RESULTS
FASTING BLOOD SUGAR IN PROTEIN CALORIE UNDERNUTRITION IN CHILDREN

The fasting blood sugar content of the children suffering from protein-calorie undernutrition is shown in Table 1. On admission the blood sugar content in kwashiorkor was within the normal range in some children while in others the fasting blood sugar content was lower than the normal. These children could therefore be grouped into two groups, normoglycemic, i.e. those having blood sugar from 50 to 110 mg%, and hypoglycemic having blood sugar below 50 mg%. There were seven cases of hypoglycemia among thirty children suffering from kwashiorkor. The blood sugar levels of the normoglycemic children suffering from kwashiorkor was more or less the same as the levels obtained after clinical recovery. Of the hypoglycemic children in this series, only 2 survived. In the latter children, the fasting blood sugar came back to normal levels after recovery. Although 7 children had hypoglycemia only 2 showed clinical symptoms of hypoglycemia shock; the other 5 cases were clinically symptomless.

In marasmus, the incidence of hypoglycemia was rare (1 out of 38 children). In most of the cases the fasting blood sugar content was more or less the same on admission as well as after clinical recovery. Even in the cases with
hypoglycemia the average blood sugar content was higher than in the kwashiorkor group with hypoglycemia. In only one case, there were symptoms of hypoglycemia.

It is generally true that the cases with hypoglycemia were more seriously ill than the normoglycemics. They had extreme lethargy, a musty feeling about their bodies, more severe diarrhoea and they were fed with extreme difficulty. An analysis of the mortality records is shown in table 2. In kwashiorkor there was a much higher mortality in the children with hypoglycemia than in the normoglycemics. The mortality in the normoglycemic kwashiorkor and marasmus was similar. For some unknown reason the mortality in the children suffering from nutritional edema was higher than either marasmus or normoglycemic kwashiorkor, even though their blood sugar content was not not found to be low.
Table I

Fasting blood sugar content in protein calorie malnutrition in children

( mg per 100 ml of plasma )

<table>
<thead>
<tr>
<th>Type</th>
<th>Time of measurement</th>
<th>Blood sugar content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwashiorkor</td>
<td>On admission</td>
<td>53.5 ± 11.2 *</td>
</tr>
<tr>
<td></td>
<td>Before discharge</td>
<td>77.9 ± 10.0</td>
</tr>
<tr>
<td>Marasmus</td>
<td>On admission</td>
<td>77.0 ± 19.3</td>
</tr>
<tr>
<td></td>
<td>Before discharge</td>
<td>84.0 ± 13.2</td>
</tr>
<tr>
<td>Nutritional Edema</td>
<td>On admission</td>
<td>71.3 ± 11.5</td>
</tr>
<tr>
<td></td>
<td>Before discharge</td>
<td>77.1 ± 12.7</td>
</tr>
</tbody>
</table>

Figures in parenthesis refer to number of cases.
Results are given as mean ± standard deviation.
* The results on admission are significantly different from those before discharge.
<table>
<thead>
<tr>
<th>Type</th>
<th>No. of Cases</th>
<th>Glycemic State</th>
<th>No. of Death</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normo- Hypo-</td>
<td>Normo- Hypo-</td>
<td></td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>30</td>
<td>23 7</td>
<td>2 5</td>
<td>9 70</td>
</tr>
<tr>
<td>Marasmus</td>
<td>38</td>
<td>37 1</td>
<td>4 1</td>
<td>10</td>
</tr>
<tr>
<td>Nutritional edema</td>
<td>20</td>
<td>20 0</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Type</th>
<th>No. of Cases</th>
<th>Glycemic state</th>
<th>No. of death</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normo- Hypo-</td>
<td>Normo- Hypo-</td>
<td></td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>172</td>
<td>140 30</td>
<td>32 21</td>
<td>22 70</td>
</tr>
<tr>
<td>Marasmus</td>
<td>211</td>
<td>207 4</td>
<td>31 1</td>
<td>16</td>
</tr>
<tr>
<td>Nutritional edema</td>
<td>142</td>
<td>140 2</td>
<td>23 1</td>
<td>20</td>
</tr>
</tbody>
</table>
ORAL GLUCOSE TOLERANCE TEST

The reaction to an oral loading dose of glucose is ascertained by sugar determination of blood at regular intervals after ingestion of the test dose. A normal, high, prolonged or low rise of the blood sugar curve would indicate, respectively normal, decreased or increased tolerance.

The response of normal children to such a test dose varies greatly. No blood sugar tolerance curve can be considered specific for a child; rather a certain range of blood sugar responses may be considered typical of a normal tolerance for them. Children have a considerably greater capacity to accommodate a sugar load than adults. From the age of 6 years on, the child's tolerance for sugar gradually decreases to the average adult capacity. A child's hyperglycemic response to a standard dose of glucose referred to kilograms of body weight, therefore, varies with age.

The average normal response of a child to the oral glucose tolerance test is a rise in the blood sugar by 30 to 40 per 100 ml, above the fasting level, reaching the peak 30 to 45 mins after ingestion. The ascending portion of the curve depends on the rate of absorption. The further course is determined by metabolic events.
set in motion by the induced hyperglycemia, and is directed towards restoring normal levels of blood sugar.

The return to fasting blood levels occurs more or less uniformly within 2 to 2½ hours. Frequently, the fading antiglycemic effect is still sufficiently strong at the end of the third hour to cause a short drop of the blood sugar below the fasting level.

The oral glucose tolerance tests were undertaken in children suffering from protein-calorie undernutrition. Only cases of classical kwashiorkor and marasmus were investigated without any preliminary feeding of carbohydrate diet. The test was carried out on twenty-two (22) children suffering from kwashiorkor and fourteen from marasmus. Glucose was administered orally in a dose of 1.75 gm per kg of body weight. But since most of these children weighed around 4 kg a standard dose of 10 g of glucose was routinely administered. Cases of kwashiorkor showed an increase of 76 ± 25 mg/ml above the fasting level in 1 hour after administration of glucose; the peak level occurred at about 1 hour, which is somewhat late in comparison to normal and the blood sugar did not return to the fasting level in two and a half hours. In the same patients the oral glucose tolerance became normal after clinical recovery.
FIG. 1

ORAL GLUCOSE TOLERANCE TEST

- ● KWASHIORKOR (on admission)
- ○ " (on recovery)

mg of glucose/100 ml of blood vs. time in hours

0 40 80 120 160
0 1 2 3

FIG. 1
FIG. 2

ORAL GLUCOSE TOLERANCE TEST
MARASMUS (on admission)

mg OF GLUCOSE/100 ml OF BLOOD

TIME IN HOURS

FIG. 2
In 3 cases of kwashiorkor there was a flat curve. The blood sugar showed a small rise of 15-20 mg per 100 ml in an hour and thereafter decreased to the fasting level or a little lower below fasting value and continued all through.

In marasmus the response to oral administration of glucose was more or less normal.

Intra Venous Glucose Tolerance Test:

The results of oral glucose tolerance test are influenced by numerous factors, mainly by intestinal absorption and other factors such as the site of blood sampling, the diet of the individual prior to the test, age, exercise, etc. The tolerance result itself had also a wide range. This makes the test rather difficult to interpret. Intravenous glucose tolerance avoids these extra complications. Hence, such tests were also undertaken in these children, in order to evaluate whether these children could handle glucose load properly.

The intravenous glucose tolerance test was carried out on 16 children suffering from kwashiorkor and 9 with marasmus. It is very difficult to draw repeated blood samples from a malnourished child, especially in marasmus. The tests usually required 5 to 6 punctures and if antecubital veins were not available, repeated femoral vein
punctures were unrealistic. However, these tests could be undertaken on 25 children only with care and diligence exhibited by the house officer, to whom I am deeply grateful. There is a higher risk of provoking hypoglycemic reactions with intravenous glucose tolerance than with the oral procedure. In our cases of kwashiorkor there was however, no such unusual effects.

When the results of oral and intravenous tests in the same patient differed, the results of intravenous test were commonly accepted. In a number of thoroughly studied clinical entities, the stress of a slowly acting oral test load brings about a positive response while the acute stress of an intravenous load fails to do so. So discriminative use of both methods in infants and children seemed advisable, i.e. first obtaining routinely the oral tolerance curve, which was followed by the intravenous procedure.

Normally, immediately after injection there is an overshoot of blood sugar to a markedly high level. Thereafter a rapid decline sets in for a period of about 1 hour and thereafter declines slowly to fasting level within 90 mins.

Glucose was injected in a dose of 0.5 g/kg body weight and blood was collected at regular intervals.
INTRAVENOUS GLUCOSE TOLERANCE TEST

KWASHIORKOR (on admission)

( on recovery )

mg of Glucose/100 mL of Blood

TIME IN MINUTES

FIG 2
FIG. 4

INTRAVENOUS GLUCOSE TOLERANCE TEST

MARASMUS (on admission)

mg of GLUCOSE/100 ml of BLOOD

0

30

60

90

120

150

TIME IN MINUTES

FIG. 4
The response of the blood sugar glucose to the load of intravenous glucose show a high degree of consistency and to enable easy visualization of the salient features the figures are illustrated graphically in fig.3. In cases of kwashiorkor, on admission the fasting blood glucose was comparatively low. Immediately after injection of the glucose the regulatory mechanism appears to function reasonably well but slightly less efficiently than in the same child, after clinical recovery. Thus, whereas in normal children and in the adequately rehabilitated child, the blood sugar returns to more or less the level of fasting, in kwashiorkor the average level of blood sugar was still 30% above the blood sugar level due to the intravenously administered glucose. The difference between these two groups of children was also pronounced at one and a half hours, when the normal and rehabilitated children reached subfasting level, but the children suffering from kwashiorkor still had blood sugar levels considerably higher than the fasting level.

The difference of this results was tested for significance, using the t distribution. The mean $K_g$ was very significantly lower in those who had had kwashiorkor than in the recovered child ($t = 4.71$, $p < 0.001$).

There was no significant correlation between $K_g$ and body weight or height on admission or after clinical recovery. There was also no direct correlation between
fasting blood sugar levels and the severity of malnutrition or the degree of hepatomegaly.

In nine children suffering from marasmus the response to the intravenous injection of glucose does not differ significantly from those found in the normal children. Only in one case of marasmus it showed a slight impairment, which becomes normal when the test was repeated after two weeks.

**Epinephrine Tolerance**

In the maintenance of blood sugar levels, mechanisms concerned with glycogenolysis and release of glucose into the circulation as well as factors concerned with the peripheral utilization of glucose are involved. Epinephrine raises blood sugar by promoting glycogenolysis and also by blocking peripheral utilization. The glycogenolytic action is mediated mainly through the enzyme phosphofructokinase. Fourteen children suffering from kwashiorkor and 8 children from marasmus were studied, on admission and the same test was repeated after 3 months in the same children. In kwashiorkor cases the ages when admitted ranged from 10 months to 25 months (mean 19 months).

Adrenaline was administered subcutaneously in a dose of 0.03 ml of a 1:1000 solution of epinephrine hydrochloride/kg, and the response was noted at regular
Figure 5 shows the epinephrine tolerance test results. The graph plots the mg of glucose/100 ml of blood over time in minutes. Two lines are depicted:

- Solid line: Kwashiorkor (on admission)
- Dashed line: " (on recovery)

The y-axis represents the mg of glucose/100 ml of blood, ranging from 0 to 200. The x-axis represents time in minutes, ranging from 0 to 150.

Fig. 5
FIG. 6

EPINEPHRINE TOLERANCE TEST
MARASMUS (on admission)

mg of GLUCOSE/100 ml of BLOOD

TIME IN MINUTES

FIG. 6
intervals. The normal response consists of a blood sugar rise of 30 to 45 mg per 100 ml during the first hour. Return of the blood sugar to pretest levels is usually completed by 2 hours after administration. Results are shown in fig. 5. Children suffering from kwashiorkor showed a mean rise of 32.5±5.1 mg/100 ml of blood sugar level. The peak occurred at about 1 hour, but the sugar level did not return to its fasting level in 2 hours. After clinical recovery the cases of kwashiorkor showed a mean hyperglycemic response of 41.4±5.0 mg per 100 ml to epinephrine administration. The curve on recovery represents a normal one, the basal level is attained within 2 hour.

In two cases suffering from kwashiorkor there was a flattened curve. The blood sugar level did not show any rise at all, and remained the same all through the period, indicating absence of response with regard to the response to epinephrine.

In cases of marasmus, it showed a mean hyperglycemic response of 45±8 mg/100 ml to epinephrine administration. The level however reached its fasting value in 150 minutes. The data were analysed for t test and were found not to be different from normal.
There appeared to be no correlation between the fasting blood sugar levels and the responses to epinephrine, on the one hand, and the severity of protein deficiency as judged by serum albumin levels, on the other. This was so both on admission and after therapy.

The possibility that the differences in epinephrine response before and after treatment are due to defective absorption of the drug from the site of injection is excluded by the observation that the pattern of rise in heart rate and systolic blood pressure following epinephrine was similar on both occasions.

The response observed in cases of kwashiorkor after treatment was similar to the response obtained in cases of marasmus after treatment.
PERIPHERAL UTILIZATION OF GLUCOSE

Since there was a delay in the return to the fasting level of intravenously administered glucose in kwashiorkor, it was considered worthwhile to investigate whether there was a defect in the peripheral utilization of glucose in this condition. The usual way to test the utilization is to estimate the concentration of glucose in the arterial and venous blood simultaneously. The difference is presumed to be due to utilization of glucose by the peripheral tissues. Such utilization is, of course, dependent on the rate of peripheral circulation. A slower rate leads to greater utilization and a rapid rate to lesser.

The estimation of sugar in simultaneously drawn femoral arterial and venous bloods in kwashiorkor and marasmus is shown in Table 3. In kwashiorkor, on admission, there was hardly any difference between the arterial and venous blood contents of sugar. In marasmus, on the other hand, the utilization was the same as on admission as after clinical recovery. The difference in the results on admission and before discharge was highly significant in kwashiorkor (p < .001). The peripheral circulation in kwashiorkor was observed to be the same on admission and before discharge. The difference in the arteriovenous oxygen content was 32% on admission and 37% before discharge.
### Table 3

**Peripheral utilization of glucose in protein calorie undernutrition in children**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Time of measurement</th>
<th>Glucose concentration</th>
<th>Δ Glucose % of Arterial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arterial venous</td>
<td></td>
</tr>
<tr>
<td>Kwashiorkor</td>
<td>On admission</td>
<td>67 ± 7.2</td>
<td>61 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>Before discharge</td>
<td>93 ± 9.1</td>
<td>71 ± 7.7</td>
</tr>
<tr>
<td>Marasmus</td>
<td>On admission</td>
<td>106 ± 5.3</td>
<td>75 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>Before discharge</td>
<td>97 ± 3.9</td>
<td>74 ± 5.2</td>
</tr>
</tbody>
</table>
**Plasma Insulin Activity**

Children suffering from protein calorie undernutrition, especially severe kwashiorkor, often have hypoglycemia (Chaudhuri, 1948; Pille, 1957; Gomez et al., 1958; Slone et al., 1961). Although glucose absorption appears to be normal except in highly edematous children some defect in the handling of glucose injected intravenously appear to be common. It therefore becomes a matter of interest to study the endocrine function of the pancreas. Insulin-like activity was measured in the plasma of children suffering from protein-calorie undernutrition. The method of utilization of glucose by rat epididymal fat pad in the presence of plasma was at first tried (Beigelman, 1960), but it was found that it was very difficult to obtain a proportional increase of glucose utilization with increasing concentrations of standard insulin. Later on, the radio-immunoassay of Yalow and Berson (1960), as modified by Hales and Randle (1963) was used to measure the plasma insulin activity of children suffering from protein calorie undernutrition. Actually the Insulin Assay Kit supplied by Radio-Chemical Centre in Amersham, England, was used as such and the directions in the Kit were explicitly followed. Results are shown in Table A.

The data are reported for normal children suffering from typical kwashiorkor and marasmus. Heparinised blood was drawn after an overnight fast of 10 hours and
plasma was immediately separated and kept in the deep freeze at -20°C. After a sufficient number of collections were made the test was done on one day. All samples were estimated within one month after withdrawal of blood in batches. The insulin activity in fasting samples of plasma of children suffering from kwashiorkor and marasmus is not lower than normal when the patients were admitted. If it is anything it is higher than normal. The difference between kwashiorkor and normal was statistically significant ($p < 0.01$). In marasmus, however, although the mean plasma insulin activity was higher than normal, the difference was not statistically significant ($p > 0.05$).
<table>
<thead>
<tr>
<th>Disease</th>
<th>Time of measurement</th>
<th>Plasma Insulin Activity units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwasborfor</td>
<td>On admission</td>
<td>31.5 ± 0.8 (20.3 - 38)</td>
</tr>
<tr>
<td></td>
<td>After Recovery</td>
<td>20.2</td>
</tr>
<tr>
<td>Marasmus</td>
<td>On admission</td>
<td>23.10 ± 6.9 (14.5 - 35)</td>
</tr>
<tr>
<td>Normal</td>
<td>(7)</td>
<td></td>
</tr>
</tbody>
</table>
HYDROXYCORTICOIDS IN THE PLASMA OF CHILDREN SUFFERING FROM PROTEIN CALORIE UNDERNUTRITION

In protein calorie undernutrition the reduction of plasma protein occurs mainly in albumin (Trowell et al, 1954; Scrimshaw et al, 1956; Gomez et al, 1950 and Waterlow et al, 1960). This is especially true for kwashiorkor and nutritional edema, although a little hypoalbuminemia is also present in marasmus. The globulins, however, are not reduced in protein calorie undernutrition in children except in terminal cases. It may, therefore, be presumed that the cortisol-binding globulin is not reduced in the malnourished children. In that event, i.e. in the presence of normal amounts of specific cortisol-binding protein, the estimation of unconjugated corticosteroids in the plasma offers an accurate estimate of adrenocortical activity (Dixon, Booth and Butler, 1967). Such an estimation would be a better index of adrenal function than, for instance, the estimation of the daily excretion of corticosteroids metabolites in the urine. The previous methods of estimation of plasma corticosteroids demanded 5-10 ml of plasma (Sweat, 1955; Abelson and Bondy, 1955; Kalant, 1958a; 1958b; Nelson and Samuels, 1952) but since it is difficult to get such large amounts of plasma in children, the more recent methods (Mattingly, 1962) employing only 1 ml of serum have made it possible for us to undertake investigations
of plasma hydroxycorticoids in children suffering from protein calorie undernutrition.

The method involved extraction of plasma methylene chloride and the extracted cortisol was estimated by its fluorescence. The results of the estimation of plasma hydroxysteroids are shown in Table 5. Not more than 4 tests plus a standard and a blank were run at one time, because the fluorescence was ready exactly 14 minutes after the addition of the fluorescence reagent. This timing was strictly adhered to, in attempts to keep non-specific plasma fluorescence as uniform as possible. Results are given as plasma cortisol but it must be emphasized that the values strictly represent compounds which include cortisol and corticosterone; but since cortisol is present in the greatest concentration, the values are presented as plasma cortisol. In the present investigation, children suffering from typical kwashiorkor and typical marasmus only are included so that if there is a difference it should be rendered more prominent. The plasma cortisol level of normal children aged between one and three years did not differ from that of adults. There was no difference between the plasma cortisol levels of children suffering from marasmus and kwashiorkor on admission of the patients. The mean levels of plasma
cortisol in both marasmus and kwashiorkor was higher than normal. After recovery the plasma cortisol level in both marasmus and kwashiorkor came down.
Table 5

Plasma cortisol level in Protein calorie Undernutrition in children

Aged 1-3 years (μg/100 ml plasma)

<table>
<thead>
<tr>
<th></th>
<th>Marasmus</th>
<th>Kwashiorkor</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.2</td>
<td>18</td>
<td>10.9</td>
</tr>
<tr>
<td>Range 6.7-24.3</td>
<td>14.8-22.1</td>
<td>8.1-16.2</td>
</tr>
</tbody>
</table>

Numbers in parenthesis refer to the number of children.
LIVER AND MUSCLE GLYCOGEN IN KWASHIORKOR AND MARASMUS

The glycogen content of the liver in kwashiorkor and marasmus is shown in Table 6. On admission, the liver glycogen content in kwashiorkor was 47% of the value obtained after recovery. The body weight at this time was 60% of the body weight after clinical recovery. However, there was no correlation between the body weight and liver glycogen content. The latter increased much earlier than a measurable increase in the former in a few cases where we could do liver biopsies three times in the course of 3 months' stay in the hospital. The soluble protein content of the liver homogenate, on admission was 70% of the figure obtained after clinical recovery. But once again, no correlation could be observed between the soluble protein and the liver glycogen contents.

In marasmus, the average weight on admission was 57% of the body weight after recovery; the loss of body weight in marasmus was not different from the loss of body weight in kwashiorkor. The hepatic glycogen content on admission was 60% of the value obtained after clinical recovery. There was a correlation between the liver glycogen content and the body weight in marasmus. The soluble protein contents of the liver homogenate on admission and after clinical recovery was comparable.
The muscle glycogen contents in both kwashiorkor and marasmus on admission of the children were about half of the values obtained after clinical recovery (Table 7). The difference in the values on admission and before discharge was statistically significant \( (p < .01) \). The glycogen contents of the muscle were much less than those of the liver. There were occasional cases where the muscle glycogen content was abnormally low \( (0.12 \, \text{g/kg}) \) on admission.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Age Months</th>
<th>Body Weight Kg.</th>
<th>Glycogen Content g%</th>
<th>Protein Content g%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwashiorkor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>12.6</td>
<td>4.1</td>
<td>2.36 ± 1.43</td>
<td>7.8 ± 1.1</td>
</tr>
<tr>
<td>On Recovery</td>
<td>12.9</td>
<td>6.8</td>
<td>4.8 ± 2.0</td>
<td>10.1 ± 1.5</td>
</tr>
<tr>
<td>Marasmus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>15.2</td>
<td>3.5</td>
<td>3.26 ± 0.50</td>
<td>10.2 ± 1.4</td>
</tr>
<tr>
<td>On Recovery</td>
<td>15.5</td>
<td>6.1</td>
<td>5.4 ± 1.6</td>
<td>11.5 ± 1.8</td>
</tr>
<tr>
<td>Disease</td>
<td>Time of Measurement</td>
<td>Glycogen</td>
<td>Disease</td>
<td>Time of Measurement</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------</td>
<td>----------</td>
<td>---------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Kwegbiokor</td>
<td>On admission (12)</td>
<td>0.51 ± 0.47</td>
<td>Marasmus</td>
<td>On admission (8)</td>
</tr>
<tr>
<td></td>
<td>Before discharge (10)</td>
<td>0.91 ± 0.41</td>
<td></td>
<td>Before discharge (5)</td>
</tr>
</tbody>
</table>
Liver is one of the most important organs for regulation of carbohydrate metabolism. In kwashiorkor, the liver is fatty but in marasmus it is not. Protein calorie malnutrition, therefore, presents a unique opportunity to study the effect of fatty liver on the metabolism of carbohydrates. Some earlier studies indicated (Waterlow & Weisz, 1956) that the glycogen content of fatty livers was increased. This suggested an inability to mobilize glycogen, and therefore, hepatic glycolytic enzymes were assayed in children suffering from protein calorie undernutrition. All the enzymes involved in glycolytic pathway could not be measured due to limitations of the biopsy material and also for other practical reasons. So certain selections were made.

THE PHOSPHORYLASE:

Animal tissues such as, liver and muscle, were shown to contain, the enzyme phosphorylase which break the glucosidic bond of glycogen, by a process of phosphorolysis, in which inorganic phosphate splits these bonds to form glucose-1-phosphate. The equilibrium constant for phosphorylase reaction in vitro is close to 3 at pH 7, and the reaction is freely reversible.
Since a mole of glycogen, either \((C_6H_{10}O_5)n+1\) or \((C_6H_{10}O_5)n\) is both a product as well as a reactant, the value of \(K\) is determined only by the ratio of inorganic phosphate to glucose-1-phosphate. At equilibrium at pH 7 and 25°C, 77 percent of the carbohydrate is present as glycogen and 23 percent as glucose-1-phosphate.

In vivo, however, glucose-1-phosphate as it is formed is removed from the system and the concentration of inorganic phosphate remains high, so the reaction is favored toward the formation of glucose-1-phosphate rather than glycogen formation.

Phosphorylase activity was assayed in vitro by measuring the inorganic phosphate liberated from glucose-1-phosphate. Determinations of the activity of phosphorylase of liver were carried out on 35 children suffering from kwashiorkor and 11 from marasmus. The test was repeated on the same children after clinical recovery. The latter values were considered to be the normal for the particular child and thus served as his own control in protein calorie undernutrition. All determinations were done in children aged between 8 months and two years and 10 months.
While carrying out determinations of inorganic phosphate by a modification of the method of Lowry and Lopes (1946) by Chatterjee and Mukherjee, it was found that some batches of trichloroacetic acid themselves gave high blank value; even the analar grades of the acid was not immune. The trichloroacetic acid, therefore, had to be distilled in vacuo to reduce the blank value. I am sorry to say this but it is difficult to rely on even the so-called 'analar' chemicals of Merck or British Drug Houses, supplied by local houses. Either the local chemical supplies temper with the chemicals or the chemical itself appears to be faulty.

In kwashiorkor the mean liver phosphorylase activity on admission was $42 \pm 26 \mu\text{mP/g/min}$. The difference of this means was tested for significance, using the t distribution. The difference was found not to be different ($t = 1.18$, $p > .2$).

In marasmus, too, like in kwashiorkor, there was no significant difference in the phosphorylase activity on admission and after recovery ($p > .5$).

Five children with kwashiorkor who died within 24 to 48 hours after admission had unusually low liver phosphorylase activity ranging between $7-11/\mu\text{mP/g/min}$. 
Glucose-6-Phosphate provides a focal point of carbohydrate metabolism since many major metabolic pathways converge or diverge from this metabolite. For example, in the liver glucose-6-phosphate may be formed from blood glucose in the post absorptive state; from glycogen by glycogenolysis in the fed animal; or from monocarbohydrate precursors via gluconeogenesis during starvation. Both glycogenolysis and gluconeogenesis provide glucose-6-phosphate which is utilized in maintenance of the blood glucose level.

In the liver cells glucose-6-phosphate may be channeled into 4 different metabolic pathways by the activity of 4 enzymes (1) Glucose-6-phosphatase - Glucose-6-phosphate may be hydrolyzed by gl-6-pase and thus glucose is made available for the other cells of the organism (2) Phosphoglucomutase - Gl-6-phosphate may be routed into storage as glycogen and phosphoglucomutase is the enzyme which channels it into this pathway. (3) Phosphohexose isomerase - Gl-6-phosphate may be converted by this enzyme into Fr-6-phosphate and this way it may enter the glycolytic pathway yeilding energy for the specialized functions of the cells. (4) Gl-6-phosphate dehydrogenase-Gl-6-F may be directly oxidized by this enzyme and metabolized via the hexose monophosphate shunt. Thus it is that Gl-6-phosphate occupies an important position in carbohydrate metabolism.
The enzyme glucose-6-phosphatase was assayed in liver homogenates of children suffering from protein calorie undernutrition. The determinations were carried out on 36 children suffering from kwashiorkor and 22 from marasmus and the test was repeated after clinical recovery. Results are shown in Table 7.

Glucose release from liver was by way of hydrolysis of a hexose phosphate. Glucose-6-phosphatase catalyzes the reaction:

Glucose-6-phosphate \rightarrow \text{glucose} + \text{inorganic phosphate}

The activity of this enzyme can easily be assayed by measuring the appearance of inorganic phosphate following the incubation with liver homogenates. The reaction is terminated by the addition of chilled trichloroacetic acid. Method depending upon the measurement of glucose released from glucose-6-phosphate have also been advised. The activity of this enzyme alters with the variation of pH. Maximal activity are found between pH 6 and 7.

In cases of kwashiorkor on admission the glucose-6-phosphatase activity showed a marked reduction. It has a value of 53% of those obtained after clinical recovery. The differences between the groups were analysed statistically by student's t-test, and was found to be highly significant (t=3.7, p<.001). The glycogen content of the liver
on admission, as shown in the previous table was only 47%. So both glycogen and glucose-6-phosphatase values are low on admission. The activities of gl-6-pase had a wide range.

In marasmus, on admission the glucose-6-phosphatase activity of the liver was not much different from those obtained after clinical recovery or in other words, it showed a normal range of activity. It is also not statistically significant as found by t test (p <.08).

GLYCOGEN SYNTHETASE

Previously it was thought that phosphorylase was the enzyme responsible for both the breakdown and synthesis of glycogen. Later on Leloir and Geldemberg (1957) showed that glucose moiety of the UDP-G was added to the glycogen primer for synthesis of glycogen and glycogen synthetase is the enzyme responsible for this conversion. It is a one way reaction. It forms glycogen by the reaction.

\[
\text{Glycogen Primer + nUDP} \underset{\text{Fyr.Kinase}}{\longrightarrow} \text{glycogen (glucose)n} + n\text{UDP}
\]

\[
\text{PEP + UDP} \underset{\text{Fyr.Kinase}}{\longrightarrow} \text{Pyruvate} + \text{UTP}
\]

\[
\text{Pyruvate + Lactic acid} \underset{\text{Lactic dehydrogenase}}{\longrightarrow} \text{Lactate} + \text{DPN}
\]

\[
\text{DPNH}
\]
Glycogen synthetase activity determined in the presence of glucose-6-phosphate represents total enzymic activity, whereas that measured in the absence of glucose-6-phosphate has been used as an indication of the independent (1) or active form (a) of the enzyme.

Glycogen synthetase was assayed by the method of Barber (1967). In the method devised, 1 ml cuvette was used for the measurement of enzymic activity. But in doing this sometimes the needle fluctuated so that correct reading was difficult to get. This disturbance is probably due to the restricted light path which can be influenced easily such as the position of the cuvette etc. But the reading was to be taken at exactly one min. interval for 6 to 10 mins. And this difference in optical density in every min was critical for evaluation of the correct result. And if more than one min is taken to adjust the cuvette expt was spoiled. So to avoid risk the assay mixture was increased to 4 time (0.78 to 3.2 ml) and all ingredients (components) of the assay mixture were increased proportionately. The reading can now be taken in 4 ml cuvette safely. So in subsequent measurements 4 ml cuvette was used. Beside this the enzyme pyruvic kinase and lactate dehydrogenase are generally added in the cuvette at the time of measurement, which also requires a proper mixing. And this task is easily carried out in 4 ml cuvette than in 1 ml cuvette.
The estimation of glycogen synthetase by this method requires a considerable amount of tissue (about 30 mg is the minimum requirement). But in liver biopsy the amount of material obtained is restricted, especially in cases of marasmus, where with great difficulty this amount of tissue can be obtained. So only a few cases could be studied. 17 children from kwashiorkor and only 3 from marasmus were studied. The results are shown in table 3.

In kwashiorkor the glycogen synthetase activity of the liver on admission did not differ much from those obtained after clinical recovery. It shows about 85% activity. The t test however is not significant (p< .2).

In marasmus, only 3 cases could be studied on admission. On recovery, only one case was done, which, however, shows a normal value. Of the three cases which were studied, the mean result on admission corresponded to the value obtained in kwashiorkor on admission. It is more probable that their result will also not be statistically different from those after recovery.

In three cases of kwashiorkor who died after 24 to 48 hours of admission the glycogen synthetase activity was not measurable at all. Though liver is the main regulatory organ for carbohydrate metabolism, muscle also plays an important role in its regulation.
Liver glycogen is reversibly convertible to blood glucose and normally serves to maintain the blood sugar level when the supply of carbohydrate from intestinal absorption is inadequate. Muscle glycogen cannot serve directly as a source of blood glucose. Muscle glycogen can become blood glucose only through first being broken down to lactic acid, which is transported by the blood to the liver, where it is converted to glucose. The glycogen of muscle and other tissues is utilized directly as a source of energy, the energy being liberated when glycogen is broken down through a long series of phosphorylated intermediates and finally oxidized to carbon dioxide and water. Although normally the per cent glycogen in liver is higher than in muscle, the total quantities in muscle and liver are about the same.

Carbohydrate metabolism in muscle is highly specialized and designed primarily for the production of ATP as a source for the contraction process. Muscle metabolism is limited largely to the breakdown of blood glucose for its carbohydrate supply.

Though the carbohydrate metabolism in muscle differs from the liver in that it cannot produce glucose directly, it undergoes the same mechanism for the formation of glycogen from glucose. This glycogen yields energy as ATP through...
glycolysis and oxidation of pyruvic acid in the tricarboxylic cycle, just as does liver glycogen.

So the same enzyme systems were also studied in muscle biopsy in protein calorie undernutrition children. The biopsy were done on 6 children suffering from kwashiorkor and 4 children from marasmus, and the enzyme phosphorylase and glycogen synthetase were studied, gl-6-phosphatase being absent in muscle. The same assay procedure was followed as those of liver. Results are shown in Table 4.

In kwashiorkor the muscle phosphorylase activity was low, about three fourth of the normal activity and returns to normal on recovery.

In marasmus also the muscle phosphorylase shows a lowered activity.

The glycogen synthetase activity in kwashiorkor was also low.

In marasmus, only a few cases was done so no conclusion can be drawn about the glycogen synthetase activity.
## Table 8

<table>
<thead>
<tr>
<th>Disease</th>
<th>On Admission</th>
<th>On Recovery</th>
<th>On Admission</th>
<th>On Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwashiorkor</td>
<td>69 ± 02</td>
<td>49 ± 26</td>
<td>84 ± 53</td>
<td>106 ± 59</td>
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<tr>
<td>On admission</td>
<td>(23)</td>
<td>(12)</td>
<td>(5)</td>
<td>(15)</td>
</tr>
<tr>
<td>On recovery</td>
<td>152 ± 76</td>
<td>34 ± 15</td>
<td>6, 1.1, 1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Marasmus</td>
<td>140 ± 48</td>
<td>32 ± 15</td>
<td>(14)</td>
<td>(8)</td>
</tr>
<tr>
<td>On admission</td>
<td>(13)</td>
<td>(14)</td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>On recovery</td>
<td>145 ± 38</td>
<td>27 ± 12</td>
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<td></td>
</tr>
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</table>
Table 9

Enzyme content of Muscle in Protein Calorie Undernutrition

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of Cases</th>
<th>Phosphorylase AMP/g/min</th>
<th>Glycogen Synthetase UDP/g/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwashiorkor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>76 ± 25</td>
<td>0.34 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>On Recovery</td>
<td>107 ± 41</td>
<td>2.2, 0.41</td>
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<tr>
<td>Marasmus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission</td>
<td>65 ± 37</td>
<td>0.4, 0.2</td>
<td></td>
</tr>
<tr>
<td>On Recovery</td>
<td>132 ± 28</td>
<td>1.5, 0.5</td>
<td></td>
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</tbody>
</table>