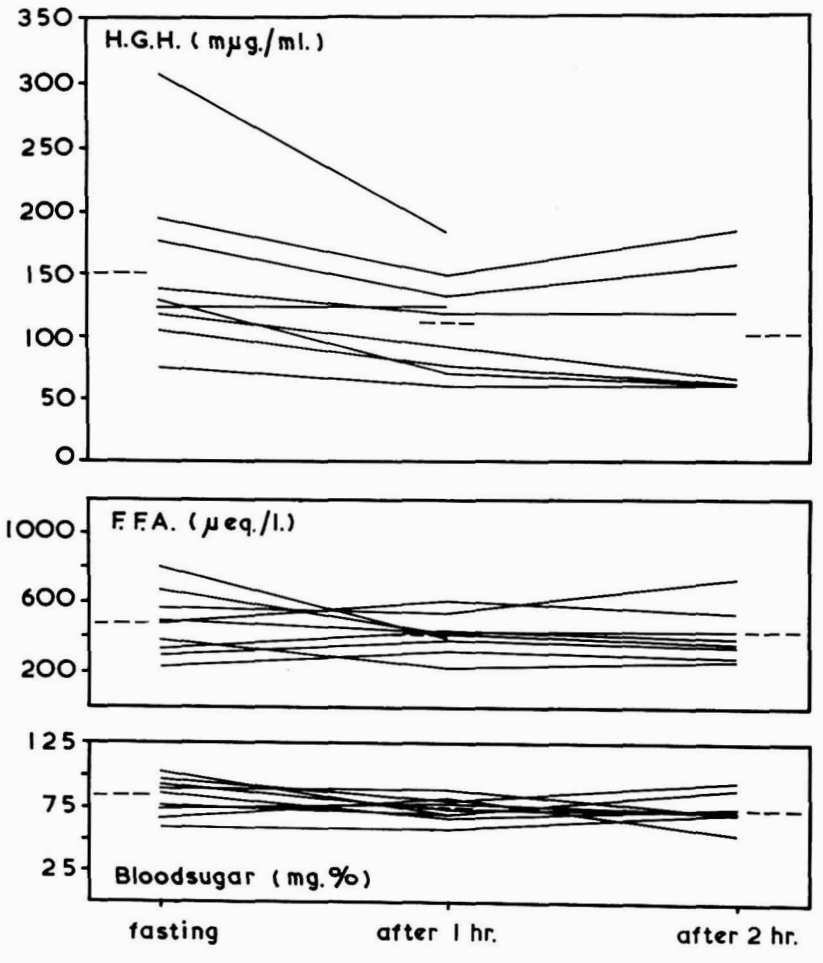

FIG. 29. Serum-H.G.H. level, serum- F.F.A. concentration and bloodsugar level in healthy individuals before, 1 hour after, and 2 hours after the ingestion of a standard meal consisting of 50 g. of carbohydrates, 50 g. of protein and 25 g. of fat.

It was concluded, that there was no correlation between growth hormone level and F.F.A. concentration in this experiment. The blood sugar did not show distinct changes. After a standard meal consisting of 50 g. of carbohydrates, 50 g. of protein and 25 g. of

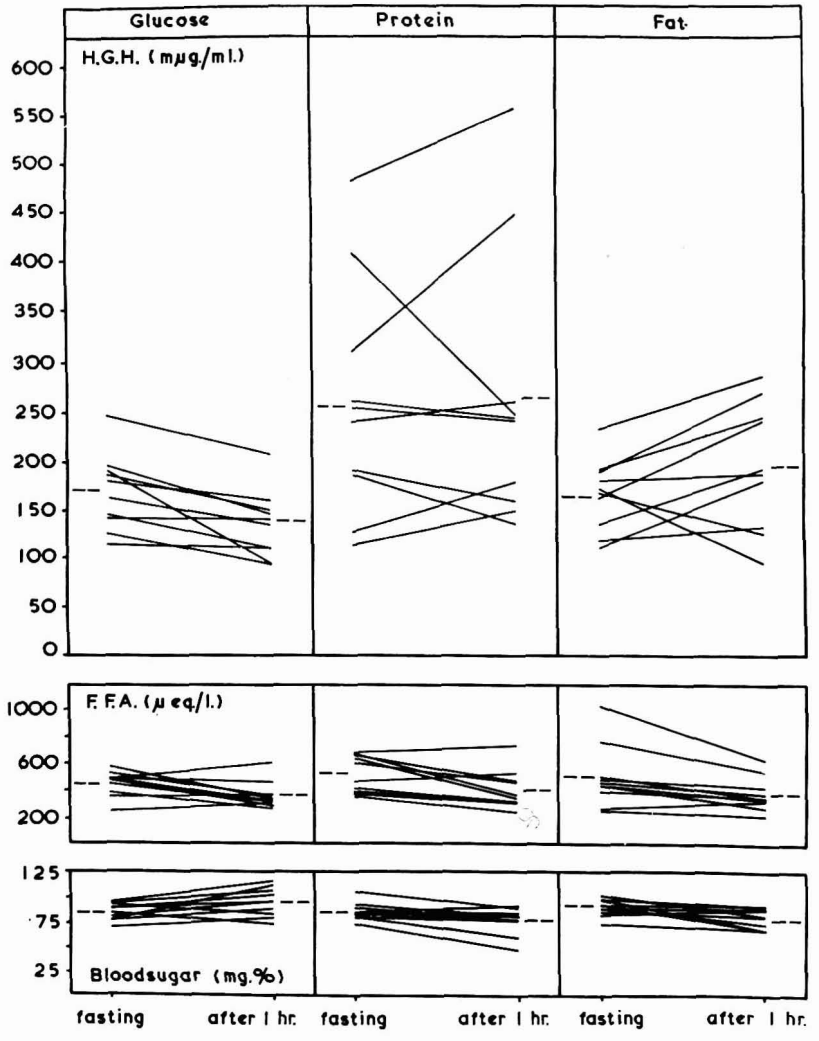

FIG. 30. Serum-H.G.H. level, serum-F.F.A. concentration and bloodsugar level in healthy individuals before and 1 hour after ingestion of either glucose (50 g.), protein (50 g.) or fat (25 g.).

fat mean growth hormone level fell within one hour in a group of nine normal individuals (Student's t-test, $P < 1\%$). Two hours post prandially some persons had a further decrease of H.G.H., others returned to the fasting level. F.F.A. and blood-sugar did not show marked changes (fig. 29).

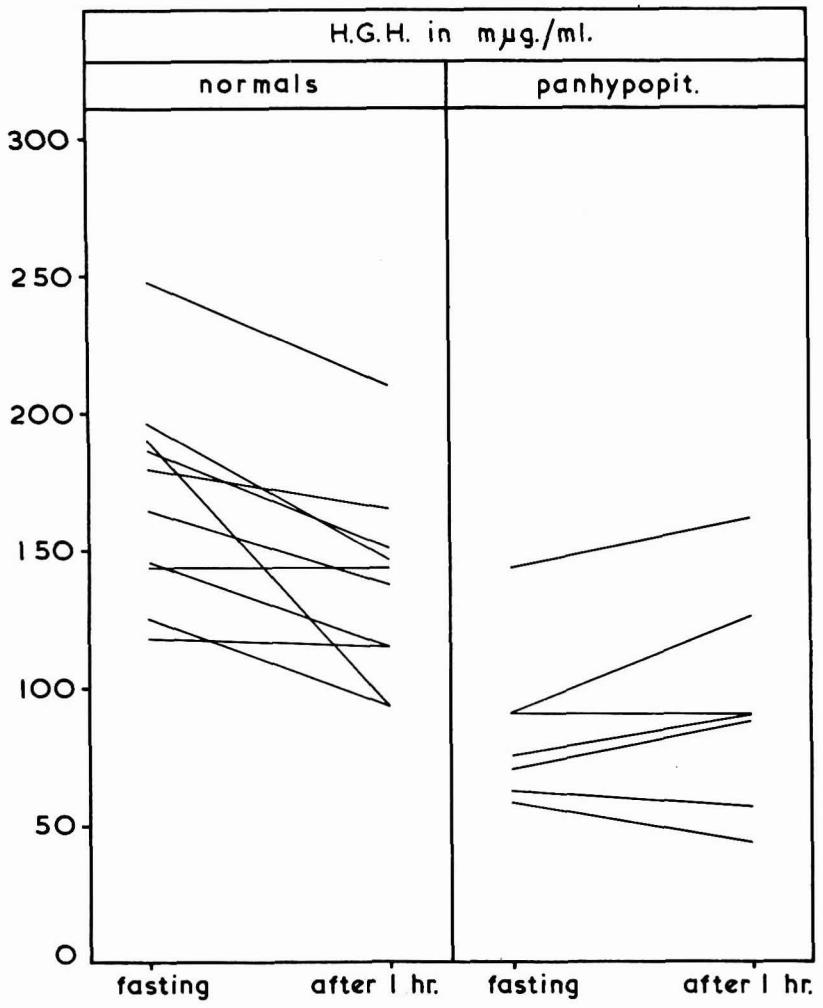

FIG. 31. Measured growth hormone levels in healthy individuals and patients with panhypopituitarism before and 1 hour after ingestion of 50 g. of glucose.

To investigate which component of food was responsible for the lowering of growth hormone level, ten individuals were given respectively 50 grams of glucose, 50 grams of protein (as eggwhite) and 25 grams of fat (as vegetable oil) on different days. After a glucose load mean growth hormone level fell in a group of ten individuals (Student's t-test, $P < 0.1\%$). In the protein and fat experiments no consistent results could be obtained in this group. F.F.A. showed a tendency to drop after glucose, after protein and after fat. Bloodsugar rose after ingestion of glucose (fig. 30). The time between sampling the blood and separating the serum from the cells was found to be important for the inhibiting factor in serum. The three high fasting and post prandial H.G.H. values in the protein ingestion experiment are caused by delay in obtaining the serum. The same individuals had fasting and postprandial H.G.H. levels similar to those of the other normal persons in the carbohydrate and fat ingestion experiments.

The effects of food intake may be explained by alterations of the 'true growth hormone' level or by alterations in the inhibiting factor. In the second case the change of the inhibiting factor would be always in the same direction. However in seven examined patients with panhypopituitarism – where the measured growth hormone level presumably consists of inhibiting factor alone – no variation in measured growth hormone level after the ingestion of glucose was found (fig. 31). The behaviour of the patients with panhypopituitarism as a group after ingestion of glucose differs from that of the normal individuals (Student's t-test, $P < 1\%$).

This favours the first possibility that the 'true growth hormone' level is lowered by ingestion of food, especially by carbohydrates. In adults the growth hormone level apparently varies inversely with the availability of readily utilisable carbohydrates calories.