had been some relatively dramatic change in water quality that coincided with the epidemic period.

The summer of 1974 was exceptionally dry, and by mid-July stream-flow in the Potomac River, from which water to the centre is derived, had fallen below the 1930–65 average. Concomitant with the decline in stream-flow, the level of algae observed in the raw water treated at the Potomac River rose (fig. 2). The epidemic period coincided with the time when the algae reached maximum levels and the river flow-rate was at its lowest. The pyrogenicity of the tap water also correlated with the algae counts (fig. 2).

Unfortunately, the levels of total viable microorganisms in the raw water were not determined, and an associated rise in the levels of gram-negative bacteria with the algae bloom and the epidemic cannot be excluded.

Discussion

Our investigations indicate that an increase in the level of endotoxin contamination of tap water used to prepare dialysis fluid was responsible for this epidemic of pyrogenic reactions. An unusual drought which caused a dramatic decline in the flow-rate and a change in the microbial flora (an algae bloom) of the river, from which water to the centre is derived, led to the increase in endotoxin. Like gram-negative bacteria, the cell walls of blue-green algae are rich in endotoxin.

Endotoxins are potent pyrogens capable of producing unequivocal febrile reactions in man after intravenous doses as small as 1 ng/kg. Di Luzio and Friedmann found that contamination of public water systems with endotoxin is common, especially if the system draws on surface water sources, as in this instance.

Control of endotoxin contamination of water used to prepare dialysate must remain within dialysis centres, since it would be impractical for water authorities to produce pyrogen-free water. Of the three forms of water treatment commonly used in haemodialysis centres, water softeners and de-ionisers do not remove endotoxin. However, experiments in our laboratory indicate that reverse osmosis can remove endotoxin from water, and we have recommended that this centre should install reverse- osmosis water treatment.

The clinical features of this epidemic and the fact that host factors played a significant role in it are also noteworthy. The decreased risk found in patients with antecedent gram-negative bacterial infections and in the Black population and the increased risk found in patients with glomerulonephritis and diabetic nephropathy demonstrate that within a given dialysis population individuals differ in their response to endotoxin. These differences in host response underscore the fact that what may represent a harmless exposure to one individual may in another cause significant illness and even death, depending on the patient's general state of health and his sensitivity to endotoxin. These differences also emphasise the importance of effective control measures to prevent these reactions. We believe that through a better understanding of the causes of these reactions and the means of their control, this significant complication of haemodialysis can be eliminated.

Requests for reprints should be addressed to S.H.H.

REFERENCES


IS GLUCAGON IMPORTANT IN STABLE INSULIN-DEPENDENT DIABETICS?

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Summary

The role of glucagon has been evaluated in the everyday regulation of carbohydrate and lipid metabolism in insulin-dependent diabetic patients. Plasma concentrations of glucagon, growth hormone, cortisol, glucose, and free fatty acids and blood concentrations of glycerol, 3-hydroxybutyrate,
acetoacetate, alanine, pyruvate, and lactate were measured in 38 fasting diabetic subjects deprived of their usual morning dose of insulin. The measurements were repeated in 25 of these patients after a further 3 hours of insulin deprivation and in 6 patients again at 6 hours. There was no correlation between the initial fasting levels of plasma-glucagon and those of the other biochemical measurements including glucose and ketone bodies. Furthermore, no correlation was found between changes in these measurements and in plasma-glucagon over a period of 3 or 6 hours. These findings suggest that glucagon is unlikely to play a role of primary importance in blood-glucose homeostasis or ketone-body metabolism in ambulant insulin-dependent diabetic patients.

Introduction
Evidence has recently been presented in support of the hypothesis that diabetes mellitus is a bihormonal disorder characterised not only by a relative or absolute deficiency of insulin but also by a relative or absolute excess of glucagon, a hormone which exaggerates the metabolic consequences of insulin lack.1-3 Observations following infusion of somatostatin into ketoacidotic diabetic subjects have implicated the gluconeogenic, ketogenic, and lipolytic properties of glucagon in the genesis of diabetic ketoacidosis.4 Although hyperglucagonaemia has been identified in less severe diabetes, its importance as a regulator of carbohydrate and fat metabolism in the symptom-free insulin-dependent diabetic and its possible role as a primary factor in the aetiology of brittle diabetes remains to be explored. In this preliminary study of insulin-dependent diabetic patients we have investigated the possible relationship between glucagon and carbohydrate and lipid metabolism. Glucose, alanine, pyruvate, and lactate were measured as indices of carbohydrate metabolism, while ketone bodies, free fatty acids, and glycerol were estimated as indices of lipid metabolism.

Patients and Methods

Patients
38 insulin-dependent diabetics were investigated. Their mean age was 53 (range 18-73) years. The weight of each patient varied between -21% and +16% of the ideal weight for their height. The mean duration of diabetes was 14 years (range 2-37 years). 15 patients were receiving treatment with long-acting insulin once daily, 12 patients with a short-acting insulin twice daily, and 11 patients with a combination of long and short acting insulins. All patients were active and ambulant and had been on their usual diet prior to study.

Test Procedures
Patients were studied after an overnight 14-hour fast and before their usual morning dose of insulin. An indwelling canula was inserted into a vein in the antecubital fossa and each patient was rested in the sitting position for at least 45 minutes. Two blood-samples were then taken at 10-minute intervals. In 25 patients a further fasting sample was taken at 3 hours, and in 6 of these subjects also at 6 hours. Blood-samples were assayed for glucagon, growth hormone, cortisol, glucose, glycerol, free fatty acids, acetoacetate, 3-hydroxybutyrate, alanine, pyruvate, and lactate.

Informed consent was obtained from all patients taking part in the study.

Assays
Blood-samples for the assay of plasma-glucagon concentration were collected with 100 K.I.U. aprotinin per ml, and plasma-glucagon was measured by a radioimmunassay5 using Medical Research Council 69/194 standards made up in glucagon-free plasma and a pancreatic-glucagon-specific (C-terminal-reacting) antisemur. Plasma-growth hormone was assayed by a double-antibody method based on that of Hales and Randle6 using growth-hormone-binding reagent. Plasma-cortisol was measured by a competitive protein-binding method (’Cortipac', Radiochemicals, Amersham). Plasma-glucose was assayed by an automated glucose-oxidase method.7 Blood lactate,8 pyruvate,9 acetoacetate,10 3-hydroxybutyrate,10 glycerol,11 and alanine12 were measured by enzymatic methods and plasma non-esterified fatty acids were measured by a radioactive-cobalt method.13 Total-ketone-body concentration refers to the sum of 3-hydroxybutyrate and acetoacetate concentrations. Plasma for glucagon, growth hormone, cortisol, and free-fatty-acid assays was separated immediately after blood-sampling and stored at -20°C so that all samples could be assayed in the same batch.

Statistics
The Kendall rank correlation coefficient was used to assess the correlation between fasting levels and changes in glucagon, growth-hormone, and cortisol and between fasting levels and changes in glucose, ketone bodies, glycerol, free fatty acids, alanine, pyruvate, and lactate. Student’s paired t test was used to examine changes in these hormones and metabolites over a 3-hour and 6-hour period.

Results
Plasma and blood concentrations of hormones and metabolites at zero and 3 hours in 25 patients and zero and 6 hours in 6 patients are shown in the accompanying table.

Initial Fasting Values
Plasma concentrations of growth hormone, glucose, and free fatty acids and blood concentrations of ketone bodies and glycerol were elevated, but the other hormone and metabolite concentrations were normal. In 38 patients there was no correlation between the fasting levels of plasma-glucagon and the corresponding levels of plasma and blood concentrations of hormones and metabolites (±S.E.M.) in fasting insulin-deprived patients at zero, 3 hours, and 6 hours.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Glucose (mg/100 ml)</th>
<th>Glucagon (pg/ml)</th>
<th>Growth hormone (ng/ml)</th>
<th>Cortisol (yg/100 ml)</th>
<th>Total ketones (mmol/l)</th>
<th>Free fatty acids (mmol/l)</th>
<th>Glycerol (mmol/l)</th>
<th>Alamine (mmol/l)</th>
<th>Lactate (mmol/l)</th>
<th>Pyruvate (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>255</td>
<td>286</td>
<td>49</td>
<td>62</td>
<td>17.2</td>
<td>11.1</td>
<td>18.5</td>
<td>0.56</td>
<td>0.66</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>232</td>
<td>232</td>
<td>49</td>
<td>57</td>
<td>12</td>
<td>7.1</td>
<td>18.5</td>
<td>0.56</td>
<td>0.66</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>232</td>
<td>232</td>
<td>49</td>
<td>57</td>
<td>12</td>
<td>7.1</td>
<td>18.5</td>
<td>0.56</td>
<td>0.66</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NS = not significant
either glucose \((r=0.27)\) (fig. 1) or total ketone bodies \((r=0.147)\).

**Changes in Hormone and Metabolite Concentrations over 3 and 6 Hours**

Plasma-glucose showed a significant fall from 255 ± 20 mg/100 ml to 232 ± 21 mg/100 ml over the 3-hour period in 25 patients. This was accompanied by a significant fall in the blood concentrations of alanine \((0.32 ± 0.02 \text{ mmol/l}} \text{ to } 0.28 ± 0.01 \text{ mmol/l})\), lactate \((0.78 ± 0.04 \text{ mmol/l}} \text{ to } 0.70 \text{ mmol/l})\), and pyruvate \((0.078 ± 0.003 \text{ mmol/l}} \text{ to } 0.061 ± 0.004 \text{ mmol/l})\). There were no significant changes in plasma glucagon or growth hormone, but plasma-cortisol fell significantly as expected from 17.5 ± 0.8 µg/100 ml to 14.4 ± 0.8 µg/100 ml.

In the 6 patients studied over a longer period of 6 hours, only alanine showed a significant fall \((0.31 ± 0.04 \text{ mmol/l}} \text{ to } 0.28 ± 0.01 \text{ mmol/l})\). Changes in plasma glucagon and glucose and changes in blood total ketones over the 3-hour and 6-hour period are shown in fig. 2. There were no significant correlations between changes in plasma-glucagon over 3 or 6 hours and corresponding changes in plasma-glucose \((r=0.117)\) (fig. 3), total blood ketones \((r=0.031)\) (fig. 3), or any of the other metabolites. Although the fall in plasma-cortisol was accompanied by a fall in the levels of glucose, alanine, lactate, and pyruvate, there was no direct relationship between individual changes in cortisol and changes in these metabolites.

**Discussion**

Many so-called stable insulin-dependent diabetics have persistently high blood-glucose concentrations. Somogyi\(^1\) drew attention to factors other than insulin which might influence blood-glucose levels in these patients. Other observers have noted abnormalities in the secretion of glucagon,\(^1\) growth hormone,\(^1\) and cortisol\(^1\) in diabetic subjects compared to non-diabetic controls. In the insulin-dependent diabetic, with little or no ß-cell function, exogenous insulin usually reaches or exceeds physiological systemic levels, promoting peripheral glucose uptake and inhibiting lipolysis from adipose tissue. However, since exogenous insulin is not secreted directly into the portal circulation its level in the portal vein is lower than normal. It is therefore less effective than endogenous insulin in inhibiting hepatic gluconeogenesis and ketogenesis. In this situation the action of hormones that stimulate hepatic gluconeogenesis and/or ketogenesis is relatively unopposed and hyperglycaemia and ketonaemia may occur in the face of apparently adequate levels of peripheral-plasma insulin.
The ability of pharmacological doses of exogenous glucagon to influence both carbohydrate and lipid metabolism is well established. Recently the consequences of more physiological changes in plasma-glucagon levels have been observed. Infusion of somatostatin in fasting normal subjects reduced plasma glucagon and insulin concentrations and this was associated with a significant decline in plasma-glucose. Simultaneous infusion of exogenous glucagon at physiological portal concentrations prevented the fall in glucose induced by somatostatin, demonstrating the importance of glucagon in maintaining resting plasma-glucose levels. In juvenile-type diabetics, deprivation of exogenous insulin was followed by an increase in peripheral concentrations of plasma-glucagon. This increase in glucagon was accompanied by progressive increases in plasma levels of glucose and free fatty acids and blood levels of 3-hydroxybutyrate and glycerol. When these patients were infused with somatostatin, glucose and ketone-body formation was retarded, possibly as a result of the concurrent inhibition of glucagon secretion.

These observations support the view that glucagon is a major factor in the regulation of carbohydrate and lipid metabolism. They also suggest the possible importance of glucagon in the aetiology of the elevated blood-glucose concentrations commonly seen in so-called stable insulin-dependent diabetics. In order to explore this possibility, patients in the present study were observed at the time of day when they would be expected to be most insulin-deprived—i.e., just before their normal dose of insulin. In such a situation any metabolic changes caused by other hormonal influences would be expected to be maximal and would be uncomplicated by the ingestion of food.

No relationship was found between the fasting levels of plasma glucagon, growth hormone, or cortisol on the one hand and fasting levels of plasma glucose and free fatty acids and blood 3-hydroxybutyrate, acetocacetate, lactate, pyruvate, glycerol, and alanine on the other. More important, neither the initial fasting levels nor changes in plasma glucagon, growth hormone, and cortisol were significantly correlated with changes in any of these metabolites when insulin deprivation was prolonged over a 3-hour or a 6-hour period. If glucagon was a major factor in plasma-glucose homeostasis and ketone-body formation in the symptom-free insulin-dependent diabetic subject, then some correlation would be expected between changes in glucagon and corresponding changes in the levels of glucose, ketone bodies, and other metabolites. Interestingly, in many of our patients plasma-glucose concentration decreased during the extra 3-6 hours of insulin deprivation. This was accompanied by a fall in blood concentrations of the gluconeogenic precursors, alanine, pyruvate, and lactate, suggesting perhaps enhanced hepatic uptake. Interpretation is, however, difficult without the use of radioactively labelled substances.

The maintenance of physiological levels of blood-glucose depends not only on insulin but also on the coordinated action of a number of other hormones. Abnormalities in the secretion of these other hormones, in particular glucagon, are found in diabetics. However, in this study of symptom-free insulin-dependent diabetic patients, we have been unable to implicate glucagon, growth hormone, and cortisol as major regulators of glucose homeostasis.

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REFERENCES


A NEW TYPE OF FAMILIAL HYPERCHOLESTEROLEMIA

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Summary Two members of a family (proband and daughter) with hypercholesterolemia have an abnormal low-density lipoprotein which fails to suppress the activity of a rate-determining enzyme for cholesterol biosynthesis (3-hydroxy-3-methyl glutaeryl-CoA reductase) in leucocytes of the patients and controls. However, the proband's leucocytes are inhibited by lipoproteins from other sources demonstrating that the mechanism for cellular regulation of the enzyme is intact. This mutant lipoprotein may have a role in the production of hypercholesterolemia.

Introduction Defects have now been described in the regulatory properties of enzymes which are associated with increased synthesis of metabolic products but without accumulation of abnormal metabolic intermediates. Thus, in lipomatosis there is impaired feedback inhibition of phosphofructokinase (E.C. 2.7.1.11.) by citrate which may be related to the increased accumulation of glyceride-glycerol found in this condition. In familial hypercholesterolemia there is impaired regulation of 3-hydroxy-3-methyl glutaryl-CoA reductase (H.M.-CoA reductase, E.C. 1.1.1.34) by lipoprotein cholesterol in fibroblasts (and other cells such as leucocytes) which may be associated with increased synthesis and accumulation of cholesterol. This enzyme is the rate-determining one in the biosynthesis of cholesterol.

It is possible that other errors in metabolic regula-