Glucose tolerance and insulin release in malnourished rats

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Summary

1. Young Wistar rats were used as an experimental model to determine the effects of protein-energy malnutrition on glucose tolerance and insulin release.

2. Malnourished rats presented some of the features commonly found in human protein-energy malnutrition, such as failure to gain weight, hypoalbuminaemia, fatty infiltration of the liver and intolerance of oral and intravenous glucose loads.

3. The rate of disappearance of glucose from the gut lumen was greater in the malnourished rats but there was no significant difference in portal blood glucose concentration between normal and malnourished rats 5 and 10 min after an oral glucose load.

4. Insulin resistance was not thought to be the cause of the glucose intolerance in the malnourished animals since these rats had a low fasting plasma insulin concentration with a normal fasting blood glucose concentration and no impairment in their hypoglycaemic response to exogenous insulin administration. Furthermore, fasting malnourished rats were unable to correct the insulin-induced hypoglycaemia despite high concentrations of hepatic glycogen.

5. Malnourished rats had lower peak plasma insulin concentrations than normal control animals after provocation with oral and intravenous glucose, intravenous tolbutamide and intravenous glucose plus aminophyllin. This was not due to a reduction in the insulin content of the pancreas or potassium deficiency. Healthy weanling rats, like the older malnourished rats, had a diminished insulin response to intravenous glucose and intravenous tolbutamide. However, their insulin response to stimulation with intravenous glucose plus aminophyllin far exceeded that of the malnourished rats. Thus the impairment of insulin release demonstrated in the malnourished rats cannot be ascribed to a 'functional immaturity' of the pancreas.

Key words: aminophyllin, glucose, glycogen, insulin, malnutrition, potassium, protein-energy malnutrition, tolbutamide.

Introduction

An impairment of carbohydrate metabolism has long been recognized as an accompaniment of undernutrition in the child and malnutrition has been regarded by some as a possible cause of diabetes mellitus in the adult (James & Coore, 1970). Clinical investigations have ascribed the glucose intolerance of infantile protein-energy malnutrition to diminished insulin release (James & Coore, 1970; Milner, 1971; Becker, Pimstone, Hansen & Hendricks, 1971) or insulin resistance (Bowie, 1964) or both (Becker, Pimstone, Hansen, MacHutchon & Drysdale, 1972). The diversity of opinions probably reflects the differences in genetic factors and dietary habits of the various populations studied. This is further complicated by the presence of infection (which in itself causes glucose intolerance) in many of the children and by the difficulty in obtaining adequate control subjects.

To eliminate some of these variables, glucose tolerance and insulin release were investigated in weanling rats fed on a low protein diet for 3 weeks.
These animals were compared with age-matched rats fed on a normal protein diet for the same period of time. Since it has been suggested by Heard, Durbin & Platt (1961) that malnutrition may cause failure of maturation of pancreatic endocrine function, weanling rats were investigated before the institution of either a low or normal protein diet.

Methods

Experimental animals

Unsexed weanling Wistar rats, 21 days old and weighing 30 g, were allowed free access to one of two diets for 3 weeks; one containing 20% protein in the form of casein, and the other 4% protein supplemented with 0·2% DL-methionine and extra dextrin. Both diets were supplemented with minerals and vitamins and were of equal caloric value. All animals were housed in an air-conditioned room (18–20°C). A full description of the malnourished rat model has been given by Stead & Brock (1972). The 20% protein-fed rats were used as the control animals (age-matched control); weanling rats (before the institution of either of these diets) were also investigated.

Chemical methods

Serum albumin was determined by the method of Doumas, Watson & Biggs (1971) and total serum protein by the method of Lowry, Rosebrough, Farr & Randall (1951). Liver fat was estimated by the decrease in weight of the dried liver after a 48 h extraction with petroleum ether (b.p. 40–60°C) (Hazlewood & Nichols, 1969) and has been expressed as a fraction of the dried liver weight. Glycogen content of the livers of starved rats was determined by the method of Good, Kramer & Somogyi (1933) and was expressed as mg of glycogen/g wet liver weight. Total body water was determined by dilution of intraperitoneally administered tritiated water (Belcher & Vetter, 1971) and has been expressed as a percentage of the total body weight. Plasma and muscle potassium concentrations were measured by flame photometry with internal lithium standardization.

Glucose concentrations were determined on 5 μl of whole blood by the glucose oxidase method of Huggett & Nixon (1957). Insulin concentrations were measured on 5 μl of plasma by the double antibody radioimmunoassay method of Morgan & Lazarow (1963), a rat insulin standard (Novo Research Laboratories, lot no. R170, 21·4 μunits/ng) being used. To detect differences in the fasting plasma insulin concentrations, a modified immunoassay on 10 μl of plasma was used. The coefficient of variation of the insulin assay at insulin concentrations between 1 and 5 ng/ml was approximately 15%. For both the glucose and insulin determinations 50 μl of whole blood was sufficient, thus making it possible to take frequent samples from the same animal. These micro methods were described in more detail by Weinkove, Weinkove & Pimstone (1974).

Plasma and muscle concentrations of potassium

Heparinized blood was collected by cardiac puncture from anaesthetized rats for plasma potassium estimation. Approximately 100 mg of gastrocnemius muscle was removed from each animal, dried for 8 days in an oven at 110°C and the fat-free dry weight of the tissue determined after extraction with petroleum ether (b.p. 40–60°C) for 72 h (Hazlewood & Nichols, 1969). Tissue potassium was determined on the nitric acid digests of this tissue (Lowry & Hastings, 1942).

Pancreatic insulin content

Starved rats (18 h) were killed by stunning and the pancreas was rapidly removed and homogenized in 10–15 ml of cold acid alcohol. After being shaken overnight at 4°C, the homogenate was centrifuged and the supernatant diluted in veronal buffer (50 mmol/l, pH 8·6). Immunoreactive insulin concentrations were determined at two dilutions and the results expressed as μg/100 g body weight.

General procedure of tests of glucose tolerance and insulin release in vivo

Animals were starved for 18 h but allowed free access to water before the tests, all of which were done in the morning on unanaesthetized rats. No animal was used for more than one provocative test. Basal blood samples were taken from the tip of the rat’s tail after warming, after which glucose, insulin, tolbutamide or aminophyllin, appropriately diluted, were given in a dose of 1 ml/100 g body weight. For the oral glucose test, glucose was administered intragastrically by means of a small polythene
catheter attached to a needle fitted to a syringe. For intravenous tests, glucose, insulin, tolbutamide or aminophyllin were injected into a superficial vein near the base of the tail using a 26 gauge needle attached to a calibrated syringe. Samples of blood (50 μl) were collected from the tip of the rat's tail at timed intervals after the completion of the intravenous or gastric load, and put into heparinized capillary tubes.

**Tolerance tests**

**Intravenous glucose.** Blood glucose and plasma insulin concentrations were estimated before (0 min) and 3, 6, 10, 15, 20, 25 and 30 min after an intravenous glucose load of 7 mmol/kg body weight. The glucose disappearance rate constant ($K_D$) was calculated according to the method of Ikkos & Luft (1957).

The glucose volume (or space), $V_G$, was calculated as described by Franckson, Ooms, Bellens, Conard & Bastenie (1962) from $V_G = Q/A - C_0$, where $Q$ = quantity of glucose injected (mmol), $A$ = theoretical concentration of glucose at time zero as calculated from $K_D$ and $C_0$ = the initial (fasting) blood glucose concentration (mmol/l).

The glucose space has been used as an estimate of the extracellular fluid space (Franckson et al., 1962) and is expressed as a percentage of the total body weight.

**Oral glucose.** Blood glucose and plasma insulin concentrations were estimated from tail blood samples before and 5, 10, 15, 30, 45, 60 and 90 min after an oral glucose load (28 mmol/kg body weight).

**Insulin.** Blood glucose concentrations were measured in rat tail blood before and 10, 20, 30, 45, 60, 90 and 120 min after the intravenous injection of soluble beef insulin (Insulin B.P., Burroughs Wellcome (Pty.) Ltd), 0.2 unit/kg body weight.

**Glucose absorption from the gut lumen**

The method described by Cori (1925) was used. Rats were killed by stunning 15 min after receiving an oral glucose load (28 mmol/kg body weight). Ligatures were tied around the oesophagus and rectum and the entire gut was removed. The bowel was opened and its contents were washed with distilled water through a cotton wool filter into a volumetric flask and the glucose concentration of this filtrate was determined. The difference between the glucose load administered and the glucose recovered from the gut lumen was used as an estimate of glucose absorption, the results being expressed as mmol/100 g body weight in 15 min. A glucose recovery rate of 80% is found with this method, in agreement with Scow & Cornfield (1954).

**Portal venous glucose and insulin concentrations after an oral glucose load**

Rats were given 28 mmol of glucose/kg body weight by intragastric tube. Five or 10 min later the animals were subjected to rapid ether anaesthesia and laparotomy. Blood glucose and plasma insulin concentrations were estimated on portal venous blood samples.

**Intravenous tolbutamide test**

Plasma insulin concentrations were estimated on tail blood samples taken before (0 min) and 3, 6, 10, 15, 30, 45 and 60 min after the intravenous injection of tolbutamide (Rastinon, Hoechst) (430 μmol/kg body weight).

**Intravenous glucose plus aminophyllin test**

Plasma insulin concentrations were estimated before and 3, 6, 10, 15, 20, 25 and 30 min after the rats were given a combined intravenous dose of glucose (7 mmol/kg) with aminophyllin (Nutrated, Propan) (140 μmol/kg body weight).

**Statistical tests and recording of results**

The Mann–Whitney U-test (Siegel, 1956) was used to test the significance of the differences between the plasma insulin in the three groups of rats. The Student's t-test (Bailey, 1959) was used to test the significance of the differences in mean values in all the other investigations. A two-tailed test was used in the determination of $P$ from the appropriate tables and $P > 0.05$ has been reported as not significant.

Only the median values of the plasma insulin concentrations are recorded in the Figures and Tables. The mean values and SEM have been provided for all other data. In all cases the number ($n$) of animals tested has been included, with the significant $P$ values given in the Tables or in the text. Individual results are available on request.
**Results**

**Nutritional status of the experimental animals** (Table 1)

Weanling rats maintained for 3 weeks on a 4% protein diet showed the following features commonly found in human protein-energy malnutrition: (1) failure to gain weight; (2) marked hypoalbuminaemia; (3) an increase in liver fat content, shown not only chemically but confirmed to be due to a mild to moderate degree of periportal fatty infiltration of the liver when frozen sections of the liver were stained with Sudan III. After 18 h starvation, the liver glycogen was significantly greater in the malnourished compared with control animals. There was no evidence of oedema in the malnourished animals, nor could occult oedema be demonstrated by the measurement of total body water. Plasma and muscle potassium concentrations did not differ significantly in the three groups of animals studied.

**Fasting blood glucose and plasma insulin concentrations** (Table 2)

Fasting blood glucose concentrations were similar in all three groups of animals but rats maintained on a 4% protein diet had significantly lower fasting plasma insulin concentrations than both 20% protein-fed and weanling rats.

**Total pancreatic insulin content** (Table 2)

Malnourished (4% protein-fed) rats had a significantly greater pancreatic insulin content than the 20% protein-fed rats, while insulin content was greatest in the weanling rats.

**Tolerance tests**

**Intravenous glucose** (Table 2). Compared with results for the 20% protein-fed animals, intravenous glucose tolerance was impaired in the 4% protein-fed animals.

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### Table 1. Nutritional status of three groups of rats studied

Statistical significance (P) of differences between measurements are placed between the values compared in this and subsequent Tables. Numbers of determinations (n) are given in parentheses. NS = not significant.

<table>
<thead>
<tr>
<th></th>
<th>20% protein diet</th>
<th>4% protein diet</th>
<th>Weanling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>93.1±2.4 (12)</td>
<td>P&lt;0.001</td>
<td>30.8±1.2 (12)</td>
</tr>
<tr>
<td>Total body water (% body wt.)</td>
<td>77.6±0.7 (8)</td>
<td>NS</td>
<td>77.7±1.1 (7)</td>
</tr>
<tr>
<td>Serum albumin (g/100 ml)</td>
<td>3.26±0.06 (12)</td>
<td>P&lt;0.001</td>
<td>2.12±0.08 (12)</td>
</tr>
<tr>
<td>Total serum protein (g/100 ml)</td>
<td>6.04±0.16 (12)</td>
<td>P&lt;0.001</td>
<td>3.98±0.14 (12)</td>
</tr>
<tr>
<td>Liver fat (% dry liver wt.)</td>
<td>4.5±0.6 (8)</td>
<td>P&lt;0.02</td>
<td>9.7±1.8 (8)</td>
</tr>
<tr>
<td>Liver glycogen (mg/g liver wt.)</td>
<td>6.5±2.3 (6)</td>
<td>P&lt;0.01</td>
<td>19.7±3.1 (6)</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>5.4±0.2 (6)</td>
<td>NS</td>
<td>4.8±0.2 (8)</td>
</tr>
<tr>
<td>Muscle potassium (mmol/100 g fat-free dry wt.)</td>
<td>47.0±0.6 (9)</td>
<td>NS</td>
<td>46.5±0.6 (8)</td>
</tr>
</tbody>
</table>

### Table 2. Fasting blood glucose, plasma insulin, pancreatic insulin content, glucose disappearance rate constant ($K_a$) and glucose space in the three groups of rats

See also Table 1.

<table>
<thead>
<tr>
<th></th>
<th>20% protein diet</th>
<th>4% protein diet</th>
<th>Weanling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.5±0.2 (12)</td>
<td>NS</td>
<td>5.6±0.5 (10)</td>
</tr>
<tr>
<td>Fasting plasma insulin (µg/l)</td>
<td>1.0 (12)</td>
<td>P&lt;0.002</td>
<td>0.3 (10)</td>
</tr>
<tr>
<td>Fasting pancreatic insulin content (µg/100 g body wt.)</td>
<td>22.3±1.4 (15)</td>
<td>P&lt;0.005</td>
<td>32.6±2.4 (15)</td>
</tr>
<tr>
<td>$K_a$ (%/min)</td>
<td>7.4±0.4 (10)</td>
<td>P&lt;0.01</td>
<td>5.3±0.6 (10)</td>
</tr>
<tr>
<td>Glucose space (% body wt.)</td>
<td>35±1 (10)</td>
<td>NS</td>
<td>34±2 (10)</td>
</tr>
</tbody>
</table>
Glucose and insulin in malnourished rats

Fig. 1. Median plasma insulin and mean (±SEM) blood glucose concentrations before (0 min) and after intragastric glucose administration (2.8 mmol/100 g body weight) in three groups of rats: ●, 20% protein-fed rats (n = 11); ▲, 4% protein-fed rats (n = 11); □, weanling rats (n = 12).

Fig. 2. Mean (±SEM) blood glucose concentrations before (0 min) and after intravenous insulin administration (0.02 unit/100 g body weight): ●, 20% protein-fed rats (n = 12); ▲, 4% protein-fed rats (n = 12); □, weanling rats (n = 12).
rats, as shown by a lower $K_c$, in spite of a similar glucose space.

**Oral glucose (Fig. 1).** Malnourished rats showed relatively mild glucose intolerance with blood glucose concentrations significantly higher than in the 20% protein-fed animals at 15 and 30 min only ($P < 0.05$ and $P < 0.001$ respectively). Weanling rats had blood glucose concentrations significantly greater than malnourished rats at 10, 15, 45, 60 and 90 min after the oral glucose load ($P < 0.05$ at 45 min; in all other cases $P < 0.001$).

**Intravenous insulin (Fig. 2).** There were no statistically significant differences between the mean blood glucose concentrations in the three groups of animals at 0, 10 and 20 min. When compared with 20% protein-fed rats, the malnourished (4% protein-fed) rats had significantly lower blood glucose concentrations ($P < 0.02$) at all time-intervals tested after 20 min and one-third of the 4% protein-fed rats originally tested did not survive the test. Weanling rats had significantly higher blood glucose concentrations than the 4% protein-fed rats at 60, 90 and 120 min ($P < 0.01$, $P < 0.02$, $P < 0.01$ respectively) after intravenous insulin administration.

**Glucose absorption (Table 3)**

The malnourished rats had a significantly greater rate of glucose disappearance from the gut lumen than 20% protein-fed controls ($P < 0.05$). There was no significant difference in the glucose absorption rate between 4% protein-fed and weanling rats. However, weanling rats had significantly higher mean portal blood glucose concentrations than the malnourished rats 5 min after oral glucose.

**Table 3. Glucose absorption and portal venous glucose and insulin concentrations after oral glucose in the three groups of rats**

<table>
<thead>
<tr>
<th>Glucose absorbed from gut lumen (mmol 5 min$^{-1}$ 100 g body wt.)</th>
<th>20% protein diet</th>
<th>4% protein diet</th>
<th>Weanling</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.89±0.09 (6)</td>
<td>$P &lt; 0.05$</td>
<td>1.32±0.15 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Portal venous concentrations of glucose (mmol/l) and insulin (µg/l) after oral glucose at time:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min              Glucose</td>
<td>10.2±1.1 (6)</td>
<td>NS</td>
<td>10.6±0.7 (6)</td>
</tr>
<tr>
<td>Insulin</td>
<td>7.3</td>
<td>NS</td>
<td>2.3</td>
</tr>
<tr>
<td>10 min             Glucose</td>
<td>12.8±0.4 (6)</td>
<td>NS</td>
<td>12.7±1.4 (5)</td>
</tr>
<tr>
<td>Insulin</td>
<td>10.6</td>
<td>$P &lt; 0.005$</td>
<td>2.1</td>
</tr>
</tbody>
</table>

**Insulin release**

**After intravenous glucose (Fig. 3).** Plasma insulin concentrations reached their peak at 3 min after intravenous glucose and were significantly greater in the 20% protein-fed rats than in malnourished animals 3, 6 and 10 min after the glucose ($P < 0.02$). There were no statistically significant differences between the weanling and 4% protein-fed rats.

**After oral glucose administration (Fig. 1).** In all three groups of rats studied the peak insulin concentration occurred 10 min after oral glucose. The 4% protein-fed rats had plasma insulin concentrations which were significantly lower than in the 20%
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FIG. 4. Median plasma insulin concentrations before (0 min) and after the intravenous administration of tolbutamide (43 μmol/100 g body weight): ●, 20% protein-fed rats (n = 8); ▲, 4% protein-fed rats (n = 9); □, weanling rats (n = 10).

After intravenous tolbutamide (Fig. 4), 4% protein-fed rats had plasma insulin concentrations which were significantly lower than the 20% protein-fed animals at 0 min (P < 0.02) and 3, 6 and 10 min (P < 0.002), 15 min (P < 0.02) and 60 min (P < 0.002) after intravenous tolbutamide administration.

Weanling rats had higher median plasma insulin concentrations than the malnourished (4% protein-fed) rats with P < 0.002 at 3 and 6 min and P < 0.02 at 10 and 15 min after the provocative load. There was no statistical significance in the difference between the insulin concentrations in these two groups after 15 min.

Weanling rats were found to have significantly higher median plasma insulin concentrations than the malnourished rats throughout the test with P < 0.002 at 3, 6 and 15 min, P < 0.02 at 10 and 30 min, and P < 0.05 at 20 and 25 min.

After intravenous glucose plus aminophyllin (Fig. 5). The 20% protein-fed rats had higher median plasma insulin concentrations than the malnourished (4% protein-fed) rats with P < 0.002 at 3 and 6 min and P < 0.02 at 10 and 15 min after the provocative load. There was no statistical significance in the

FIG. 5. Median plasma insulin concentrations before (0 min) after the intravenous administration of glucose (0.7 mmol/100 g body weight) plus aminophyllin (14 μmol/100 g body weight): ●, 20% protein-fed rats (n = 9); ▲, 4% protein-fed rats (n = 7); □, weanling rats (n = 10).
Discussion

**Experimental model**

Weanling rats maintained for 3 weeks on a 4% protein diet supplemented with methionine, vitamins and minerals had many of the features commonly found in human protein-energy deficiency, namely failure to gain weight, hypoalbuminaemia and fatty infiltration of the liver. The absence of oedema in these animals, as shown by the measurement of total body water, is not surprising in view of the reported difficulty of producing oedema in rats (Widdowson & McCance, 1957; Ramalingaswami & Deo, 1968), and was of value in that glucose, insulin and other drugs could be administered according to body weight without correction for any difference in body water content.

It is not possible to ascribe all the differences between the malnourished (4% protein-fed) rats and the normal (20% protein-fed) rats to the difference in their dietary protein intake. Though the two diets have the same food energy content per g, the malnourished rats reduced their intake to a level just sufficient to maintain body weight. As with malnourished infants these animals suffer protein-energy deficiency and not pure protein deficiency.

**Glucose tolerance**

In common with malnourished infants, the malnourished rats had glucose intolerance, more marked after intravenous glucose administration than after oral glucose loading. This discrepancy between the oral and intravenous glucose tolerance test in malnourished animals has been quoted as evidence for diminished glucose absorption in malnutrition by Platt, Heard & Stewart (1964). In the present study, however, glucose disappearance from the gut lumen was greater in the 4% protein-fed rats than in the 20% protein-fed controls. Despite the rapid glucose absorption from the gut lumen, the malnourished rats did not show the expected early increase in portal venous glucose concentration. These findings are consistent with those of Lifshitz, Hawkins, Diaz-Bensussen & Wapnir (1972), who have reported an increase in the transport of glucose in perfused intestinal segments of malnourished rats with an increased utilization of glucose by the intestine. Kershaw, Neame & Wiseman (1960) have also found that semi-starvation enhanced the rate of disappearance of glucose from the small intestine in vivo. Thus, far from impairing absorption, protein-energy deprivation appears to enhance the rate of glucose absorption as has also been reported with amino acids (Kirsch, Saunders & Brock, 1968).

**Insulin resistance**

If the rate and the degree of the drop in blood glucose concentration after the administration of exogenous insulin is used as a criterion of insulin sensitivity (Cerasi & Luft, 1969), the malnourished rats were not insulin resistant (Fig. 2). The finding of a low fasting plasma insulin concentration with normal fasting blood glucose concentration (Table 2) in these animals may be cited as corroborative evidence, since insulin resistance as defined above is often associated with fasting hyperinsulinaemia (Berson & Yalow, 1965; Porte & Bagdade, 1970; Goldfine, Kahn, Neville, Roth, Garrison & Bates, 1973; Olefsky, Farquhar & Reaven, 1973).

The evidence for insulin resistance in human protein-energy malnutrition has been indirect, being based on the measurement of the glucose disappearance rate after the administration of glucose plus insulin (Bowie, 1964; Alleyne, Trust, Flores & Robinson, 1972) or the presence of sustained insulin secretion in the face of glucose intolerance (Becker et al., 1972). In other experimental animal models of protein-energy deficiency (Heard & Henry, 1969) glucose plus insulin was given to assess insulin sensitivity. In the present experimental rat model the administration of insulin alone produced a normal rate of fall of blood glucose concentration but the hypoglycaemia was prolonged. Heard (1966) has also reported prolonged hypoglycaemia after insulin administration to malnourished pigs and spontaneous hypoglycaemia has been reported as a complication of kwashiorkor in humans (Slone, Tait & Gilchrist, 1961).

Possible explanations of the prolonged hypoglycaemia after insulin administration to malnourished rats include impaired hepatic extraction of insulin, impaired release of gluconeogenic and glycogenolytic hormones such as glucagon and adrenaline, or failure of the liver to respond to these hormones due to a deficiency of gluconeogenic or glycogenolytic enzymes. Impaired hepatic degradation of insulin is not the cause of the persistent hypoglycaemia since the rate of disappearance of endogenous insulin after an intravenous glucose load
(see Fig. 3) and exogenous insulin clearance by the isolated perfused liver (H. S. Sacks & B. L. Pimstone, unpublished work) are similar in 4% protein-fed and 20% protein-fed rats. Alleyne & Scullard (1969) have reported that malnourished children showed an increase in their hepatic activities of one of the key gluconeogenic enzymes, glucose 6-phosphatase, normal concentrations of liver phosphorylase, and a normal hyperglycaemic response to glucagon administration. B. L. Pimstone & D. J. Becker (unpublished work) have shown elevation of plasma glucose and cyclic AMP after glucagon administration in human protein-energy malnutrition. This would favour glucagon deficiency as a cause of the failure of malnourished rats to correct insulin-induced hypoglycaemia and may also account for their normal fasting blood glucose concentrations in the face of low fasting plasma insulin concentrations and elevated liver glycogen (Table 1). The role of adrenaline in this context has not been studied.

**Insulin release**

Malnourished rats had a greater pancreatic insulin content than the well-nourished control rats. Thus the poor insulin response of the former to oral and intravenous glucose appeared to be due to a defect in insulin release. Although glucose is still accepted as the most important stimulus of insulin release, other natural and pharmacological agents are capable of doing so (Mayhew, Wright & Ashmore, 1969). If the poor insulin response of the malnourished rats was due to a defect in some pancreatic 'glucoreceptor', it was hoped that some other agent acting via a different mechanism might improve it. Tolbutamide directly stimulates the beta cells of the islets of Langerhans (Basabe, Lopez, Viktora & Wolff, 1971) and has an action on the early release of insulin which is independent of the blood glucose concentration and is unaffected by mannosephulose, which blocks the insulin response to glucose (Loubatières-Mariani, Loubatières & Chapel, 1973). However, this stimulus failed to 'normalize' the insulin release of malnourished rats (Fig. 4). Aminophyllin has been shown to induce insulin release in rats unresponsive to intravenous glucose (Turtle, Littleton & Kipnis, 1967) and caffeine, another methylxanthine alkaloid, released insulin in a neonatal diabetic child who did not respond to glucose or tolbutamide (Pagliara, Karl & Kipnis, 1973). Caffeine has also been shown to improve the glucose-induced (Lambert, Junod, Staffacher, Jeanrenaud & Renold, 1969) as well as tolbutamide-induced (Lambert, Jeanrenaud, Junod & Renold, 1969) insulin release in foetal pancreatic tissue. As with tolbutamide, glucose plus aminophyllin failed to elevate the plasma insulin concentrations of malnourished rats to values similar to those of the 20% protein-fed control animals.

Potassium deficiency has been reported to occur frequently in protein-energy malnutrition (Mann, Bowie & Hansen, 1972) and early potassium supplementation has been shown to improve, although not normalize, their insulin responses to intravenous glucose (Mann, Becker, Pimstone & Hansen, 1975).

In rats loss of total body potassium has been shown to be reflected by reduced muscle potassium concentrations (Whang & Welt, 1963), which was found to be normal in this study (Table 1). Thus potassium deficiency cannot be invoked as the cause of their diminished insulin release.

Since weanling rats placed on a 4% protein diet failed to grow, the possibility that pancreatic endocrine function failed to mature in these rats was also considered. Weanling rats before the institution of the low protein diet showed little or no difference from the older malnourished rats in their insulin response to intravenous glucose (Fig. 3) and intravenous tolbutamide (Fig. 4). However, the younger weanling rats were shown to have a much greater insulin response to intravenous aminophyllin than the malnourished rats (Fig. 5). For this reason it was thought unlikely that the deficiency in insulin release of the malnourished rats was due to immaturity of pancreatic endocrine function.

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