THE EXOCRINE PANCREAS

AND

PROTEIN-CALORIE MALNUTRITION.

THESIS

SUBMITTED FOR THE DEGREE OF

DOCTOR OF MEDICINE

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BY

GILBERT O. BARBEZAT, M.B., Ch.B.

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THIS THESIS IS DEDICATED TO

MY TEACHERS,

MY PARENTS,

and

WYN.

"Efforts at investigation always

go to prove that the subject is

not as simple as it looked".

Cicily D. Williams. (1940).

(Trans. roy. Soc. trop. Med. Hyg., 34, 85.)

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1. The selection of the patients and their clinical management.
2. The development and execution of the duodenal intubation technique.
3. The performance of the pancreatic function tests (PFT).
   (a). Introducing the tube on the night prior to the test.
   (b). The full PFT in every detail.
4. Examination of the x-ray of the abdomen.
5. The microscopy of the duodenal juice.
6. Blood taking by venipuncture.
7. The modifications to and the establishment of all the enzyme assay methods. The lipase method, however, was developed in conjunction with Mr. Bernd Lehmann.
8. All the trypsin and chymotrypsin assays throughout the study, and a small number of the assays of amylase, lipase and ribonuclease.
9. The modifications to and the development of the colour index method.
10. The correlation of all the data after each PFT, including the calculation of the final units.
11. The histological study in toto, including the review of the records and the selection of the slides.
12. All the parametric statistical analyses including the computation of means, standard deviations, correlation coefficients, regression lines and t-tests.
13. All the non-parametric statistical analyses not listed in Tables V, W, and X. (Pages205 to 207). The results that are shown in these tables were obtained with the aid of an electronic computer; the author prepared the data for the computer analysis and punched all the computer cards. Spot checks were performed on the computer

analysis. Each value for U (see page 198) was determined from tables by the author.

14. The colour photography.

TABLE OF CONTENTS

SECTION I.

REVIEW - CHAPTER 1.

PROTEIN-CALORIE MALNUTRITION, GASTROINTESTINAL FUNCTION, AND THE PANCREAS.

A. Protein-calorie malnutrition (P-C.M.) 2

B. The focus on the gastrointestinal tract in P-C.M. in man. 3

C. The investigation of pancreatic function in human P-C.M. 6

1. The analysis of duodenal juice. 6

2. Tolerance tests. 12

3. Serum enzyme estimations. 13

4. Histology. 15

D. Nutrition and the pancreas in experimental animals. 19

1. The effect of diet on the pancreas. 19

(a) Duodenal enzyme studies. 19

(b) Studies on whole pancreas. 21

(c) Studies on pancreatic tissue slices. 23

(d) Radio-active isotope studies. 23

(e) Studies on serum. 24

(f) Histology. 24

2. The relation between the pancreas and a fatty liver. 26

(a) The effect of exclusion of pancreatic tissue or of its exocrine secretion. 26

(i) Pancreatectomy. 26

(ii) Ligation of the pancreatic duct. 26

(iii) Complete pancreatic fistula. 27

(b) The effect of induced pancreatic dysfunction 27

(i) Nutritional deficiency. 27

(ii) Ethionine toxicity. 27

(c) Lipocaic. 28

(d) Anti-fatty liver factor. 28

CHAPTER 2.

THE PHYSIOLOGY OF THE EXOCRINE PANCREAS.

A. Embryology and structure. 30

B. The pancreatic acinus and enzyme production. 31

1. Pancreatic protein turnover. 31

2. The synthesis and secretion of enzymes. 31

3. Enzyme function. 32

(a) Amylase. 32

(b) Lipase. 33

(c) Trypsin 34

(d) Chymotrypsin. 35

(e) Carboxypeptidase. 35

(f) Ribonuclease. 36

C. Fluid secretion. 37

D. Bicarbonate in pancreatic juice. 38

E. Electrolytes. 39

F. Enzyme inhibitors. 40

G. The control of pancreatic secretion. 41

1. Hormonal control. 41

(a) Secretin 41

(b) Pancreozymin. 44

2. Nervous control. 46

(a) Parasympathetic. 46

(b) Sympathetic. 48

CHAPTER 3.

THE ASSESSMENT OF EXOCRINE PANCREATIC FUNCTION.

A. The analysis of duodenal aspirates. 49

1. Dual aspiration techniques. 49

2. Pancreatic stimulation. 50

(a) Secretin. 50

(b) Pancreozymin. 50

(c) Parasympathetic effect. 51

(d) Enzyme determinations after a test meal. 51

B. Tolerance tests. 52

C. Serum enzymes. 52

D. X-ray studies. 52

1. Straight X-ray abdomen. 52

2. Opacification media. 52

3. Pancreatic visualisation with retropneumo-peritoneum and pancreatography. 53

E. Radio-isotope studies. 53

1. Scanning techniques. 53

2. Absorption of iodine-^{131} 53

F. Stool examination. 54

1. Microscopy. 54

2. Faecal trypsin. 54

G. Glucose tolerance test. 54

Summary of Review. 56

SECTION II.

ORIGINAL INVESTIGATIONS

CHAPTER 4.

AIMS OF THE INVESTIGATION.

A. Pancreatic function tests in children. 59

1. Technique. 59

2. Enzymes. 59

3. Volume. 59

4. Bicarbonate. 59

5. Colour Index. 60

B. Pancreatic function in normal children. 60

C. Pancreatic function in kwashiorkor. 60

D. Pancreatic function in marasmus. 60

E. Pancreatic function after the treatment of acute P-C.M. 60

F. Pancreatic function in children with chronic prolonged

malnutrition following an acute episode of P-C.M. 61

G. Pancreatic calcification. 61

H. Microscopy of the duodenal aspirate. 61

I. Pancreatic histology in P-C.M. 61

CHAPTER 5.

CLINICAL MATERIAL AND WARD MANAGEMENT.

A. Clinical material. 62

1. Kwashiorkor. 62

2. Marasmus. 64

3. Assessment of cure. 66

4. "Five-year follow-up" children. 68

5. "Controls". 68

B. Ward Management. 71

1. General. 71

2. Diet. 72

(a) Children with kwashiorkor and marasmus. 72

(b) "Five-year follow-up" children and "controls". 72

3. Medication. 72

(a) Children with kwashiorkor and marasmus. 72

(i) Antibiotics and chemotherapy. 72

(ii) Supplements. 73

(iii) Miscellaneous. 73

(b) "Five-year follow-up" children and "controls". 73

C. Problems encountered. 74

1. Marasmic-kwashiorkor. 74

2. Infectious diseases. 74

3. Secondary pathology. 74

4. Death. 74

D. Histology. 75

CHAPTER 6.

METHODOLOGY.

A. Pancreatic function test (PFT). 76

1. Period of fasting. 76

2. Sedation. 76

3. Duodenal intubation. 76

4. Gastric intubation. 77

5. X-ray. 77

6. Aspiration of juices. 78

(a) Apparatus. 78

(b) Procedure. 78

7. Difficulties encountered in performing PFT's in children. 80

(a) The clinical condition of the patient. 80

(b) Sedation. 81

(c) Duodenal intubation. 81

(d) X-ray. 81

(e) Aspiration of juice. 81

(f) Stimulation. 82

B. Volume. 83

C. pH. 83

D. Enzyme assays. 83

1. General. 83

2. Amylase. 84

(a) Apparatus. 84

(b) Modifications. 84

(c) Reproducibility. 85

(d) Difficulties. 85

3. Lipase. 85

(a) Modifications. 86

(b) Reproducibility. 87

(c) Calculation of units. 87

(d) Difficulties. 88

4. Ribonuclease (RNase). 88

(a) Apparatus. 88

(b) Modifications. 88

(c) Reproducibility. 89

(d) Difficulties. 90

5. Trypsin. 90

(a) Apparatus. 90

(b) Modifications. 90

(c) Calculation of results. 91

(d) Reproducibility. 92

(e) Difficulties. 92

6. Chymotrypsin. 93

(a) Modifications. 93

(b) Calculation of results. 94

(c) Reproducibility. 94

7. Calculation of results of enzyme assays. 94

E. Colour Index. 95

(a) Apparatus. 95

(b) Modifications. 96

F. Microscopy of the duodenal aspirate. 96

G. Histology. 96

H. Serum proteins. 97

I. Haemoglobin. 98

J. Packed cell volume. 98

K. Weight. 98

L. Height. 98

M. X-rays. 98

CHAPTER 7.

RESULTS.

A. Pancreatic function tests. 100

1. Volume. 100

(a) Basal secretion. 100

(b) Response to stimulation. 100

(i) General. 100

(ii) Relation to body weight. 101

(iii) Response to stimulation in the different clinical groups. 101

(c) Comparison between groups. 103

(i) Basal test. 103

(ii) Secretin test. 104

(iii) Pancreozymin test. 104

(iv) Mean of secretin and pancreozymin tests. 104

(v) Response to stimulation. 104

(d) Summary - Volume. 104

2. pH. 105

(a) Basal secretion. 105

(b) Response to stimulation. 105

(c) Comparison between groups. 106

(i) Basal test. 106

(ii) Secretin. 106

(iii) Pancreozymin test. 106

(iv) Mean of secretin and pancreozymin tests. 106

(v) Peak. 106

(vi) Response to stimulation. 106

(d) Summary - pH. 107

3. Enzymes. 107

I. Amylase. 107

(a) Basal secretion. 107

(b) Response to stimulation. 107

(c) Comparison between groups. 108

(i) Basal test. 108

(ii) Secretin test. 108

(iii) Pancreozymin test. 108

(iv) Mean of secretin and pancreozymin tests. 109

(v) Peak values. 109

(vi) Response to stimulation. 109

(d) Summary - Amylase. 109

II. Lipase. 110

(a) Basal secretion. 110

(b) Response to stimulation. 110

(c) Comparison between groups. 111

(i) Basal test. 111

(ii) Secretin test. 111

(iii) Pancreozymin test. 111

(iv) Mean of secretin and pancreozymin tests. 111

(v) Peak values. 112

(vi) Response to stimulation. 112

(d) Summary - Lipase. 112

III. Ribonuclease. 112

(a) Basal secretion. 112

(b) Response to stimulation. 113

(c) Comparison between groups. 113

(i) Basal test. 113

(ii) Secretin test. 113

(iii) Pancreozymin test. 113

(iv) Mean of secretin and pancreozymin tests. 113

(v) Peak values. 114

(vi) Response to stimulation 114

(d) Summary - RNase. 114

IV. Trypsin. 114

(a) Basal secretion. 114

(b) Response to stimulation. 115

(c) Comparison between groups. 115

(i) Basal test. 115

(ii) Secretin test. 115

(iii) Pancreozymin test. 116

(iv) Mean of secretin and pancreozymin tests. 116

(v) Peak values. 116

(vi) Response to stimulation. 116

(d) Summary - Trypsin. 117

V. Chymotrypsin. 117

(a) Basal secretion. 117

(b) Response to stimulation. 117

(c) Comparison between groups. 118

(i) Basal test. 118

(ii) Secretin test. 118

(iii) Pancreozymin test. 118

(iv) Mean of secretin and pancrezymin tests. 118

(v) Peak values. 118

(vi) Response to stimulation. 118

(d) Summary - Chymotrypsin. 119

VI. Pancreatic function after protein repletion in marasmic children. 120

VII. Recovery of pancreatic function in P-C.M. 121

(a) Acute kwashiorkor. 121

(b) Chronic P-C.M. 121

VIII. Enzyme output in relation to serum albumin concentration. 127

IX. Enzyme output in relation to height and weight. 130

X. The effect of sex on pancreatic enzyme deficiency in P-C.M. 130

XI. Diet and enzyme output. 131

XII. Giardiasis and the recovery of pancreatic function. 131

4. Colour Index. 133

(a) Basal secretion. 133

(b) Response to stimulation. 133

(c) Comparison between groups. 134

(d) Summary - Colour Index. 134

5. X-ray of the abdomen. 134

6. PFT as a diagnostic aid. 135

(a) Fibrocystic disease of the pancreas. 135

(b) Diarrhoea of uncertain origin. 136

B. Microscopy of the duodenal aspirate. 137

1. Kwashiorkor. 137

2. Marasmus. 138

3. "Five-year follow-up" group. 138

4. Recovered kwashiorkor. 139

5. Controls. 139

Conclusion. 139

C. Histology. 141

1. Pancreas. 141

(a) Autolysis. 141

(b) Acinar structure. 141

(c) Zymogen granules. 142

(d) Ducts. 143

(e) Connective tissue. 144

(f) Inflammatory cells. 144

(g) Islets of Langerhans. 145

(h) Illustrative plates. 146

2. Liver. 149

Summary - Histology. 150

Summary of Results. 151

CHAPTER 8.

DISCUSSION.

A. The assessment of exocrine pancreatic function and structure in children with P-C.M. 154

B. Duodenal juice microscopy. 162

C. The clinical significance of this study. 164

D. Unanswered problems. 164

Conclusions. 167

APPENDIX.

A. Laboratory methods. 169

1. Amylase. 169
2. Lipase. 170
3. Ribonuclease. 172
4. Trypsin. 173
5. Chymotrypsin. 174
6. Colour Index. 176

B. Detailed results of pancreatic function tests. 177

1. Volume. 178 to 182
2. pH. 183 to 187
3. Enzymes. 188 to 192
4. Colour Index. 193 to 197

C. Statistical methods. 198

D. Results of detailed statistical analysis. 204

BIBLIOGRAPHY 208

FIGURES.

		Opposite page
1.	Lipase - comparison of units per ml, units per minute and units per minute per Kg body weight.	95
2.	Volumes - response to secretin and pancreozymin.	100
3.	Relation of volume output to body weight after secretin and pancreozymin stimulation.	101
4.	Volumes - comparison between groups (ml/min.).	102
5.	Volumes - comparison between groups (ml/min./Kg.).	103
6.	pH Values - response to secretin and pancreozymin.	105
7.	pH Values - comparison between groups.	106
8.	Amylase - comparison between groups.	108
9.	Lipase - output before and after the treatment of kwashiorkor.	111
10.	Ribonuclease - comparison between groups.	113
11.	Trypsin - output before and after the treatment of kwashiorkor.	115
12.	Chymotrypsin - comparison between groups.	118
13.	Chymotrypsin - output before and after the treatment of kwashiorkor.	119
14.	Response to treatment - total enzyme output before and after protein repletion. (Related to time).	123
15.	Response to treatment - total enzyme output before and after protein repletion. (Related to serum albumin concentration).	124
16.	Relation between serum albumin concentration and total enzyme output.	128

17. Relation between serum albumin concentration and total enzyme output in the different clinical groups. 130

18. Colour Index - response to secretin and pancreozymin. 134

19. Colour Index - comparison between groups. 135

PLATES.

		Opposite page
1.	The first step in duodenal intubation.	76
2.	X-ray of abdomen with 1 tube in the duodenum and the other in the stomach.	77
3.	Apparatus for the aspiration of duodenal juice.	78
4.	A pancreatic function test in progress.	79
5.	Juice obtained during a pancreatic function test.	133
6.	Histology : Normal pancreas.	Page 146
7.	Histology : The pancreas in severe kwashiorkor.	147
8.	Histology : The pancreas in fibrocystic disease.	148

TABLES.

1.	Duodenal enzymes in P-C.M. (Review).	8
2.	Clinical data : Kwashiorkor.	63
3.	Marasmus.	65
4.	Kwashiorkor after treatment.	67
5.	"Five-year follow-up".	69
6.	"Controls".	70
7.	Relation between pancreatic volume output in ml. per minute and body weight in Kg.	102
8.	Rate of recovery - acute kwashiorkor.	122
9.	Repeated PFT's in "5 - year follow-up" children.	124
10.	The relation between serum albumin concentration in grams per 100 ml. and enzyme output in units per minute per Kg. body weight.	129
11.	Diet and enzyme output.	132
12.	Duodenal juice microscopy.	140
13.	Histology : Autolysis.	141
14.	Acinar structure.	142
15.	Zymogen granules.	143
16.	Ducts.	143
17.	Connective tissue.	144
18.	Inflammatory cells.	145
19.	Islets of Langerhans.	145
20.	Liver.	149

APPENDIX.

Table			
A.	Volume :	Kwashiorkor.	178
B.		Marasmus.	179
C.		"Five-year follow-up".	180
D.		Kwashiorkor after treatment.	181
E.		"Controls".	182
F.	pH :	Kwashiorkor.	183
G.		Marasmus.	184
H.		"Five-year follow-up".	185
J.		Kwashiorkor after treatment.	186
K.		"Controls".	187
L.	Enzymes :	Kwashiorkor.	188
M.		Marasmus.	189
N.		"Five-year follow-up".	190
O.		Kwashiorkor after treatment.	191
P.		"Controls".	192
Q.	Colour Index :	Kwashiorkor.	193
R.		Marasmus.	194
S.		"Five-year follow-up".	195
T.		Kwashiorkor after treatment.	196
U.		"Controls".	197
V.	Statistics :	Comparisons within each group.	205
W.		Comparisons between each group - Enzymes.	206
X.		Comparisons between each group - Volume, pH, Colour Index.	207

SECTION 1.

REVIEW.

CHAPTER 1.

Protein-calorie malnutrition, gastrointestinal function, and the pancreas.

A. Protein-calorie malnutrition.

The evolution of knowledge of nutritional disorders in childhood has been rapid since Dr. Cicily Williams first described kwashiorkor in 1933.(411). It is now generally recognised that kwashiorkor is only one facet of the broader concept of protein-calorie malnutrition (P.C.M.). (205). This term includes a spectrum of clinical conditions including kwashiorkor, marasmic-kwashiorkor, nutritional marasmus, and nutritional dwarfism. (206, 327).

Kwashiorkor is characterised clinically by growth failure, oedema, skin lesions, hair changes, muscle wasting with retention of subcutaneous fat, psychomotor changes, and a fatty liver. (48, 206). At the other end of the spectrum, children with marasmus also have retardation of growth, but are wasted with no subcutaneous tissue (206, 392); they do not have skin lesions, are not oedematous, and typically do not have a fatty liver. Nutritional dwarfism may manifest itself simply by growth retardation or merge to a greater or lesser degree with the other more dramatic presentations. Children with marasmic-kwashiorkor show features common to both extremes of the spectrum, any combination of signs being possible (302).

Kwashiorkor stems from a diet deficient in quantity and/or quality of protein but with adequate or near adequate carbohydrate calories. A diet deficient in all nutrients leads to marasmus. (393, 396, 392).

As kwashiorkor is usually the most dramatic variant of this group of conditions, it has attracted most attention; consequently a large proportion of the literature on nutritional disorders in childhood focuses on this end of the spectrum.

B. The focus on the gastrointestinal tract in P-C.M. in man.

With the recognition of kwashiorkor as a clinical entity (411) came the increasing awareness of gastrointestinal symptoms in malnourished children. Diarrhoea is important in the morbidity and mortality of this condition and has been given a varying degree of pre-eminence by different investigators. (48, 132, 133, 141, 163, 203, 214, 242, 317, 327, 334, 345, 351, 388, 369, 394, 413, 57, 368, 349, 44, 90). Numerous causes have been postulated for this diarrhoea, including infection (48, 214, 334, 414), imbalance or maldistribution of bowel flora (345), vitamin deficiency (pellagra (350, 366, 135) and vitamin A deficiency (204)), lactose intolerance (90, 44, 45), intestinal atrophy (351, 349) and directly or indirectly to pancreatic deficiency. (204, 132, 327, 366, 368, 10, 374, 394, 57, 140, 85). That these factors, to a greater or lesser degree, play some part in the aetiology of diarrhoea is not doubted, but most investigators agree there are many unknown factors. The vicious circle enveloping dietary deficiency and gastrointestinal upset, particularly diarrhoea, is a notorious one (48), and it is often difficult to decide which is the primary factor. There is no doubt that malnourished children are more predisposed to diarrhoea, particularly in severe and recurrent episodes.(214, 413).

The description of the stools in many reports on kwashiorkor suggest a malabsorption syndrome with maldigestion (steatorrhoea and sometimes creatorrhoea). (132, 135, 141, 242, 366, 369, 368, 411, 90, 140, 57). This is so severe in some cases that specific foodstuffs may be recognised in the stool (368). Malabsorption has been proven by balance studies in many parts of the world (141, 142, 165, 413); these studies are well reviewed by Waterlow (394). However, the nitrogen balance studies do show that adequate nitrogen is absorbed, and that the retention of nitrogen is extremely

good. (395). Individual results, nevertheless, reveal a very big range of nitrogen absorption, with some patients showing a percentage absorption only a little greater than 30%. (142, 165, 413). This may correlate with the severity of the illness, although the control of the diarrhoea and the amount of oral intake are important factors in the interpretation of these results. (163).

The fat balance studies show a greater degree of dysfunction with greater regularity (as reviewed by Waterlow (394) and Scrimshaw (327)). The mean fat absorption of patients on admission in the study by Gomez was only 50% (141). The degree of steatorrhoea has been said to vary depending upon the type of food ingested, particularly its fat content. (48, 368). In these balance studies, it was usually found that the absorption improved significantly with protein repletion therapy.

With steatorrhoea there is malbasorption of vitamins. This may be adding insult to injury as the diets may already be poor in vitamins. Children suffering from kwashiorkor have been shown to have low blood levels of carotenoids and tocopherol, which are fat soluble, compared with normal controls (368) and to poorly absorb vitamin A palmitate. (327). The malabsorption of vitamin A has been associated with the development of mucosal changes in the gut (204, 357) and ocular defects (41, 368, 269). The deficiency in other vitamins which are mainly water soluble is probably less affected by gastrointestinal disorder.

The pancreas plays a vital role in digestion, and it is known that deficiency of this organ may lead, among others, to the above-mentioned symptoms. (11, 64, 70, 71, 96, 105, 175, 218, 219, 228, 244, 252, 261, 263, 296, 316, 347, 368, 373). It has been said that some digestion can occur in pancreatic deficiency states, but that this takes place at a slower rate;

this may even permit the splitting of fat which is found in the stools in excessive quantities as free fatty acids. (132, 392). The absorption is, nevertheless, impaired. The function of this organ in states of maldigestion and malabsorption, therefore, warrants critical appraisal.

A constant feature in kwashiorkor is the fatty infiltration of the liver.(47, 57, 85, 172, 348, 368, 374, 259, 10, 48, 132, 133, 369, 392, 388, 366, 327, 411, 204). Even in the so-called "adult kwashiorkor", this has been reported. (28, 249, 244, 383). The pancreas, liver and small intestinal mucosa are the tissues in the body with the most rapid protein turnover (356); it is not surprising, therefore, that in states of protein depletion they should be severely affected. The link between these three organs involves more than this common factor. There is strong evidence in man and in experimental animals that inadequate pancreatic function is associated with the presence of a fatty liver. Most authors do not venture to say whether this association is coincidental or not and simply record their findings. (19, 28, 120, 407, 11, 259, 244, 132, 204, 205, 327, 366, 369, 394, 10). Davies, however, has expressed the view that kwashiorkor is fundamentally a pancreatic disorder (85), and Trowell et al record the pancreatic acinar cell changes as "the most constant and persistent lesion in kwashiorkor". (368). Véghelyi supported this view by his experience with children suffering from nutritional oedema during World War 11. (374). Others have also suggested a link between these two findings. (47, 57, 172, 249, 392). The finding of a fatty liver in the presence of a normal pancreas has been described (47, 259, 388), but one must not overlook the fact that the dietary therapy prior to death may bring about striking changes in pancreatic morphology in a short period of time. (368). In the study by Bras et al (47) the liver changes were mild in the few cases with a normal pancreas, and Manson-Bahr records one such case as an exception to the rule. (259). A normal pancreas in P-C.M. is often

associated with under-nutrition (marasmus) and these cases must be distinguished from kwashiorkor in this respect. (265, 23, 392, 255, 267, 172, 404). Waterlow comments that a reduction in pancreatic enzyme activity resulting from pancreatic atrophy may be one of the rare examples of a biochemical difference between protein malnutrition and general under-nutrition. (394). Children with marasmus characteristically do not have a fatty liver. The fact that kwashiorkor and marasmus may merge in many cases (206) makes the interpretation of these findings very difficult.

C. The Investigation of Pancreatic Function in Human P-C.M.

The pancreas is an inaccessible organ and the investigation of its functional capacity is difficult. (96, 175, 412). The investigation of pancreatic dysfunction has until recently been very limited. The difficulties experienced are accentuated in children (66) as the patients are much smaller, laboratory methods often have to be modified to micro-methods because of small samples for analysis, and the co-operation of the patient is often necessary for the success of the procedure. (107). The degree of investigation to which one can submit a sick child is also limited. Consequently, the investigation of pancreatic functions in P-C.M. has been restricted both in type and extent of procedure. These can be resolved into 4 main groups.

(1). The analysis of duodenal juice.

(2). Tolerance tests.

(3). Serum enzyme estimations.

(4). Histology.

(1). The analysis of duodenal juice.

This direct method of assessing the function of the exocrine pancreas by examining its secretion into the duodenum has been performed by

many investigators (Table 1). Many reviews on kwashiorkor quote the results obtained by this method of investigation. (48, 89, 327, 368).

Aspiration of duodenal content presents certain difficulties besides duodenal intubation which, with practice, becomes a relatively straightforward procedure. (12, 131, 267). The problem of gastric contamination of the duodenal content is an important one. (95, 107). In all the work to date on P-C.M., this has not been stressed. Most investigators have ignored this contamination completely, while others have taken precautions to minimise it by continuously watching the aspirate and discarding that which is obviously contaminated (267) as judged by pH change or inspection. Despite these precautions, the pH of the juice in the study where this precaution was taken (267) ranged from 6.6 to 7.6 with a mean of 7.2. Contamination with gastric juice inhibits optimum enzyme activity (95, 119), makes any pH or bicarbonate estimations completely unreliable, lowers enzyme concentration by dilution of the duodenal content, and precludes any attempt at measuring total enzyme output from the pancreas over a given period of time. Andersen (12) measured volume output and McDougall (267) and Badr El-Din et al (23) measured the pH in the duodenal juice, but their results are of no real value because of this factor. Banwell et al in their study on adults (27, 28) collected pure duodenal juice by keeping the stomach empty with a separate tube; they were thus able to measure volume and bicarbonate adequately. Waterlow has pointed out that no measurements of the volume of juice in kwashiorkor have yet been made. (395). It has been stressed by Dreiling and Janowitz (107), who have had vast experience with pancreatic function tests, that enzyme concentration per se cannot be used as a parameter in the assessment of pancreatic function; the range is wide, the variation is great, and no satisfactory lower limit of normal can be found. Total enzyme secretion, especially when expressed per kilogram body weight does permit the establishment of a statistically valid

DUODENAL ENZYMES IN P-C. M.

Investigator	Date	Locality	Aspiration Method	Stimulation	Diagnosis	Controls	Volume	pH	Enzymes: Amylase	Enzymes: Lipase	Enzymes: "Trypsin"	Reference
Andersen	1942	U.S.A.	Single tube	Nil	Marasmus + Others	Yes	Measured	-	D	D	D	12
Maddock et al	1943	U.S.A.	Not Specified	Secretin	"Chronic Nutritional Disturbance"	Yes	-	-	N or I	N or I	N	255
Carvalho et al	1948	Brazil	Single tube	33% Mg SO4 Warm Milk	"Nutritional Deficiency with oedema"	?	-	-	-	D	D	57
Véghelyi	1948 1950	Hungary	?	Nil	"Nutritional oedema"	Yes	-	-	D	D	D	374 375 377
McDougall	1950	U.S.A.	Single tube some Dual Tube	Nil	"Extreme Malnutrition" + Others		-	Yes	N	N	N	267
Thompson and Trowell	1952	Uganda	Single tube	Nil	Kwashiorkor	Yes	-	-	D	D	-	362
Altmann	1953	S.Africa	Single tube	Nil	"Malignant Malnutrition"	Yes	-	-	-	-	N	10
Gomez et al	1954	Mexico	Single tube	Nil	"3rd Degree Malnutrition" with oedema	Yes	-	-	D	D	D	140
Bate and James	1956	England	Not Specified	Nil	"Malnutrition in Infancy"	Yes	-	-	-	-	N or D	31
Badr El-Din and Aboul Wafa	1957	Egypt	Single Tube	Nil	Kwashiorkor Marasmus "Growth Failure"	Yes	- - -	Yes Yes Yes	N or D N N	- - -	O or D O or D N	23
Kerpel-Fronius	1960	Hungary	Single Tube	Nil	"Athrepsie" "Nutritional oedema"	?	- -	- -	N D	N D	N	224
Banwell et al	1963 1964	Uganda	Dual Tube	Secretin and Pancreozymin	Adults with Diabetes and Malabsorption	Yes	D	D	D	-	-	27 28

N = Normal D = Decreased I = Increased

TABLE 1.

normal range. For this to be done, an accurate measurement of volume is needed. Most workers using the modern dual tube technique with secretin and pancreozymin stimulation in adults express the enzyme activity either as total output for a given period of time (74, 353, 261, 352, 95) or total output per kilogram body weight for a given time period. (4, 107, 53). Bank et al (26), however, in their series found that expressing the results in terms of total output did not significantly alter the conclusion drawn from results expressed as mean concentration.

A standard pancreatic stimulus is necessary for the accurate assessment of pancreatic function. (96, 107). Not one of the tests performed on children with kwashiorkor used a parenteral form of pancreatic stimulation. (56, 57, 374, 377, 362, 10, 140, 23). Morrison has said that under such basal conditions the only criterion of any significance is a repeated absence of enzyme function (281) while Andersen has said that the finding of "appreciable quantities" of enzymes disproves the diagnosis of pancreatic deficiency (12); this latter study was made with particular reference to fibrocystic disease of the pancreas. The point is clear that the concentration of enzymes in the basal aspirate is an unreliable parameter in the measurement of pancreatic function. In one study (57) intra-duodenal 33% magnesium sulphate was used to "stimulate bile flow". As $MgSO_4$ is an acid salt ($MgSO_4 + 2(H^+ + OH^-) \rightleftharpoons Mg(OH)_2 + H_2SO_4$) this may act as an acid on the duodenal mucosa to produce secretin and pancreozymin. (100, 246, 303, 358, 390). Warm milk was introduced into the duodenum in the same study and this would also act as a stimulus to the pancreas. (156). These stimuli are, however, very arbitrary and no standardisation is possible. In the study by Maddock et al (255) secretin was used as a stimulus in cases of "chronic nutritional disturbance". The dose used was 2 clinical units per kilogram body weight which is double the dosage used in adults on a unit per weight basis. (261, 95).

Unfortunately clinical details of these children were not provided.

In many instances it will be noticed that malnourished children were tested as part of a larger series of assorted cases, particularly fibrocystic disease of the pancreas. (12, 255, 267, 31). These tests were all performed on non-specific low weight children mostly of the marasmic type. The criteria for labelling these cases with the diagnosis of marasmus are not stated. The variation in the age of these patients is great (31, 267, 12), with many children falling outside the usual kwashiorkor age group. (367).

Bate et al claim that pancreatic depression may be expected in gastro-entiritis when weight loss exceeds 7% of body weight (31); this may well be due to dehydration. Doubt has been expressed whether hydration affects volume output of the pancreas (192) but Shwachman (337) and Andersen (12) regard dehydration as a contra-indication to performing pancreatic function tests. Without pancreatic stimulation this factor is accentuated.

Gómez et al (140) in their study showed a significant rise in duodenal enzymes after treatment of their children with 3rd degree malnutrition. No controls were studied, so it is not possible to say whether the enzyme concentration on recovery was normal or only much improved.

Thirty of the 41 adult patients with malabsorption tested by Banwell et al in Uganda had abnormal pancreatic exocrine function as judged by a full pancreatic function test. (28). Many of these patients were also diabetic (27) and had pancreatic calcification. Their youngest patient was 8 years old, and 25% were less than 25 years of age. These findings were strongly related to malnutrition as judged by clinical signs and a low serum albumin concentration. Shaper (332) in Uganda, and Ratnaike and Rajasuriya (309) in Ceylon have also associated pancreatic deficiency, calcification and diabetes with protein malnutrition. This type of pancreatic deficiency

presents not uncommonly in the adolescent or young adult. Marks et al (263) discussing the syndrome of "silent pancreatitis" in which there are signs and biochemical findings of pancreatic deficiency in the absence of pain, suggest that this may be related to nutritional deficiency in childhood as described by Davies. (85). This link between protein deficiency in childhood and adult pancreatic disease has never been the subject of careful investigation.

The spectrum of enzymes assayed is limited in most of the series published. Only 5 investigators have assayed the 3 most important enzyme parameters (amylolytic, lipolytic and proteolytic) in the same patients. (12, 255, 267, 140, 224). Of these studies only 1 (140) deals with kwashiorkor as such, although 2 of the cases recorded by Kerpel-Fronius did have nutritional oedema. (224). All enzymes appear to be affected in severe kwashiorkor.(140, 362, 57).

The methods for "trypsin" assay used in these studies (12, 255, 267, 10, 140, 31, 23) are now outdated (112) but do, nevertheless, give a fair index of the pancreas' ability to digest protein. Gelatin is used as a substrate; this is digested by all proteolytic enzymes and thus measures total proteolytic activity and not only trypsin,which is a specific endopeptidase acting on peptide bonds whose carboxyl group is contributed by an amino acid that has a positively charged side group (e.g. arginine or lysine). (289, 288, 406). It has been stated by Johnstone (208) that false positive results may be found using a gelatin substrate owing to the presence of gelatinase.

The theory of parallelism of enzyme secretion postulated by Babkin (20, 21) states that amylase, lipase and trypsin are always secreted in parallel by the pancreas. This theory has exceptions, particularly in states of malnutrition (107) and nutritional imbalance induced in animals.(190, 191, 253). It would, therefore, seem desirable to spread the enzyme net a little

wider with more refined methods to try to determine if any enzyme is more sensitive to P-C.M. in the human subject.

Despite the rather crude methods used to date, there is strong evidence from the analysis of duodenal juice that P-C.M. does lead to pancreatic deficiency, particularly in kwashiorkor. This may lead to permanent damage in later life.

(2). Tolerance Tests.

This method estimates the ability of the digestive enzymes to hydrolyse a given protein (usually gelatin or casein) by measuring the level of α-amino nitrogen or a specific amino-acid in the blood after an oral protein load. This, therefore, gives an indication of proteolytic activity as well as the ability to absorb the products of digestion.

Numerous investigators have applied this method to malnourished children (31, 66, 140, 265, 404). Bate and James (31) claim this is a more sensitive index of proteolytic activity than estimation of duodenal trypsin.

The only study conducted in children with kwashiorkor was that of Gómez (140) who found a delay in the absorption of unmodified protein (gelatin) by these children during the acute illness as compared with the same children after treatment.

The other studies have been performed on children with marasmus or non-specific under-nutrition. West et al (404) and Matsaniotis (265) found the absorption of casein to be normal in their series; it must be emphasised that the total serum protein of the children in the latter study was normal and that the albumin/globulin ratio was greater than 1. In both these studies the factor of absorption was also considered by feeding the children a casein hydrolysate; this was well absorbed, and this excluded a mucosal absorption defect. Doing a simple tolerance test without regard for

the absorption factor may give some false low serum nitrogen levels.

Christensen et al (66) found a correlation between the amount of gelatin absorbed and the amount of tryptic activity in the duodenal content. The methods and detailed results of the duodenal trypsin assays are not specified. Defective gelatin absorption was found in malnourished children by Bate and James. (31). In these 2 studies the absorption factor was assessed giving the patients pancreatin orally with the gelatin. In the latter study, one child was found with low blood amino-acid levels after oral gelatin who also had a proven malabsorption factor; he had had a previous bowel resection and had a flat glucose tolerance curve.

False high serum amino acid values may be obtained in liver disease. (66). This may be due to the inability of the liver to metabolise the absorbed amino-acid resulting in prolonged high blood levels. This test may, therefore, be unsuitable in children with kwashiorkor who are known to have liver pathology with upset liver function. (392).

(3). Serum enzyme estimations.

The level of serum enzymes believed to be of pancreatic origin, may be of value in the assessment of pancreatic function. This applies particularly to pancreatitis, especially during an acute attack. The provocative serum enzyme tests have been useful in the diagnosis of malignant disease or outflow obstruction of the pancreas; in this test the serum enzymes are measured after "provoking" the pancreas with secretin and/or pancreozymin. (26, 105, 261, 353).

Serum amylase and lipase or esterase have been studied in kwashiorkor. Dean and Schwartz (89) and Srinivasan and Patwardhan (348) found low values for amylase and esterase and lipase. Mukherjee and Werner (282) were able to demonstrate a low serum amylase concentration which correlated significantly with the serum albumin concentration, but the lipase

was only reduced in 1 out of 15 cases. Demaeyer and Vanderborght (92) and Sénécal et al (328) found low serum amylase values in kwashiorkor, although both these studies indicate a wide range of results with a broad overlap with normal controls. In marasmus the serum amylase was found to be normal in most cases with some having a reduced concentration. However, Sénécal et al (329) in a further publication state that serum amylase concentration is of little diagnostic significance in the borderline or mild case of kwashiorkor; only half of such cases showed abnormally low results.

The concentration of amylase in the serum rises progressively with treatment, (89, 92, 348, 328) and has been shown to rise concomitantly with the serum albumin concentration.(282). A failure of this enzyme to rise with treatment indicates a poor prognosis or the presence of some complicating factor. (329). The lipase or esterase in serum also improves with protein repletion. (89, 348).

The advantages in studying the serum amylase and lipase concentrations are evident; serum is easily obtainable and assay methods are relatively simple. No lengthy clinical procedure is necessary.

The disadvantages are, nevertheless, great:-

(a). By many assay methods the normal level of serum esterase or lipase is low. (63, 337, 363, 364). A rise in these enzyme concentrations is easily detectable, but a lowered value is always of doubtful significance. It is probably because of a similar difficulty that a lowered serum trypsin concentration is not quoted in any literature.

(b). Amylase assays in serum are subject to the criticism of their being non-specific with regard to the pancreas. Parotid abnormalities may raise the serum concentration (329) as may peritonitis, intestinal obstruction, uraemia and liver disease. (82, 308, 363). There is also little doubt that the liver is an important organ in amylase synthesis. (35, 77, 101,

197, 268, 314). As the liver is affected in kwashiorkor the low serum amylase values in this clinical state may to some extent be due to the liver pathology. It has been shown that the amylase of pancreatic origin moves with the γ-globulin fraction of the serum proteins on electrophoresis, whereas the iso-amylases move with the albumin fraction. (35, 268, 314). Determining electrophoretic mobility may thus be of some help in determining the source of origin of the enzyme, except that in very low values this would be technically difficult.

(e). Janowitz and Dreiling (197) have stated that in states of high carbohydrate utilization, the low plasma amylase values found are not dependant on pancreatic function. Besides the pancreas, salivary glands and liver, striated muscle, adipose tissue, and the fallopian tubes can all produce amylase.

It may be said then, that although serum amylase and lipase concentrations are of value in the diagnosis of inflammatory and obstructive lesions of the pancreas, they are of doubtful, and, at their best, non-specific value in states of pancreatic hypofunction.

(4). Histology.

Jackson (196) is reported to be the first person who described the atrophy of pancreatic acinar cells in inanition. This observation has since been confirmed by numerous investigators (47, 55, 85, 172, 259, 332, 377, 392, 22, 54, 46), and Trowell et al (368) have described these changes as the most constant lesion in kwashiorkor.

The changes in the acinar cell are typical; they consist of a disappearance of zymogen granules, decreased cellular cytoplasm with preservation of the nucleus, and a crowding together of the cells with the loss of normal acinar architecture. Cloudy swelling of the cell may be seen

as an early sign of cellular degeneration. A range of severity of these changes may be seen from patient to patient, and even sometimes in parts of the same pancreas.

Some workers have stressed the interlobular fibrosis seen in kwashiorkor (85, 259, 377), whereas, many have recorded the finding of fibrosis as a less common accompaniment of the basic pancreatic atrophy. (46, 47, 54). With gross acinar atrophy there may be a relative excess of connective tissue which may be confused with a true fibrosis. Campbell (55), working here in Cape Town, found a true fibrosis in only 1 of the 34 pancreases examined histologically; in 2 severely affected cases the connective tissue was pronounced, but this was only a relative excess. Pancreatic fibrosis in P-C.M. has been found in Europe (Hungary) (377), Africa (85), and in the East (259) and West Indies .(47). It would be difficult to incriminate any secondary factor in the aetiology of this fibrosis.

The changes in the ducts are as a rule not marked, if present at all. A slight dilatation of the ducts has been recorded.(85, 46, 47, 368). Microcysts are also described (368, 85, 134) but this is a non-specific feature; Baggenstoss (24) has described microcysts in cases of severe infection among other conditions. Véghelyi (377) described cystic changes in his patients and reproduced these in rats fed on a protein-deficient diet. Most workers in this field, however, do not stress these changes or do not record them at all. Gillman and Gillman (134) alluded to a similarity between this condition and fibrocystic disease of the pancreas, but a better understanding of mucoviscidosis in recent times has clearly separated this from the changes of protein deficiency. Davies in 1948 (85) drew a distinct distinction between the pancreatic changes of kwashiorkor and those of mucoviscidosis.

The Islets of Langerhans are typically not affected in the pancreatic atrophy of malnutrition (377, 172, 55), although Davies claimed that in advanced cases the Islets may be affected (85); he also described an apparent hypertrophy of the Islets.

Considerable care is required in interpreting the findings of a histological study of the pancreas. The vast majority of specimens in humans are taken at autopsy; the post-mortem interval may then be a lengthy one. In tropical countries especially, adequate refrigeration is necessary until the autopsy is performed. (85). The problem of autolysis is decreased in kwashiorkor as this takes place at a decreased rate owing to enzyme deficiency. (368). It is surprising how few writers on the subject of pancreatic histology in malnutrition comment on the problem of autolysis of the tissue.

An important factor in the interpretation of the histological changes in the pancreas in P-C.M. is the amount of treatment received prior to death and its duration. The pancreas regenerates very quickly with adequate dietary treatment (368, 47); this may occur in a patchy distribution at first. This may account for the failure to demonstrate the pathological changes in the pancreas in some cases. Unfortunately the inaccessibility of the pancreas in the human subject makes serial biopsies impossible.

It has been demonstrated that the pancreas in infancy and childhood may normally show significant differences from the adult gland. (113, 227). The connective tissue element is usually more abundant at a younger age, becoming progressively less with advancing age. The study by Emery (113) details all the criteria for the assessment of the maturity of the gland. It is possible that in P-C.M., particularly in kwashiorkor, the pancreas fails to develop and retains the characteristics of infancy or may show retrogressive changes. Caution must be exercised in the diagnosis of pancreatic fibrosis in

a child. Véghelyi (377) observed that the younger the child, the earlier the symptoms of pancreatic deficiency appeared after protein depletion; this may reflect a greater protein need or a degree of immaturity.

It is not clear whether the pancreas is significantly affected in children with marasmus. Some investigators record a sparing of the pancreas in marasmus (172, 393) and others describe changes which are less severe than those of kwashiorkor. (47, 305, 392). The spectrum included in P-C.M. makes a clear-cut differentiation of the extremes of kwashiorkor and marasmus difficult. Pancreatic pathology may be detectable on histology in the early stages of malnutrition. Mild changes resembling those found in kwashiorkor were seen in Jamaican children who were malnourished, some having subclinical kwashiorkor. (46, 47). Walt et al could find no pathological pancreatic changes in children dying with kwashiorkor, even though some died within 24 hours of admission. (388). No details of the histology are given in this study.

There is a statistically significant link between histological changes in the pancreas and the presence of a fatty liver. (46). This subject has already been discussed.(page 5).

Shaper (332) has described pancreatic changes in adults which he attributes to prolonged malnutrition. The histology in the 7 cases examined in this study all showed varying degrees of fibrosis. Ivy (195) has equated the secretory capacity of the pancreas with the amount of pancreatic acinar tissue. The functional changes described by Banwell et al (27, 28) in malnourished adults from Uganda, may thus be a reflection of true structural damage. These adults have thus probably reached the stage described by Brock (50) of "irreversible structural damage" as a result of prolonged malnutrition.

It is clear that protein deficiency in the human results in

pancreatic acinar atrophy. This is more marked in kwashiorkor than in the other clinical presentations of P-C.M. Care is required in the interpretation of histological findings, and this is of very limited practical value as all sections are taken from post-mortem material.

D. Nutrition and the pancreas in experimental animals.

Experimental animals have been used extensively in the study of pancreatic function, both under physiological and various predetermined laboratory conditions. Because of the difficulty experienced in studying the pancreas in humans, the contribution of the animal work is a valuable one. However, one must never be distracted from the fact that man remains the best experimental subject for work applicable to man, as species specificity plays an important role in many investigations. (408). In this section the effect of diet on pancreatic function will be discussed with particular reference to experimental P-C.M.

1. The effect of diet on the pancreas.

(a). Duodenal enzyme studies.

Varying the dietary constituents of experimental animals has been shown to have an effect on the enzymes secreted by the pancreas. (2, 42, 153, 194, 229, 253, 310, 379, 390).

A diet poor in protein leads to a diminution in pancreatic enzyme output. (2, 229, 379, 245). Véghelyi and Kemény in an excellent paper (379), were able to show a specific order of disappearance of enzymes in rats fed on a methionine-deficient diet and in the later stages also given carbon tetrachloride. Ribonuclease and an unknown fraction (probably carboxypeptidase), trypsin, lipase and chymotrypsin disappeared in this order. Amylase remained constant. The enzymes in the juice were estimated by paper electrophoresis;

this method, however, only gives an indication of enzyme concentration and gives no information regarding activity. Restricting a single amino acid from the diet may give a warped image of total protein deficiency. It has been shown by Boissonas (43) and Hokin (186) that methionine is not essential for amylase synthesis. This may explain the persistance of amylase on a purely methionine-deficient diet.

Adams et al (2) demonstrated a kwashiorkor-like condition in rodents fed cassava, a diet which leads to kwashiorkor in humans. This was accompanied by a 75% reduction in duodenal tryptic activity.

Carbohydrate is not only a poor supply of basic raw materials for enzyme synthesis but is also a poor stimulus to secretin and pancreozymin production by the small intestinal mucosa. (42, 194, 390). Wang and Grossman (390) in a very ingenious study on dogs showed that duodenal instillation of carbohydrate (as starch, maltose or dextrose) resulted in a slight increase in volume and bicarbonate output and no change in the enzyme output from the pancreas. When peptones and amino-acids were introduced into the duodenum, there was a large increase in enzyme output and a moderate increase in volume and bicarbonate output from the pancreas. It would appear that Ivy was correct in 1930 when he claimed that carbohydrate excites pancreatic secretion only through "appetite secretion"; for this to occur, the animal must eat "voluntarily and with appetite". (194). This hardly describes the eating habits of a child with kwashiorkor.

Lyman and Wilson (253) working on both intestinal content and pancreatic tissue in rats found that dietary deficiencies in single essential amino acids reduced the pancreatic enzyme reserve, with certain enzymes being affected more by one particular amino acid deficiency than another. (e.g. Phenylalanine, histidine and methionine deficiencies were most effective in

depressing intestinal protease levels). The ability of the pancreas to renew active enzyme was also impaired by such a deficiency.

Guth et al (160) could not demonstrate an adaptation of individual enzymes to the type of food ingested, but only lipase was estimated in this study.

Nonconformity of lipase in this respect was confirmed by Reboud et al (310) although these authors found that starch-rich and casein-rich diets increased amylase and chymotrypsin activity respectively.

(b). Studies on whole pancreas.

It has been shown that the enzyme content of pancreatic juice parallels that existing in pancreatic tissue. (153). In small animals such as the mouse and rat, examination of pancreatic tissue is more easily accomplished than that of pancreatic juice. These two methods of investigating pancreatic function, therefore, supplement each other.

Studying enzyme activity in homogenised pancreas Grossman et al were able to demonstrate an adaptation of the composition of the chief pancreatic enzymes to the predominant constituent of the diet. (153). Substitution of the hydrolysed form of the starch and casein in a similar study showed a rise in amylase with the former and a drop in trypsin with the latter (154); the explanation that this is due to a difference in the stimulatory effects of these two foodstuffs is unlikely as carbohydrate is known to have a poor stimulatory effect and amino acids a strong stimulatory effect.(390). An adaptation of enzymes to dietary constituents was also found in a more recent study by Howard and Yudkin. (191).

It must be explained that in all these studies, the minimum amount of casein in the diet was 10% by weight. Wachstein and Meisel (386) have shown that a synthetic diet containing 7.5% casein results in the pancreas

having an "almost normal" appearance histologically; at dietary levels of casein below 7.5% definite pancreatic atrophy is seen. In the studies mentioned then, the pancreas must have had sufficient protein with which to manufacture enzymes and maintain its own integrity. Different results may be expected when the building blocks for enzyme synthesis (amino-acids) are restricted to a subminimal level.

Magee and Anderson (256) suggested that in the rat an adequate stimulus for the release of pancreozymin (e.g. the presence of valine in the duodenum) seemed more important for lipase and trypsin production than the nutritional sufficiency (presumably biological value) of the protein consumed. They claimed, without any justification from the literature, that the changes produced in the pancreas in persons dying from starvation are different to those found in kwashiorkor, and that the latter syndrome does not result from a diet deficient in any of the essential amino-acids, but of certain proteins only. This hypothesis is convincingly disproved by the work of Sidransky and Farber (340); they produced the typical pancreatic atrophy of malnutrition in rats by feeding them a diet balanced in amino-acids (including valine) but deficient or devoid in threonine only. Similarly, Véghelyi and Kemény (379) produced histological changes in the pancreas with accompanying enzyme deficiency in rats fed a diet deficient only in methionine. More recent studies by Magee and Hong showed that varying the dietary amino-acids led to changes in the pancreatic enzymes (257, 190), and alternating high and low dietary protein content decreased the weight of the pancreas but did not decrease its enzyme content. (258). Furthermore, Hansen et al (164) have demonstrated that cure can be initiated in children suffering from kwashiorkor by feeding them an amino-acid mixture; this is probably the basic unit of protein required in these patients. It is clear that although defective stimulation may be a factor in the poor pancreatic enzyme production in

kwashiorkor, the basic deficiency of amino-acids is of primary importance.

(c). Studies on pancreatic tissue slices.

Using pigeon pancreas slices in vitro, Hokin has studied enzyme synthesis in this tissue under varying experimental conditions.(184, 186, 185, 187). The increase in total amylase activity was found to be greatest using a tissue incubation medium containing a complete amino-acid mixture, least in a medium without amino-acids, and intermediate in serum. (184). Of the 16 amino-acids found in crystalline α -amylase, 10 have been found necessary and also sufficient for maximum amylase synthesis. Methionine is not necessary for amylase synthesis. (186, 43). Amino-acid deficiency under these experimental conditions can, therefore, lead to impaired enzyme synthesis.

(d). Radio-active isotope studies.

The incorporation of labelled valine and iso-leucine into total pancreatic protein was studied in rats force-fed on a diet depleted or devoid of certain amino-acids. (340). The decreased uptake in animals fed a threonine-devoid diet suggests an impaired ability to synthesize protein. However, in animals fed on a methionine-devoid diet, the uptake of isotope was greater than in controls. A similar phenomenon has been observed in rats treated with ethionine, and the resultant increased amylolytic and proteolytic activity was inhibited by methionine. (338). Despite this a methionine-deficient diet leads to pancreatic atrophy. (379).

From these studies it can be gleaned that specific amino-acid deficiencies affect enzyme synthesis by the pancreas. Depending on which amino-acid and to what degree it is lacking, turnover may be either increased or decreased.

(e). Studies on serum.

Fujino (128) has demonstrated a lowering of the serum esterase and amylase in malnourished experimental animals. A reduction in lipase, amylase, trypsin and chymotrypsin in the pancreas was also found. The decrease in enzymes is attributed to a disorder in the bio-synthesis of enzyme protein. Details of the methods used are not given in this paper.

(f). Histology.

The histological changes brought about by malnutrition in the experimental animal closely resemble those found in humans.

The most striking feature is acinar atrophy. (2, 93, 127, 153, 230, 243, 245, 250, 271, 319, 339, 378, 379, 384). Deo et al (93) give an account of the acinar changes in force-fed rhesus monkeys which bears the essence of the findings of most of the other workers. The earliest change is loss of zymogen granules and basophilic chromidial material from the acinar cells. Although the acinar pattern could still be distinguished after 4 weeks depletion, the changes became progressively worse with advancing time. By 10 weeks there was marked shrinkage and partial disruption of the acini. The cells lost most of their cytoplasm and acini became composed mostly of naked nuclei.

Similar acinar changes are found on electron microscopy. (243, 401). In addition, dense bodies were seen in the cytoplasm; there was also some loss of ribosome material and a widening of spaces between the ergastoplasmic membrane. Weisblum et al (401) recorded a spontaneous return toward normal after 12 days on a protein-free diet; this may represent a raiding of protein stores or may have been an adaptive phenomenon. It is interesting to note that in this same study rats fed a protein-free diet ate more than those given a protein-free diet plus ethionine; in the former the pancreas showed more severe protein depletion, probably because of the

necessity of enzyme production. This concept may be significant in the difference between marasmus and kwashiorkor, the latter having a greater calorie intake and more depleted pancreatic protein.

Some observers record a sparing of the ducts in the pancreas (250, 384) while others comment on a relative increase in the ducts. (2, 93, 271). Kristal (230) describes the late stages of pancreatic atrophy as a mass of dilated tubules embedded in adipose tissue. However, by using histochemical methods, Adams et al (2) claim that at least half of the apparently newly formed ducts might be shrunken acini, and they produce some convincing evidence in support of this. A relative increase in the ducts is probably the most accurate interpretation of these findings. Volk and Lazarus (384) noted that ductular proliferation only occurred in animals treated with steroids.

Some workers have observed vacuolation of the pancreas and the formation of cysts. (271, 378, 379).

The Islets of Langerhans are not affected in nutritional pancreatic atrophy (93, 153, 250, 271, 339, 377) although Véghelyi records an increase in the number and size of the islets (378), which is probably only of relative significance. Special stains were not used in the study of the islets in most cases.

Véghelyi consistently described fibrous tissue proliferation in his clinical cases and in experimental animals.(378, 377, 379). Many of his experimental animals received carbon tetrachloride which adds another variable to the experimental conditions. Adams et al (2), Deo et al (93) and McPhedran and Lucas (271) failed to demonstrate significant fibrosis, even using special connective tissue stains in the last-mentioned study.

From histological studies, McPhedran and Lucas (271) claim that

in the rat dietary deficiency is more deleterious than alcohol to the pancreas as well as to the liver. This may be of great significance in its clinical application; the Coloured population in the Cape who have a heavy alcohol intake and who are often malnourished for prolonged periods have a high incidence of pancreatitis.

2. The relation between the pancreas and a fatty liver.

Fatty infiltration of the liver is a very complex and lengthy subject and there are many factors involved in its pathogenesis. It will not be discussed here in any detail except to review the experimental link between pancreatic pathology and the presence of a fatty liver.

(a). The effect of exclusion of pancreatic tissue or of its exocrine secretion.

(i). Pancreatectomy. Since 1924 it has been observed that complete removal of the pancreas results in the development of a fatty liver. (8). This has been confirmed in many reports. (114, 115, 37, 59, 60, 102, 216, 217, 276, 277, 278, 279, 313, 370, 415). This effect may take as little as 3½ weeks to develop, but at least 16 weeks are required for it to be a constant feature. (217). The fat is often patchy in distribution. (59). Spontaneous regression of the fatty liver may occur if the animal survives for a long enough period. (217).

(ii). Ligation of the pancreatic duct. Fatty infiltration has been found in the liver of animals with ligated pancreatic ducts, thus preventing the exocrine secretion of the pancreas from reaching the duodenal lumen. (34, 70, 276). van Prohaska et al could not confirm this, but their experiments were performed over a limited period of time, and many estimations were made on liver biopsy specimens. (370). Owing to the frequent patchy distribution of the fatty deposits and the prolonged time often required before a fatty liver is consistently found, the findings of van Prohaska et al

are subject to criticism.

(iii). Complete pancreatic fistula. Berg and Zucker (34) were able to demonstrate fatty infiltration of the liver in dogs with total pancreatic fistulae, and found the changes to resemble those produced by prolonged inanition. Colwell (73) and van Prohaska et al (370) were unable to confirm these findings; their experimental data is, however, open to criticism as detailed above.

(b). The effect of induced pancreatic dysfunction.

(i). Nutritional deficiency. As already discussed, it is clear that the pancreas may be deleteriously affected by dietary imbalance or insufficiency. Such induced pancreatic pathology has been linked with the development of a fatty liver in experimental animals. (2, 34, 93, 127, 153, 250, 339). These findings may be reproduced by force-feeding diets deficient in one or more amino-acids. (319, 340). Whether the liver or the pancreas is affected first is not clear; Kristal (230) found some grossly pathological livers with no noticeable change in the pancreas while McPhedran and Lucas (271) found pancreatic lesions with normal livers. Volk and Lazarus (384) have attributed the hepatic alterations to the dietary imbalance and not primarily to the pancreatic pathology.

(ii). Ethionine toxicity. Administration of ethionine leads to pathological changes in the pancreas (118, 213, 226, 283, 338, 385) and these changes are often accompanied by fatty infiltration of the liver. (88, 116, 137, 138). It is doubtful whether the pancreatic lesions under these circumstances result in the fatty liver as the onset of the fatty change is extremely rapid. (137). The changes in the pancreas produced by ethionine toxicity have been found to be similar to those produced by dietary imbalance or insufficiency as judged by electron microscopy. (177, 178, 401). These changes may be produced by the competitive inhibition of methionine by

ethionine (343), and the liver changes may have the additional factor of hypoglycaemia playing a role, as administration of glucose has been reported to prevent the development of the fatty liver. (116).

(c). Lipocaic.

van Prohaska, Dragstedt and Harms have shown that raw pancreas can prevent fatty infiltration of the liver in depancreatised dogs maintained with insulin. (370). These workers then postulated the existence of a hormone, which they labelled lipocaic, produced by the pancreas and playing a role in fat metabolism. (102). It is not present in pancreatic juice and can prevent or reverse the accumulation of fat in the livers of depancreatised dogs. (103). Kaplan and Chaikoff (217) have criticised the work of Dragstedt and his colleagues on the grounds of the experiments being conducted over a too short time period and also that too much evidence was obtained from liver biopsy specimens. Entenman et al (115) extracted lipocaic from pancreatic tissue and found it a poor source of anti-fatty liver factor.

(d). Anti-fatty liver factor.

Chaikoff, Entenman, Kaplan and Montgomery have put forward their concept of the anti-fatty liver factor in a series of papers. In depancreatised dogs maintained with insulin, fatty infiltration of the liver may be prevented by the oral administration of raw pancreas (114, 115, 217, 277), pancreatic juice (276, 278), hydrolysed casein or free methionine (60) or even crystallized trypsin added to the food. (279). Lean meat and unhydrolysed casein were ineffective in this respect. The plasma choline in dogs given oral pancreatic juice was raised to a far greater level than could be accounted for by the choline present in the administered juice itself (278), and brain, which contains more lecithin and choline than pancreas, is ineffective in preventing fatty infiltration of the liver. (370).

It seems likely, therefore, that the ability to hydrolyse protein

in the gut, thus making it available for absorption and body metabolism, is a most important factor in the prevention or treatment of the fatty liver. The hydrolysate of a complete protein acts in a similar way. This function of protein hydrolysis is normally performed to a major extent by the pancreas in its secretion of proteolytic enzymes. The dietary and pancreatic deficiencies may thus act in a complementary fashion in the production of a fatty liver. More recent studies have confirmed this concept (161, 312), although a basic quantity of protein is necessary in the diet; mice given a high fat, low protein diet developed a fatty liver despite pancreatic extract given orally. (43a).

In children with kwashiorkor, the basic cause of the fatty liver appears to be the lack of protein. This is aggravated by a deficiency of pancreatic enzymes, as the little protein ingested is not used under optimal conditions.

CHAPTER 2.

The physiology of the exocrine pancreas.

In order to understand the pathology of the pancreas and the investigation of pancreatic function, a basic knowledge of the physiology of this organ is essential.

A. Embryology and Structure.

The pancreas arises as two entodermal outgrowths (the dorsal and ventral buds) from the cephalic limb of the primitive gut when the embryo is as small as 3 to 4 millimetres in length (i.e. about the 4th to 5th week of gestation). (18, 293). The ventral bud rotates around the right side of the duodenum, often having a common opening into the duodenum with the hepatic diverticulum. By the 7th week, the ventral and dorsal parts of the pancreas interlock intimately, with fusion of their ducts. (18, 42). The adult pancreas is derived chiefly from the dorsal bud which gives rise to all the gland except the head; most of the head is derived from the ventral bud. Acini begin to appear in the 3rd month as terminal and side buds from the ducts. The Islets of Langerhans also differentiate from the ducts at about the same time. There is evidence that insulin or pre-insulin is produced by the embryonic pancreas. (323).

The pancreas is enveloped in a poorly developed capsule. Septa extend inwards from the capsule to divide the pancreas into lobules. The main duct (duct of Wirsung) virtually acts as the backbone of the organ. (162). A system of branching ducts link the main ducts with the acini. The bulk of the gland is composed of acini which produce the exocrine enzyme secretion. The Islets of Langerhans, which have an endocrine function, are scattered unevenly throughout the gland. The pancreas is adequately supplied with blood and lymph vessels. (130). The nerve supply to the organ is from both the para-

sympathetic and sympathetic systems.

B. The pancreatic acinus and enzyme production.

(1). Pancreatic protein turnover.

The pancreas, the liver and the small intestinal mucosa are the tissues which show evidence of having the greatest protein turnover in the body. (405, 356). This high protein turnover represents principally the metabolic activity of the exocrine portion of the pancreas. (405). In young animals protein is used for new cell formation to a greater extent than in mature animals. (78). However, the major turnover of protein in the pancreas is concerned with the production of digestive enzymes. (1, 83, 84, 117, 148, 91, 212, 231, 284). The turnover of pancreatic enzyme protein is approximately three times greater than that of serum protein. (1, 231). It is well known that the serum albumin concentration is depressed in severe P-C.M. (266); that the pancreatic enzymes should be depleted under such circumstances is, therefore, to be expected.

(2). The synthesis and secretion of enzymes.

Excellent work has been performed by Siekevitz and Palade using electron microscopy techniques in tracing the steps in the synthesis of enzymes by the acinar cell. (292, 341, 342). Radioactively labelled amino-acids were found to be incorporated into the ribosomes as α -chymotrypsinogen within 1 to 5 minutes after injection in experimental animals; this site has been postulated as that of enzyme synthesis. This has been confirmed by other workers regarding ribonuclease synthesis by the mouse pancreas. (280). From the ribosome the enzyme migrates to the centrosphere (Golgi) region of the cell where it is concentrated into a smooth-surfaced vacuole. This vacuole gives rise to the zymogen granule which ultimately moves to the apical portion of the acinar cell and is then discharged into the duct system. The formation

of the mature zymogen granule takes about 15 to 45 minutes. (292, 391). This sequence of events is followed by the proteolytic enzymes, but the other enzymes produced by the acinar cell may differ in their journey to the ductules. (292, 239). It has been demonstrated that enzyme protein is synthesised from amino acids and not from plasma proteins per se. (212, 284). Enzymes may be stored for a short period in zymogen granules. (68).

The secretion of enzyme depends on the stimulus provided - either hormonal or neurogenic. (117, 148, 240). Enzyme synthesis is also increased after stimulation of the pancreas by neural and hormonal mechanisms. (117, 341).

(3). Enzyme function.

Digestion is defined by White et al (406) as "the sum of the enzyme hydrolyses of large molecules - polysaccharides, proteins, lipids, nucleic acids - to smaller components which can be absorbed and then metabolised". The pancreas is an important source of the hydrolytic catalysts. As already discussed (page 3), children with severe P-C.M. show clinical evidence of maldigestion. Knowledge of the action of the enzymes necessary for normal digestion is, therefore, necessary for the understanding of the pathophysiology of digestion and for the analysis of enzyme activity in the digestive juices.

(a). Amylase. The activity of amylase was probably the first recognised enzymatic reaction. (295). Amylases are glycosidases, i.e. they hydrolyse glycoside links in polysaccharides. They may be divided into α- and β-amylases, the former having an endo-amylase activity (they split glycosidic links in the middle of polysaccharide chains), and the latter an exo-amylase activity (these work from the non-reducing end of the polysaccharide, splitting off maltose residues one at a time). (99). It is the α-amylase which is found in pancreatic juice and saliva. The modern tendency is to pinpoint the site of action of the enzyme, thus being able to give it a specific

name:- thus α-amylase becomes α-1, 4 - glucan, 4 - glucanohydrolase. These α-amylases can hydrolyse glycogen, starch and residual polysaccharides of starch (amylodextrin) to give glucose, maltose and products that no longer give a colour with iodine. (406). Of all the substrates which are hydrolysed by amylase, starch has stood the test of time and is the standardly recommended substrate for the assay of pancreatic amylase activity. (69). The optimum pH for pancreatic amylase activity is 6.7 to 7.2. Amylase has an absolute requirement for chloride ion, and is stimulated by calcium ions.(406). As already discussed (page 14) serum amylase is only partially of pancreatic origin.

(b). Lipase. The pancreas produces lipase and one or more esterases. True lipases hydrolyse fats into long chain fatty acids and glycerol; simple esterases catalyse reversibly the hydrolysis of simple esters of lower alcohols and fatty acids. (63, 94, 406). Esterases can hydrolyse esters in solution, whereas pancreatic lipase acts exclusively on emulsified esters as it acts at the interface of the emulsified globules. (94). Bile plays an important part in emulsifying the contents of the bowel. (252). Emulsified triglycerides are, therefore, recommended as a substrate for lipase assay. (69). The optimum pH for lipase activity is 8.0 . (129). It has been demonstrated that exclusion of pancreatic juice from the bowel in experimental animals decreases the utilization of both saturated and unsaturated fats, but does not affect the absorption of oleic and palmitic acids. (218, 219). Deficiency of lipase leads to steatorrhoea, one of the classical manifestations of pancreatic dysfunction (252); owing to the tremendous functional reserve of the pancreas, more than 90% of the pancreas must be non-functioning before steatorrhoea occurs. (11, 299). Lipase, which is probably of pancreatic origin, is found in small quantities in the serum (363, 364); methods for its

detection in the serum are not very reliable. (69).

(e). Trypsin. This enzyme is synthesised and secreted by the pancreas as its inactive precursor trypsinogen, sometimes known as a zymogen. (112, 173, 289). Enterokinase, produced by the intestinal mucosa and present in intestinal juice, was found in 1899 to activate trypsinogen to trypsin.(335). By a process of autocatalysis, trypsin itself also promotes the activation of trypsinogen. (420). The position of cleavage and residue release in zymogen activation is known. (289). Trypsinogen is not activated by bile as previously thought. (112, 173). The activation is enhanced by the presence of calcium ions. (173). As enterokinase is present in intestinal juice, pancreatic juice aspirated from the duodenal lumen contains trypsin in its active form and is thus suitable for direct use in enzyme assays. In many older publications the activity of "trypsin" is equated with total proteolytic activity. (12, 23, 31, 112, 140, 255, 267, 10). As trypsin is a specific enzyme which is available in crystalline form (291) and with a known molecular weight and amino-acid sequence (289), this is a misleading generalisation and should be avoided. Trypsin is an endo-peptidase which hydrolyses only peptide bonds whose carboxyl group is contributed by an amino-acid that has a positively charged side group (e.g. arginine or lysine). (288, 289, 406). Besides the proteolytic activity of trypsin on substrates such as casein (119), gelatin (13, 87), albumin (122, 123), serum (143) and denatured haemoglobin (17), this enzyme possesses an esterase activity. (324). This is demonstrated in the hydrolysis of benzoyl arginine ethyl ester (BAEE) which is a specific substrate; using this substrate, the specific activity of a sample of trypsin may be determined (expressed in terms of micro-moles of substrate split). (225, 398). Synthetic substrates, particularly BAEE, are recommended by modern authorities for the assay of trypsin activity. (69). The optimum pH for this enzyme is 7.8. (406).

(d). Chymotrypsin. The synthesis, intracellular transport and storage of chymotrypsinogen have been closely studied, right from its origin in the ribonucleoprotein of the acinar cell to its passage into the pancreatic duct system. (292, 342). Its chemical composition is known (289, 409, 410) and it is available as a crystalline substance. (232). This enzyme is also synthesised and secreted in its inactive precursor form (chymotrypsinogen) and undergoes activation in the gut. (108, 287, 406). Duodenal juice is thus fully activated. The activation of the zymogen, initially by trypsin and then also by chymotrypsin, has been studied (38); the position of cleavage and the residue release on activation have been determined. (289). The similarity between trypsin and chymotrypsin, particularly at their active sites, is striking. Chymotrypsin acts preferentially on peptide bonds whose carboxyl group is adjacent to an amino-acid that has a 6 - carbon ring in its side group (e.g. tyrosine or phenylalanine). (288, 289, 406). It possesses an esterase activity towards synthetic substrates such as tyrosine ethyl ester (TEE) and acetyl-tyrosine ethyl ester (ATEE). (325). The latter is recommended by modern authorities for the assay of chymotrypsin activity(69); specific activity may be determined using synthetic substrates as micro-moles of substrate split may be accurately determined. (225, 398). The optimum pH for chymotrypsin activity is 8.0. (129).

(e). Carboxypeptidases. These are exo-peptidases, also secreted in the precursor form as procarboxypeptidase. They are called exo-peptidases because they exclusively hydrolyse the last peptide bond in a polypeptide chain. Carboxypeptidase-A acts preferentially on peptide bonds adjacent to terminal amino-acid residues with a 6 - carbon ring side group (e.g. Tyrosine, tryptophan, phenylalanine); carboxypeptidase - B acts

selectively on those adjacent to terminal residues whose side groups end in NH_2 (e.g. lysine or arginine). (289, 406). Their action is, therefore, complementary to the endopeptidases chymotrypsin and trypsin. Little work has been done on these enzymes in clinical medicine, although specific substrates are now available for their assay. (69). Procarboxypeptidase-A is composed of 3 fractions, the second of which has some endopeptidase properties and can hydrolyse ATEE; this property is not possessed by carboxypeptidase-A. (222, 288). The optimum pH for carboxypeptidase activity is 7.5. (406). Carboxypeptidase-A requires the presence of zinc for its activity. (289).

(f). Ribonuclease. (RNase). This enzyme was first crystallized by Kunitz (234) and through a series of experiments, the complete amino-acid sequence of the enzyme has been determined by Hirs. (180, 181, 182, 183). It is synthesised by the ribosome fraction in the pancreatic acinar cell. (280, 342). RNase may be more widely distributed through the pancreatic cell than the proteolytic enzymes which are probably localised in zymogen granules. (284). The enzyme acts on ribonucleic acid (RNA), hydrolysing phosphodiester linkages of the pyrimidine nucleotides in pentose nucleic acids. (97). The products of digestion are nucleotides and nucleosides (406); they are not precipitated by glacial acetic acid or uranyl acetate, and they readily diffuse through collodion or cellophane membranes. (16, 234). These principles are important in the laboratory assay of the enzyme. It has been said that magnesium ions inhibit RNase activity (241, 307); this is a pseudo-inhibition applicable only to turbimetric assay methods (97), and magnesium ions actually potentiate RNase activity in other assay methods. (58, 97). It is a very stable enzyme with an optimum pH for its activity at 7.7. (234).

Note:- Typical of most enzymes, those found in pancreatic juice lose their activity upon standing at room temperature (12, 251,

318), particularly after dilution. (87), 159). Amylase (87) and RNase (234) are relatively more stable than trypsin.(12). Lipase is unstable in the presence of trypsin. (318). The effect of these factors may be minimised by keeping the enzyme solutions at 0° to 4°C. (318, 251) and performing the enzyme assay as soon as possible after collection of the specimens.

C. Fluid secretion.

The fluid secretion of the pancreas is important as a vehicle for enzymes, bicarbonate and electrolyte output besides its contribution to the fluid content of the bowel during digestion. The volume of juice obtained by duodenal drainage increases with advancing age, from an average of 3 to 5 ml. per hour in infants to as much as 20 or 30 ml. per hour in older children. (286). The origin of the fluid secretion of the pancreas is as yet uncertain, but it is known that the intercalated ducts play an important part in fluid production. (198). Dogs rendered diabetic by alloxan were found to have vacuolization of the intralobular ducts in the pancreas; the acinar cells were normal. Upon stimulation with a secretin-pancreozymin mixture, the enzyme response in these dogs was normal but not the volume response. This points to the small duct cells as the source of pancreatic juice as distinct from enzyme production by the acini. (155). Giving anti-diuretic hormone to dogs decreases pancreatic volume flow. (297). The hydration of the patient, particularly in children, is important in assessing pancreatic function as the volume output is affected by hydration. (12, 286). It is interesting to note that the pancreas contains osmo- and baro-receptors, which constitute part of the afferent side of the antidiuretic system. (193). As the weight of the pancreas increases almost proportionally to body weight it is possible that

volume output may also parallel body weight. The weight of the pancreas is approximately 1 gram per kilogram body weight. (397). Hypothermia decreases pancreatic volume output (380); it is, therefore, necessary to preserve normal body temperature during a pancreatic function test.

D. Bicarbonate in pancreatic juices.

It is well known that pancreatic secretion is alkaline (361) and that bicarbonate is of prime importance in the maintenance of a pH suitable for optimum digestion. (252). Diamond found the concentration of bicarbonate in pancreatic juice under basal conditions to be 26 milli-equivalents per litre (m. equiv./L); this was found to rise to 90 m. equiv./L or more after stimulation with secretin. (95, 106). In dogs Hart and Thomas found a relation between the bicarbonate concentration and the rate of secretion provided the rate of secretion remained below 0.05 ml. juice per minute per kilogram body weight. (171). At higher rates of flow the bicarbonate concentration attained a constant maximum characteristic of the animal; the maximum ranged from 135 to 148 millimoles per litre. Bicarbonate and chloride, the two main anions in pancreatic juice, vary inversely in concentration the one with the other; their total concentration is always of the order of 150 m. equiv./L (174, 198, 315). Prolonged secretion of pancreatic juice through a traumatic pancreatic fistula in a human subject resulted in a drop in serum sodium, chloride, and calcium, but not of bicarbonate. (192). This suggests that bicarbonate is produced by the pancreas. Bicarbonate production is probably a function of the epithelium of the pancreatic ducts, particularly the intercalated ducts, as these have been shown to contain carbonic anhydrase, which is necessary for bicarbonate production.(198, 260). The acini do not contain carbonic anhydrase. (260). The submaxillary and parotid glands have intercalated ducts and secrete bicarbonate, whereas the

sweat glands and the sublingual glands neither have intercalated ducts nor produce bicarbonate in their secretions. (198). Besides producing a milieu of near optimum pH for the pancreatic enzymes, the alkalinity of the pancreatic juice renders zymogen granules more soluble, thus enabling them to liberate their enzymes more easily. (188). The presence of bicarbonate in the intestine is also important in the digestion of fat. (71).

The complete assessment of pancreatic function, therefore, needs to include the bicarbonate output although this probably is a test of the function of the duct cells rather than the acinar cells. In conditions where the acinar cells are primarily affected (e.g. P.C.M.) one would expect the enzyme output to be affected to a far greater extent than the bicarbonate output.

E. Electrolytes.

The literature on the electrolyte content of pancreatic juice is well reviewed by Janowitz and Dreiling (198) and Haverback . (174). The principle anions in pancreatic juice are bicarbonate and chloride as discussed above. The main cations are sodium, potassium and calcium. Sodium and potassium are present in quantities isotonic with serum; their concentration is unaffected by the rate of flow. (29, 298). The concentration of calcium is lower than that of serum (3 to 4 mg. per litre), but it varies in a parallel fashion with serum calcium; administration of parathyroid extracts to dogs increases the concentration of calcium in their pancreatic juice.(179).

Because of the importance of electrolytes in the activation and action of many enzymes, they play a valuable role in digestion. The measurements of electrolytes in human studies are difficult as, ideally, the pylorus should be occluded to prevent any possible contamination of electrolyte-rich juice from the stomach, or the pancreatic duct should be cannulated. This is clearly impractical for routine use.

F. Enzyme inhibitors.

The pancreas synthesises a trypsin inhibitor (72, 157, 173) which has been crystallized by Kunitz. (233). This inhibitor appears to be present in greater concentrations in whole pancreas compared with zymogen granules.(188). Trypsin inhibitor is soluble in trichloracetic acid whereas trypsin is not (72, 157); this facilitates separation of the two substances.

It has been found that in the rat, trypsin and chymotrypsin inactivate the inhibitor (157) although this has not been confirmed in humans (173) or cattle. (157). An extract of duodenal mucosa leads to inactivation of the inhibitor in pancreatic juice (215); this has been thought to be due to the activation of trypsinogen and chymotrypsinogen to their respective active forms which in turn attack the inhibitor (157) or possibly to a direct effect of enterokinase. (161). The latter explanation has been disproved by Mars et al. (264). It seems difficult to understand how trypsin can both inactivate and be inactivated by the same chemical substance. It would appear that there is some as yet undefined factor in the intestinal mucosa or which is secreted into the lumen of the gut which inactivates the inhibitor or enables some other substance to render the inhibitor ineffective. Despite the apparent confusion as to the mechanism of its action, it is clear that the pancreas and its secretion contain a trypsin inhibitor and that the effect of this inhibitor is largely lost in the gastrointestinal tract. Its function appears to be to prevent the pancreas from the hazards of autodigestion and yet not to prevent normal proteolytic enzyme activity in the gut. The assay of trypsin, therefore, from fully activated juice collected from the gut should not be significantly affected by trypsin inhibitor.

Trypsin and chymotrypsin, but not their zymogens, may be inactivated by diisopropylfluorophosphate (199, 200, 201, 202); the exact mechanism of

this inhibition has been determined. (289). There are also certain naturally occurring inhibitors which may have some effect in the diet, e.g. that found in soybean (235, 236, 237, 238), egg albumin (252), colostrum and lima bean. (72). It is interesting that Ascaris lumbricoides which is a very common intestinal parasite in malnourished children also produces a trypsin inhibitor. (72). Structural analogs of specific substrates have also been used to inhibit enzymes. (220, 221).

G. The control of pancreatic secretion.

The pancreas is controlled by hormonal and neural mechanisms. These mechanisms have been separated in this section purely as a matter of convenience for description, but this separation must be regarded as an artificial one. Grossman has emphasised that co-operative neuro-hormonal actions are likely; this cooperation may involve the release of hormone, their action on the pancreas or possibly both. (158).

(1). Hormonal control.

(a). Secretin. In 1825 Leuret and Lassaigne observed that acid applied to the duodenal mucosa resulted in an increased flow of pancreatic juice; they presumed that a similar mechanism would apply when acid chyme passed over the duodenal surface. (246). This observation was confirmed by Claude Bernard (36) and Dolinski. (100). Popielski in 1901 postulated the existence of a local reflex between the duodenum and the pancreas to explain this phenomenon. (303). Then, in 1902, Bayliss and Starling published their classical paper demonstrating the presence and action of secretin, the first hormone described; this hormone is produced by the mucosa of the upper small bowel, particularly the duodenum, and is transmitted by the circulation to the pancreas. (33).

Secretin originates from the small intestinal mucous membrane

(361) although a substance with similar activity, but of doubtful physiological significance, has been found in the gastric antrum. (42). Its secretion is stimulated by the presence of acid in the duodenum (194); in the dog a pH near 4.0 is required as a threshold stimulus. (358). Food also acts as a stimulus, different foodstuffs having different stimulatory potency; in general it is found that proteins are good stimulants whereas carbohydrates are poor stimulants. (359, 360, 365, 390). Duodenal irritation (e.g. by a rubber tube) also increases pancreatic secretion (107, 387) possibly by a hormonal mechanism; this factor may be operative during pancreatic function tests employing duodenal aspiration.

Secretin is a protein of molecular weight about 5,000 (42), which is very stable at low temperatures (6) and not destroyed by heat over a short period. (33). This makes it suitable for storage and sterilisation and is of great benefit for its clinical use. The mucosal homogenate from which secretin is extracted has to be freed from contamination by vasopressor substances, pyrogens, cholecystokinin, enterocrinin and pancreozymin. (125). Methods have been evolved to obtain secretin in a clinically usable form.(4, 144). The method of Crick, Harper and Raper is fairly simple, gives a reasonable yield and is suitable for producing secretin for routine clinical use. (79). This method is used by the Boots Pure Drug Company for their commercially available preparation. More recently, Jorpes and Mutt have produced a highly refined secretin preparation which is well suited for scientific investigations. (211).

The respiratory rate of the pancreas in vitro is increased by secretin, but enzyme secretion is not affected. (86, 98). This observation is compatible with in vivo experiments on animals and humans in which it is

demonstrated that secretin stimulates the pancreas to produce a juice large in quantity (29, 74, 151, 156, 248, 254), rich in bicarbonate (29, 67, 74) and poor in enzymes (67, 74, 122, 124, 148, 166, 389), a function which Babkin has labelled "hydrelatic". (21). Any increase in enzyme concentration is a temporary one owing to a flushing out of the ducts. (67, 124, 148). A cholegogue effect has also been ascribed to secretin. (4, 121).

Secretin is highly potent, concentrations in the micro-gram range being sufficient to stimulate the pancreas in dogs. (145). The standard dosage given to humans for clinical purposes is of the order of 1 unit per kilogram body weight. (95, 106, 124, 139, 261). In vitro experiments in dogs have shown that the hormone may be inactivated in serum (146) but this factor does not appear to have any clinical significance. Giving extremely large doses (6.4 units per kilogram) led to a generalised collapse in dogs. (67). Sun and Shay recommended doing skin tests for secretin sensitivity before injecting the hormone intravenously claiming that slightly over 0.5% of their patients showed a positive reaction. (352). This precaution is not taken routinely by most workers and clinical reactions of any severity are rare using the recommended dosage of secretin, even using the older preparations. (4). Burton et al in a large series found side effects in 0.7% of their patients using secretin in a dose of 1.7 units per kilogram body weight. None of these were of a serious nature, and none were on an allergic basis; they did, however, take the precaution of withholding the hormone from asthmatics. (53). Unfortunately secretin must be given intravenously as it is digested by the enzymes in the gut.(5).

It has been observed since 1904 that acid in the duodenum inhibits gastric secretion.(346). This has been confirmed in more recent studies. (417, 14, 15). That secretin plays a role in this inhibition has been demonstrated by the fact that intravenously administered secretin inhibits gastric

acid output. (7, 136, 150, 223, 210, 285, 418, 419). This factor will help to prevent the contamination of duodenal juice by gastric juice after secretin has been administered during a pancreatic function test.

It is clear that secretin plays an important part in pancreatic function and the process of digestion. To assess the "hydrelatic" function of the pancreas the "secretin effect" must be produced. As the hormone is now freely available for clinical investigation, a standard stimulus may be applied to the pancreas. This is invaluable for the assessment of pancreatic function.

(b). Pancreozymin. After the discovery of secretin there was considerable debate as to whether secretin was responsible for enzyme production by the pancreas or whether this was purely a neurogenic function. Harper and Vass in 1941 postulated the existence of a second hormone.(166). They showed that secretin did not excite the pancreas to produce enzymes and yet if food was placed in the duodenum of cats after section of all extrinsic nerves to the small intestine, the enzyme output of the pancreas was increased. Two years later Harper and Raper isolated this enzyme which they called pancreozymin.(167). This work was confirmed by Greengard et al.(147).

The stimulus for pancreozymin secretion by the duodenum varies with different foodstuffs, being strongest with peptones, amino-acids and soap (Na oleate), intermediate with acid, and weakest with fats and carbohydrates.(390). Pressure increase in the duodenum does not increase enzyme output.(166). A topical anaesthetic applied to the intestinal mucosa prevents the release of pancreozymin.(354).

Pancreozymin is a protein which is thermo-stable, thus facilitating sterilisation. It is destroyed by pancreatic juice and hence needs to be administered intravenously for clinical investigations. The distribution of pancreozymin in the small intestine is similar to that of

secretin. It is not present in extracts of gastric mucosa (167) although a pancreozymin-like effect has been produced by gastric stimulation in cats. (39). Extraction of the hormone from intestinal mucosa has been successfully accomplished, although several contaminants create difficulties. Most commercially available pancreozymin contains cholecystokinin which causes contraction of the gall bladder (42, 109, 139, 169, 211), an enterokinetic substance (probably substance P, a plain muscle stimulant)(42, 169) and a pepsigogue.(42, 402). Vasopressor substances are easily destroyed.(167). The pancreozymin produced by Greengard and Ivy was reputed to be free of secretin, cholecystokinin and vasopressor activity (149), but it has unfortunately never been commercially available. The extraction method devised by Crick et al (79) yields pancreozymin which has considerable cholecystokinetic properties and is relatively crude (261, 262); this extraction method is used by the Boots Pure Drug Company for their product. A more refined preparation is now available from Sweden using the method of Jorpes and Mutt in its extraction. This, however, also contains cholecystokinin.(211).

Pancreozymin acts directly on the acinar cell; in vitro experiments have shown an increase in the cell respiratory rate and an increase in enzyme production after pancreozymin. (86, 98). The actual incorporation of radioactively labelled amineacids into pancreatic protein is enhanced 1 hour after pancreozymin injection (400), indicating a more rapid enzyme synthesis. Triglycerides and phospholipids are also formed at a greater rate by the acinar cell 45 minutes after pancreozymin; simultaneous studies on liver homogenates showed no such effect, thus suggesting an organ specificity. (399). Harper and Mackay performed the classical experiments showing the histological depletion of cell granules containing enzyme from the acinar cell

after pancreozymin injection; this did not occur after secretin.(168). The volume is characteristically only slightly raised after pancreozymin, but the cholecystokinetic effect seen with most preparations used makes this difficult to interpret. Some workers have described an increased volume output after pancreozymin.(403). The "in vivo" effect of the hormone in humans confirms the prior laboratory tests.(109, 389). Using Babkin's terminology, pancreozymin has a primarily "ecbolic" effect. (21).

When injected, this hormone often causes untoward reactions. Burton et al experienced this in 13% of their large series of patients. However, only 3% were of any severity and only 1% led to discontinuation of the procedure. All reactions were transient.(53). Commonly listed reactions were headache, nausea, vomiting, abdominal cramp, and venospasm during injection. Some workers recommend skin sensitivity testing, but Sun and Shay found no positive skin reactions to pancreozymin in about 200 patients.(352). Slow injection of the hormone, well diluted, minimises reactions.(53). The usual dose given for clinical purposes is of the order of 1.5 units per kilogram body weight.(261, 53).

To provide a standard stimulus for the ecbolic function of the exocrine pancreas, pancreozymin has provided a valuable tool for the gastroenterologist. This would appear to be of particular importance where enzyme output is to be critically appraised, as in malnutrition.

(2). Nervous control.

(a). Parasympathetic. Pavlov and his pupils were the first to show that the vagus is the secretory nerve to the pancreas.(294). It is of interest that from the earliest experiments difficulties were experienced in the interpretation of these results. Stimulation of the vagus also causes the stomach to produce an acid secretion which in turn may enter the

duodenum and stimulate the pancreas by hormonal mechanisms. Later experiments by Pavlov, using dogs with the pyloric antrum occluded, showed that the vagus does stimulate the pancreas to produce a secretion low in volume and rich in enzymes - it has an ecbolic effect.(21). This has subsequently been confirmed.(151). With neurogenic stimulation (both parasympathetic and sympathetic) there are also the difficulties of fluctuating blood pressures, local vasoconstriction or vasodilatation, cerebal cortical factors and the effect of anaesthesia; these are all important variables which make scientific interpretation of the results difficult, particularly in humans.

Histological depletion of pancreatic cell granules after stimulation of the vagus has been demonstrated.(168, 331). Atropine inhibits this effect, but does not affect the action of pancreozymin.(168). The effect of vagal stimulation on the rate of secretion is not important. (166).

It is well known that parasympatheticomimetic drugs stimulate the pancreas to produce enzymes.(74, 98, 86, 117, 184, 189, 192, 240, 248, 306, 307, 322, 340, 375). This provides a convenient way of stimulating the gland. They do not affect volume flow to any degree.(248). Their action on the pancreatic cell has been investigated in vitro showing an increased respiratory rate and an increase in enzyme output.(86, 98). Similar findings have been found in in vivo studies; after an initial measurable loss of pancreatic protein, the extent of C^{14} labelled amino-acid incorporation into pancreatic protein is increased 4 hours after pilocarpine injection.(117).

The consensus of opinion is that vagotomy does not detrimentally affect pancreatic secretion, provided the normal hormonal mechanisms are operative.(9, 30, 80, 104, 316). However, the pancreatic response to induced

hypoglycaemia is depressed in these cases, probably because of gastric hypo-acidity with resulting failure of hormone stimulation from the duodenum.(104, 299, 316, 326).

Some inhibitory fibres have been described in the vagus (81, 355), but these are of minor importance.

In practice, Werner and Mutt have tried to minimise the effect of vagal stimulation during pancreatic function tests in children by ensuring adequate sedation.(403). An argument could be advanced for the use of atropine in this respect.

Although the parasympathetic nervous system stimulates the pancreas, the difficulty in applying a standard stimulus makes it unsuitable for the accurate assessment of pancreatic function in humans. However, because of its being controlled to some degree by involuntary higher centres, it will probably play some part in all pancreatic function tests.

(b). Sympathetic. The effect of sympathetic nervous stimuli on the pancreas is by no means clear. They have been described as having an effect similar to that of the vagus (32, 331), their extirpation resulting in decreased enzyme and bicarbonate output.(336). Harper and Vass found sympathetic stimulation to be either inhibitory or without effect. (166). The present position regarding the effect of the sympathetic nervous system on the pancreas is summed up by Gregory who claims that "nothing can be said as to the possible physiological significance of these fibres".(151).

CHAPTER 3.

The assessment of exocrine pancreatic function.

The investigation of pancreatic function in human protein-calorie malnutrition has already been discussed (page 6). In this chapter improvements on the older methods are briefly considered and newer techniques are presented, constantly bearing in mind their application to children with P-C.M.

A. The analysis of duodenal aspirates.

In chapter 1 the need for separate aspiration of duodenal and gastric juice is stressed, and the desirability of a standard stimulus to the pancreas discussed. It is important, however, to determine whether these modifications are possible in sick children, and whether the information gained by doing more elaborate pancreatic function tests justifies the time and trauma to both patient and investigator.

(1). Dual aspiration techniques.

This has been recommended in adults by Lim since 1924 . (247). Véghelyi recorded the successful use of a dual tube technique in children in 1949.(375). Jones thought this modification unnecessary (209), but his opinion is heavily outweighed by that of modern authorities who recommend simultaneous continuous aspiration of both gastric and duodenal juices by separate tubes. (3, 95, 53, 74, 107, 261, 352). The rationale behind the use of this technique is discussed on page 7 . The duodenal intubation is the troublesome procedure as far as the investigator is concerned; simply passing a gastric tube does not add to the difficulty of the procedure in most cases. The method has been used successfully in children with no recorded ill effects. (131, 267, 375).

(2). Pancreatic stimulation.

(a). Secretin. Chiray is reported as being the first to use secretin for the study of pancreatic secretion in man in the year 1926.(65). Since then this has been recognised as a major factor in the assessment of pancreatic function (95, 107) and has been used by all the leading authorities on pancreatic function in adults. (3, 95, 53, 74, 107, 261, 352). Secretin has rarely been used in children and in most cases on limited numbers of patients with various gastrointestinal disorders. (75, 131, 255, 376, 403). In fibrocystic disease of the pancreas, there is no response to secretin stimulation (131, 255), whereas other children showed a characteristic response. The details of volume output are very scanty in all the reported series. Secretin is safe for routine use although occasional reactions have been reported.(page 43). As the dose of secretin is calculated proportional to body weight, children may be given the standard pancreatic stimulus.

(b). Pancreozymin. This is not used as commonly as secretin in the assessment of pancreatic function. Reactions to this hormone are more common than with secretin (page 46) and it has been doubted whether the test yields any additional information to that provided by the secretin test.(412). However, many investigators use this stimulation as part of their routine pancreatic function tests in adults (26, 27, 53, 107, 139, 261, 352) in combination with secretin; it is not used on its own. Where the ecbolic function of the pancreas is being particularly investigated (as in P-C.M.) it would appear to be of importance as it is primarily a stimulant of ecbolic function. Only one report could be found in which pancreozymin was used in children.(403). This was done on small mentally defective children using a single tube for aspiration; these showed the expected response with no ill

effects recorded. Most investigators administer secretin first, followed by pancreozymin.(26, 53, 107, 261). Some inject pancreozymin first.(139, 352). The volume response to pancreozymin is variable, so in children where small volumes are expected, it is logical to give secretin first followed by pancreozymin. The usual time taken for the secretin test is 80 minutes (95, 106), so if one injects the pancreozymin before this time has elapsed, there should be a combined hydrelatic and ecbolic effect on the pancreas. Using pancreozymin also enables the investigation of biliary function owing to the presence of cholecystokinin in the pancreozymin preparation.(262).

These tests probably offer the most direct information concerning pancreatic function, as all the other tests involve one or more additional variable factors.

(c). Parasympathetic effect. Parasympatheticomimetic drugs have been used for their ecbolic effect on the pancreas.(74, 304, 375). However, it is difficult to standardise this stimulus, and the effect is not localised to the pancreas. With the availability of pancreozymin, there is no need to resort to these drugs. Insulin-induced hypoglycaemia has also been used to stimulate the pancreas in man. (124, 126, 299, 300). Here again, the action is non-specific, but in addition the effects of hypoglycaemia are potentially dangerous, particularly in malnourished children. (224).

(d). Enzyme determinations after a test meal. This method has been suggested as a physiological approach to the assessment of pancreatic function. (252, 381). However, in malnourished children who have a very variable gastric emptying time and who more often than not have diarrhoea, this assessment would in all probability yield results of dubious significance.

B. Tolerance Tests.

This method of investigating pancreatic function has been discussed on page12 . Here again, the diarrhoea and liver disease found in malnourished children would tend to invalidate this method.(66).

C. Serum Enzymes.

The value of serum enzyme determinations in the assessment of pancreatic function in P-C.M. has already been discussed (page 13). The view expressed is in agreement with that of Sherlock that the determination of enzymes in blood is an unreliable parameter.(69). Similar criticism applies to the determination of enzymes in urine.(105, 412).

D. X-ray Studies.

(1). Straight x-ray abdomen.

This is of value in the determination of pancreatic calcification in all age groups (28, 152, 207, 309, 332); many of these reported cases were believed to be a result of malnutrition. This sign always indicates advanced pathology. If an x-ray is taken during the course of a routine pancreatic function test to determine the position of the gastric and duodenal tubes, the x-ray should be examined critically for calcification of the pancreas. The duodenal tube usually outlines the head of the pancreas.

(2). Opacification media.

Experimental studies in dogs have indicated that zinc is concentrated by pancreatic acinar cells.(333). The rationale behind this method is to prepare a halogenated, soluble and non-toxic zinc compound in which the halogen acts to produce radio-opacity and the zinc to direct the compound to the pancreas. As yet this is not a functional proposition mainly owing to

heavy metal toxicity.

(3). Pancreatic visualisation with retropneumoperitoneum.(275), and pancreatography (111) are procedures being developed for the detection of tumours of the pancreas. They have no place in the investigation of the pancreatic atrophy of P-C.M.

E. Radio-isotope studies.

(1). Scanning techniques.

The pancreatic uptake of methionine is great owing to its rapid protein turnover. The sulphur in the methionine molecule may be replaced by radio-active selenium - 75 to produce selenomethionine; the uptake of this compound by the pancreas may be determined by scanning. (40, 421). Unfortunately the liver also takes up methionine and sometimes obscures the picture; this uptake by the liver is increased in starved patients.(40) The use of radio-active studies in children must always be viewed very critically. The information derived from such a test in a malnourished child may show an atrophic pancreas; the uptake of selenomethionine may be increased or decreased and its functional significance is questionable. Furthermore, the presence of an enlarged liver in children with kwashiorkor would lead to pronounced interference. This method of investigation is, therefore, not favoured in such cases. A similar test is being attempted using labelled zinc, but this has not met with much success.(273).

(2). Absorption of iodine-^{131}.

Triolein labelled with I-^{131} may be fed to patients, and the residual radio-activity measured in the stool. The difference will have been absorbed, this necessitating the action of lipase to split the triolein. (64, 347). Besides the disadvantage of working with radio-active material,

this test relies to a large degree on normal absorption. The presence of severe diarrhoea is thus a contraindication to its use, thus making it an unreliable test in children with P-C.M.

F. Stool examination.

(1). Microscopy.

The presence of fat, starches or muscle fibres in the stool in the absence of other gastrointestinal disorders suggests pancreatic exocrine insufficiency.(170). The stools described in kwashiorkor fit this description (368), but this is a non-specific finding, particularly in the presence of severe intestinal hurry. Steatorrhoea usually precedes creatorrhoea in severe pancreatic dysfunction.(175).

(2). Faecal trypsin.

This has been described as a screening test for pancreatic deficiency (170) and used in the investigation of the diarrhoea of P-C.M. by Altmann.(10). The results correlate poorly with duodenal trypsin assays, and the test is non-specific.(208). It is thus of doubtful value for scientific investigations.

G. Glucose tolerance test.

This test detects involvement of the Islets of Langerhans secondary to destruction of the exocrine portion of the pancreas (i.e. when it is used as a test of exocrine pancreatic function and not in the diagnosis of diabetes mellitus per se). It is used by many investigators (26, 75, 175) but is by no means the most sensitive index of exocrine pancreatic function. As the Islets of Langerhans are typically not affected in the pancreatic atrophy of acute P-C.M. (page 17) this test is of little value in the assessment of exocrine

pancreatic function in this condition in small children.

Considering all the available methods for measuring exocrine pancreatic function in children with P-C.M., the most suitable appears to be the measurement of pancreatic response to a standard stimulus (using secretin and pancreozymin), using a dual tube technique for the collection of the juice, and accurately measuring the output of enzymes, bicarbonate and the volume of juice.

Summary of Review.

The general concept of protein-calorie malnutrition (P-C.M.) has been reviewed with particular reference to pancreatic function and the gastro-enterological disturbances associated with this syndrome. Study of the literature has revealed that a diet deficient in protein or certain essential amino-acids may lead to functional and structural disturbances in the pancreas. This has been demonstrated in children with P-C.M. and in protein depleted experimental animals.

The methods employed for the investigation of pancreatic function in malnourished patients have, however, been relatively crude according to present day standards. In studies on the duodenal aspirate the concentration of a limited number of enzymes were estimated under basal conditions; adequate drainage of the stomach was not performed, and a standard hormonal stimulus was not applied. No information could be found concerning the volume and bi-carbonate output from the pancreas in malnourished children, and the effect of prolonged malnutrition has not been investigated. In adults, well stan-dardised pancreatic function tests are described and extensive experience has been gained in their use.

The high fat content of the liver in children with kwashiorkor has been associated with pancreatic deficiency. It would appear that the association arises because both these organs have a rapid protein turnover and are thus sensitive to protein deficiency.

From the literature on the physiology of the pancreas information on the action of enzymes, their production by the pancreas and their secretion into the duodenum may be obtained. Factors governing the fluid and electrolyte secretion of this organ are also presented. This knowledge may be applied

to the study of pancreatic function in P-C.M.

Numerous methods are available for studying the pancreas, but few may be successfully applied to the study of pancreatic function in children with P-C.M. From the available information, the most suitable technique in this condition is that using the aspiration of both gastric and duodenal juices and stimulating the pancreas with secretin and pancreozymin.

SECTION 2.

ORIGINAL INVESTIGATIONS.

CHAPTER 4.

Aims of the Investigation.

A. Pancreatic Function Tests in Children.

(1). Technique.

A test was evolved to assess exocrine pancreatic function in children; the technique was safe and sufficiently reliable for the satisfactory interpretation of the results obtained. Duodenal and gastric juices were aspirated simultaneously by continuous suction, and the duodenal juice was collected under basal conditions and after pancreatic stimulation with secretin and pancreozymin. In this way, both the ecbolic and hydrelatic responses of the pancreas could be assessed.

(2). Enzymes.

The activity of a broad spectrum of enzymes was assayed in the duodenal aspirate; these were amylase, lipase, trypsin, chymotrypsin and ribonuclease. Methods were modified for the determination of these enzymes. By stimulating the pancreas with hormones the most sensitive enzyme in the assessment of the ecbolic pancreatic function in children suffering with P-C.M. was sought.

(3). Volume.

The dual-tube technique used permitted the assessment of volume output from the pancreas. In this way the hydrelatic effect of secretin on the pancreas could be estimated. The relation between volume output and the body weight of the patient was investigated.

(4). Bicarbonate.

The bicarbonate output from the pancreas could also be estimated

using a dual-tube technique. As only a small volume of juice could be obtained after the standard stimulation in children, and most of this juice was used for the enzyme assays, the pH of the juice was measured as a reflection of the bicarbonate concentration.

(5). Colour Index.

Cholecystokinin being present in the pancreozymin preparation used in the tests, the colour index of the juice was measured; this gave an indication of the contractability of the gall-bladder and of the pigment in the bile .

B. Pancreatic Function in Normal Children.

To establish normal values for the pancreatic function tests(PFT's)as indicated previously, a group of children who were in good health and in a satisfactory nutritional state were examined. These children provided some standard with which the sick children could be compared before and after treatment.

C. Pancreatic function in kwashiorkor.

Children suffering with acute kwashiorkor were subjected to PFT's to investigate the functional capability of the pancreas in this condition.

D. Pancreatic function in marasmus.

Children with nutritional marasmus in a similar age group to those with kwashiorkor (as mentioned above) were investigated using the same PFT technique.

E. Pancreatic function after the treatment of acute P-C.M.

The ability of the pancreas to recover its exocrine function after a

nutritional insult was assessed by repeating the PFT's after cure had been initiated in these patients. The rate of recovery was also investigated in a limited number of patients.

F. Pancreatic function in children with chronic prolonged malnutrition following an acute episode of P-C.M.

Children who had been hospitalized with kwashiorkor 6 to 7 years previously, and who are known to have been chronically malnourished since their discharge from hospital, were investigated. A limited number of these children who were among those found to have abnormal PFT's were reinvestigated after dietary repletion in order to determine whether they would recover their pancreatic function.

G. Pancreatic calcification.

As part of the PFT an x-ray of the patient's abdomen was taken. This was critically examined for evidence of pancreatic calcification.

H. Microscopy of the duodenal aspirate.

A specimen of duodenal juice was examined under the microscope in every child to determine the presence of any helminths, flagellates, ova, fungi, cells or crystals.

I. Pancreatic histology in P-C.M.

A retrospective study of pancreatic histology was made using sections obtained from another group of children who died with P-C.M. and who came to autopsy. Differences between marasmus and kwashiorkor were looked for as was the presence of pancreatic atrophy and a fatty liver.

CHAPTER 5.

Clinical Material and Ward Management.

A. Clinical Material.

(1). Kwashiorkor.

Fourteen children with this syndrome were selected from patients attending the Outpatients Department of the Red Cross War Memorial Children's Hospital, Rondebosch, Cape. (Table 2). Every child was examined personally by the investigator, and the criteria for the diagnosis of kwashiorkor were:- (a) underweight for age; (b) the presence of clinically detectable oedema, and (c) the presence of skin lesions (hypo- or hyperpigmentation, "crazy-paving", flaking or ulceration). Additional features such as psychomotor changes, sparse, thin or discoloured hair, hepatomegally, "moon-face", anaemia and associated infections weremoted but not considered necessary for the diagnosis to be made. (48, 206). A full general and dietary history was taken from the mother in every case. The urine was examined for protein and a slide of the urinary sediment was examined microscopically. Each child was submitted to an x-ray of the chest.

Children were excluded from this study who showed evidence of having:- (a) an illness too severe to permit the performance of pancreatic function tests (PFT's); (b) a chest x-ray showing any evidence of tuberculosis or the presence of a pneumonia; (c) renal disease; (d) a history of recent contact with or clinical evidence of any infectious disease; (e) herpes stomatitis; (f) dehydration estimated as equivalent to more than 5% of the body weight; and (g) the presence of meningitis.

There were an equal number of males and females and their mean age was 25 months (standard deviation (S.D.) 10.10). All these children

TABLE 2.

CLINICAL DATA - KWASHIORKOR.

Patient No.	Age Months	Sex M or F	Oedema o to +++	Skin Lesions o to +++	Weight Kg.	% Expected Weight %	Height inches	% Expected Height %	Albumin g. per 100 ml.	Haemoglobin g. per 100 ml.	Packed Cell Volume %
1	22	M	+++	++	9.665	80.54	30.375	89.34	1.31	10.60	26.75
4	26	F	++	+	6.920	54.92	27.500	79.14	1.65	9.25	30.00
7	20	F	+++	++	9.670	84.09	29.000	89.23	1.70	9.75	26.50
9	18	F	+++	+	6.370	57.91	27.750	86.72	1.82	13.05	38.50
10	48	F	+++	+	9.900	60.00	31.750	77.44	1.73	13.90	39.00
14	24	M	+	+++	8.910	73.03	29.500	85.51	1.54	12.20	40.75
15	22	M	+++	++	6.970	58.08	26.875	79.04	0.70	9.55	25.50
16	18	M	+	+	9.520	84.25	30.250	93.80	1.86	10.45	30.00
17	45	M	++	+	10.280	66.32	31.625	79.56	1.27	10.45	33.00
18	10	F	+	++	5.360	59.56	24.625	87.95	1.93	9.80	30.50
23	25	F	++	+++	6.750	54.44	27.375	79.93	1.16	7.25	25.00
27	28	F	++	+	9.180	71.16	30.750	86.62	2.61	9.80	31.50
31	25	M	++	++	9.120	72.38	30.125	86.69	2.27	11.50	35.50
35	20	M	+	+	9.470	80.94	29.625	89.77	1.81	10.70	35.05
Mean:-	25	7 M 7 F	++	++	8.435	68.40	29.080	85.05	1.67	10.59	31.97
S.D. ± :-	10.10				1.595	11.07	2.017	5.09	0.47	1.67	5.19

had both oedema and skin lesions. Their mean weight was 8.435 kg.(S.D.1.595), and their mean percentage expected weight (expressed as a percentage of the 50th Boston percentile) was 68.40%, (S.D. 11.07). Only 2 children had weights above the 3rd Boston percentile weight for age; both these children dropped well below this level after they had lost their oedema. Their mean height was 29.08 inches (S.D. 2.017) and their mean percentage expected height (also expressed as a percentage of the 50th Boston percentile) was 85.05% (S.D. 5.09). The serum albumin concentration was depressed in every child, the mean value being 1.67 grams per 100 ml. (S.D. 0.47). Compared with the "control" group this was a highly significant difference ($P < 0.001$). Anaemia was not a striking feature in this group; the mean haemoglobin value was 10.59 g. per 100 ml. This concentration was not statistically significant compared to that of the "control" group ($P > 0.05$).

(2). Marasmus.

Seven children with this syndrome were selected from the same source as those with kwashiorkor. Similar criteria to those stated were applied in excluding children from this group, but in addition children who were too small (< 4.1 kg.) and/or too young (< 10 months) were also excluded. The diagnosis of marasmus was dependant upon the following features:- (a) underweight for age; (b) gross wasting with absence or virtual absence of subcutaneous tissue; and (c) the absence of oedema and skin lesions.(206).

Details of the clinical data on these patients may be found in Table 3. Their mean age was 20 months (S.D. 9.79), and mean weight 6.004 kg. (S.D. 1.287). This weight represents 52.61% of their expected weight (S.D. 7.32), which is significantly less than that of the "controls" ($P < 0.001$) and the children with kwashiorkor ($P < 0.01$). It is interesting that their percentage expected height of 88.11% (S.D. 6.74) was not significantly less than

TABLE 3.

CLINICAL DATA - MARASMUS.

Patient No.	Age Months	Sex M or F	Oedema o to +++	Skin Lesions o to +++	Weight Kg.	% Expected Weight %	Height inches	% Expected Height %	Albumin g. per 100 ml.	Haemoglobin g. per 100 ml.	Packed Cell Volume %
2	15	M	0	0	6.385	60.24	29.250	94.35	1.88	6.25	20.0
3	23	F	0	0	4.900	40.83	25.500	75.56	2.49	8.35	25.5
8	10	M	0	0	5.110	54.95	27.250	95.61	2.71	12.20	34.5
26	39	F	0	+	7.820	52.48	32.875	84.84	2.16	11.55	35.0
29	25	F	0	0	7.430	59.92	30.750	89.78	1.60	4.84	18.5
46	13	F	0	0	4.442	44.87	25.750	86.55	-	6.50	-
47	17	F	+	0	5.940	55.00	28.375	90.08	2.05	8.15	25.0
Mean:-	20	2M 5F			6.004	52.61	28.540	88.11	2.15	8.26	26.4
S.D. ±	9.79				1.287	7.32	2.674	6.74	0.40	2.74	7.01

the children with kwashiorkor ($P > 0.20$). The mean serum albumin concentration of the patients in this group was 2.15 g. per 100 ml. (S.D. 0.40), this being significantly lower than the "control" children ($P < 0.001$), and although generally higher than the children with kwashiorkor, this difference was not statistically significant ($P > 0.05$). The children with marasmus were significantly more anaemic than those with kwashiorkor ($P < 0.05$), the mean haemoglobin concentration being 8.26 g. per 100 ml. (S.D. 2.74). One child had minimal oedema, and one other had mild "crazy-paving" of the skin over her legs; in all other respects, these two children had the typical features of marasmus.

(3). Assessment of cure.

The criteria for the initiation of cure in children with kwashiorkor were basically those defined by Brock et al.(49). There was a marked improvement in their general condition; their appetites improved; their oedema disappeared followed by a steady gain in weight; the skin lesions were healed; the psychomotor changes were reversed and the children became playful and happy.

As may be seen in Table 4, eleven children were submitted to repeat PFT's after cure had been initiated. By this time their mean serum albumin concentration had risen to 3.78 g. per 100 ml. (S.D. 0.27), which was almost identical to that of the "control" children. The two patients who had a serum albumin concentration of < 3.5 g. per 100 ml. at the time of repeat testing were both children who had to be discharged prematurely because of the presence of an infectious disease in the ward. The time taken for this improvement to be clinically established ranged from 23 to 40 days. Two children who were admitted with a diagnosis of marasmus were also subjected to repeat PFT's after partial recovery.

TABLE 4.

CLINICAL DATA - KWASHIORKOR AFTER TREATMENT.

Patient No.	Test No.	Age Months	Sex M or F	Weight Kg.	% Expected Weight %	Albumin g. per 100 ml.	Haemoglobin g. per 100 ml.	Packed Cell Volume %	Time Treated Days	Giardiasis Treatment Yes or No	Diet
1	5	23	M	9.160	74.47	3.84	11.90	36.00	35	Yes	M, C
7	12	21	F	9.800	83.76	3.69	7.40	22.50	28	Yes	M, CR
10	13	49	F	11.070	66.29	3.69	10.35	31.50	23	Yes	M, CR
14	19	25	M	9.025	71.63	4.08	10.75	34.00	26	No	M,C,Mx
16	20	19	M	9.865	85.78	3.54	8.65	27.00	28	Yes	M,C,CR
15	21	23	M	6.480	52.68	3.44	8.10	19.50	31	-	M,C,CR
17	22	46	M	11.395	72.12	3.31	10.40	31.00	32	No	M,C,Mx
27	34	29	F	10.920	82.73	4.21	10.80	33.50	23	No	M, Mx
23	37	26	F	9.410	74.68	3.86	12.30	37.50	42	Yes	M, C, Mx
31	44	27	M	11.775	91.28	3.96	11.35	31.50	48	No	M, Mx
35	45	21	M	11.675	98.11	3.91	10.80	33.75	37	No	M, Mx
Mean:-		28	7 M 4 F	10.052	77.59	3.78	10.25	30.70	32		
S.D. ±		10.05		1.56	12.54	0.27	1.56	5.57	7.88		

Diet:- M = Milk, C = Casilan Cream, CR = Chicken and Rice, Mx = Mixed diet.

(4). "Five-year follow-up" children.

These children drawn from a similar cross section of the population as the children with kwashiorkor and marasmus, are part of a study being undertaken on the long term effects of protein-calorie malnutrition. They all had kwashiorkor 6 to 7 years ago and were all hospitalized for the treatment of this condition. Five to six years after their discharge from hospital, their nutritional status was re-assessed and found to be unsatisfactory. Their dietary history and social conditions revealed that they had been chronically malnourished. The mean age of these children at the time of testing was 102 months (S.D. 11.14).

Their poor nutritional state was reflected in their weight which, expressed as a percentage of their expected weight, was 67.99% (S.D. 12.71) of normal; this weight deficit was similar to that of the children with kwashiorkor. Their growth was also stunted as they only attained a height which was 85.57% of that expected for their age (S.D. 5.83). The serum albumin concentration in these children was also depressed, but was not significantly lower than the "controls" ($P > 0.10$). None of these children was anaemic, their mean haemoglobin concentration being 12.93 g. per 100 ml. (S.D. 0.86). Details of these clinical data may be found in Table 5.

(5). "Controls".

The children used as controls were drawn from a number of children who were part of a separate field study. These children had been fed a nutritious dietary supplement since birth. They were visited every week by a senior social worker, and had close medical supervision.

As will be seen from Table 6, eight tests were performed on 7 children in this group. Their mean age was 23 months (S.D. 5.09), which fell between the mean ages of the children with kwashiorkor and those with marasmus.

TABLE 5.

CLINICAL DATA - " 5-YEAR FOLLOW-UP".

Patient No.	Age Months	Sex M or F	Weight Kg.	% Expected Weight %	Height inches	% Expected Height %	Albumin g. per 100 ml.	Haemoglobin g. per 100 ml.	Packed Cell Volume %	Period since kwashiorkor Years
39	97	M	19.574	78.30	44.750	89.95	4.02	13.93	41.50	7
40	95	M	15.692	63.79	40.500	82.23	3.40	13.45	41.50	7
41	103	F	20.474	76.97	45.875	89.51	3.77	12.28	33.25	6
42	88	M	19.012	82.66	44.375	93.52	2.66	12.15	36.50	6
43	103	M	22.556	86.42	45.000	88.45	3.43	12.65	40.00	7
49	120	M	16.650	55.13	-	-	3.76	14.13	41.00	7
50	118	F	14.773	48.28	39.750	73.61	2.99	12.75	39.00	7
51	108	F	17.727	63.54	43.250	82.78	3.14	13.20	40.00	7
52	100	F	14.777	57.05	42.500	83.74	3.69	13.40	39.50	7
53	87	M	15.455	67.79	41.000	86.32	3.03	11.35	34.00	6
Mean:-	102	6 M 4 F	17.669	67.99	43.00	85.57	3.39	12.93	38.63	6.7
S.D.± :-	11.14		2.662	12.71	2.19	5.83	0.43	0.86	3.01	

TABLE 6.

CLINICAL DATA - "CONTROLS".

Patient No.	Age Months	Sex M or F	Weight Kg.	% Expected Weight %	Height inches	% Expected Height %	Albumin g. per 100 ml.	Haemoglobin g. per 100 ml.	Packed Cell Volume %
25	21	F	9.625	82.26	30.375	92.05	4.16	12.75	37.50
28*	21	M	12.824	107.76	30.750	91.79	3.41	13.50	37.00
30	21	M	10.720	90.08	32.000	95.52	3.83	9.30	30.00
32	16	F	9.930	93.68	29.000	93.55	3.07	11.35	32.00
33	27	F	10.400	81.25	35.500	101.43	4.10	14.18	40.00
36	25	M	11.080	87.94	32.500	93.53	4.07	12.85	37.25
38	33	F	12.964	92.60	35.500	96.60	3.75	11.20	34.00
48*	22	M	14.006	116.72	32.250	94.85	3.40	11.30	33.00
Mean:-	23	4 M 4 F	11.444	94.04	32.23	94.92	3.72	12.05	35.09
S.D.$^{+}_{-}$:-	5.09		1.609	12.31	2.32	3.10	0.40	1.56	3.36

* Same patient

Most of these children had attained a satisfactory weight for their age, the mean being 11.444 kg. (S.D. 1.609). This corresponded with 94.04% (S.D. 12.31) of their expected weight for their age. Their mean height was 32.23 inches (S.D. 2.32) and their mean percentage expected height was 94.92% (S.D. 3.10). The mean serum albumin concentration in these children was 3.72 g. per 100 ml. (S.D. 0.40). Only one child in this group had a haemoglobin concentration of $<$ 10 g. per 100 ml; the mean haemoglobin concentration was 12.05 g. per 100 ml. (S.D. 1.56). Two children in this group had a serum albumin concentration which was slightly lowered, and another 2 children tended to be underweight for age. In other respects they were physically well and they were thus included in the "control" group.

B. Ward Management.

(1). General.

The children were all admitted to the metabolic research unit at the Red Cross War Memorial Children's Hospital. Their medical care was directed by the investigator, advice being also given by other members of the research group. Nursing care was ably provided by nurse aids, who are specially trained in metabolic work and who perform no other nursing duties besides those of the unit; an experienced trained sister was in charge of the ward of which the unit forms part. Patients were all seen at least twice a day by the investigator. They were weighed daily, and their serum proteins, haemoglobin and haematocrit were checked weekly. All patients had their stools examined microscopically and cultured for pathogenic organisms. Schooling facilities were available for the older children who were hospitalized for any length of time.

(2). Diet.

(a). Children with kwashiorkor and marasmus. On the day of admission feeds of ½ strength Darrow's and 2½% dextrose solution were given in the standard quantity of approximately 150 ml. per kg. body weight per 24 hours. Occasionally similar fluid was given intravenously in moderately dehydrated patients. From midnight on the night prior to the PFT they received tap water only. Following the test, feeding was commenced with a mixture of full cream milk and ½ strength Darrow's and 2½% dextrose solution, the concentration of milk being gradually increased until full strength milk was reached approximately 5 to 7 days after admission. Sucrose was added to these feeds. In some children where diarrhoea was persistent, a lactose free formula (Casilan cream) was administered, often with a dramatic effect in controlling the diarrhoea. When solid feeds could be taken, usually 10 to 14 days after admission, these were given as a full mixed diet; milk was also given in generous quantities in addition to the mixed diet. In a limited number of patients (Table 4) a diet consisting of chicken, rice and milk was given.

(b). "Five-year follow-up" children and "controls". These children were offered a normal diet on the day of admission until 6 p.m. From then on they were given ½ strength Darrow's and 2½% dextrose solution until midnight, and then tap water only until 6 a.m. They were discharged to their homes on the day of the test or the following day. The 2 children in this group who were followed up over a longer period, spent half their stay in hospital at a convalescent home where they received an adequate diet; the remainder of the time was spent in our unit where a high protein diet was given.

(3). Medication.

(a). Children with kwashiorkor and marasmus.

(i). Antibiotics and chemotherapy. All children received

actual test. The patient was restrained with hands and feet loosely held with "stockinette". A Rusch x-ray tube (size 14) was used for the intubation. The length of tube first introduced was assessed using the rough formula of 2 x the length of the sternum plus 1 inch. The tube was lubricated using KY jelly or xylocaine anaesthetic jelly; the local anaesthetic minimised the discomfort of advancing the tube. The naso-gastric route was used to introduce the tube. Once the tube was in the stomach it was strapped in position to the upper lip. The patient was then turned on his right side where he remained until the x-ray was taken the following morning. The tube was marked at intervals of approximately 1 to 1½ inches commencing at the nose, one mark for each hour of the night. (Plate 1). The night nurse then advanced the tube one mark per hour. The following morning at 8 a.m. bile was aspirated from the tube by a syringe indicating that the tube had probably entered the duodenum.

(4). Gastric intubation.

A second Rusch tube was introduced into the stomach via the free nostril.

(5). X-Ray.

One straight x-ray of the abdomen was taken in the supine position. (Plate 2). Care was taken to shield the gonads with lead rubber in every child. One x-ray usually provided the answer as to the position of the tube, but in the rare instances where there was any doubt, a lateral plate was taken or the patient was screened under an image intensifier. Minor adjustments to the positions of the tubes were sometimes necessary to ensure that the tip of the duodenal tube was between the 3rd part of the duodenum and the duodeno-jejunal flexure, and that the gastric tube be in the dependent part of the stomach.

Procaine Penicillin 300,000 units intramuscularly daily and Sulphadiazine suspension 100 to 200 mg. per kilogram body weight daily in divided doses for 1 week. In circumstances where another antibiotic was indicated, the choice was decided by appropriate culture and sensitivity reports. Tetracycline was the commonest "second choice" antibiotic.

(ii). Supplements. All children received potassium chloride in solution, 0.5 g. being given 3 times a day. A Ferrous gluconate and vitamin preparation was given to all patients except those who received a chicken and rice diet. (A separate concomittant haematological study was performed on these patients). Some patients required intramuscular iron injections for which imferon was prescribed. Magnesium sulphate, calcium gluconate and dextrose were added to the intravenous regime of patients requiring intravenous rehydration. Ostelin forte in a dose of 600,000 units was given to children with rickets.

(iii). Miscellaneous. Coincidental infections and infestations were treated as indicated:- Piperazine citrate was given for infestation with *Ascaris lumbricoides* and mepacrine for *Giardia lamblia*; Mycostatin was used in the treatment of moniliasis; nitrofurantoin ointment was applied to impetigenous skin lesions; scabies was treated with benzyl benzoate and lice with dicophane application. Chloral hydrate was used as sedation.

(b). "Five-year follow-up" children and "controls". No medication was given to these children before the PFT was performed. Those requiring treatment for any incidental infection or infestation were treated as outlined above after the test was completed; this included treatment for giardiasis, impetigo, ascariasis and lice.

C. Problems encountered.

(1). Marasmic-kwashiorkor.

In selecting patients for this study it was found that the majority of children with P-C.M. were what could most accurately be labelled as marasmic-kwashiorkor or nutritional dwarfs. The full-blown cases of kwashiorkor were less common, but were sufficiently typical not to present a diagnostic problem. The children with marasmus, however, were difficult to select. Those fitting the typical description of marasmus were usually very young (6 months or less) and very small (4 kg. or less); this made them unsuitable for PFT's if any accurate quantitative results were to be obtained. Older children, untainted with some signs of kwashiorkor were rare. Mild skin lesions or oedema sometimes became apparent in children with typical marasmus 2 or 3 days after their admission to the ward.

(2). Infectious diseases.

During the investigation there were four outbreaks of infectious diseases (measles thrice, and varicella once). When this occurred, the ward had to be cleared and fumigated before any further investigation could continue. This led to many patients being lost to the series, particularly as regarded repeat testing after cure.

(3). Secondary pathology.

By carefully selecting the patients before admission, this factor was reduced to a minimum. One child developed an abscess in the ligamentum teres which was drained surgically and was subsequently found to have non-pulmonary tuberculosis. Repeat tests were not done on this child.

(4). Death.

Two children died during the period of investigation. These were:- (a) a child with marasmus who died in pulmonary oedema, and (b) one

child with kwashiorkor, who developed a pneumonia while in hospital and deteriorated rapidly and died. Both these children came to autopsy and the clinical diagnoses were confirmed. There was no evidence that the investigations performed contributed to the cause of death in either child.

D. Histology.

The slides for the retrospective histological review were taken from the histology records of the Department of Pathology of the Red Cross War Memorial Children's Hospital. All the autopsy notes of children coming to post-mortem examination during the years 1962 to 1965 inclusive were examined. From these a group were selected who had a clinical and pathological diagnosis of kwashiorkor or marasmus. Any child with a diagnosis of marasmic-kwashiorkor, tuberculosis, herpes stomatitis, syphilis or any secondary intraabdominal pathology was excluded. Children below the age of 10 months were also excluded. A number of slides taken from children succumbing with fibrocystic disease of the pancreas and some with normal pancreatic histology were also studied. The sections of the liver were examined in every case. Notes of the clinical, pathological and histological findings were available in every case.

Plate 1.

The first step in duodenal intubation. (Note the markings on the tube).

CHAPTER 6.

Methodology.

A. Pancreatic Function Test (PFT).

Experience was gained in performing duodenal intubations on convalescent children and by doing peroral small intestinal biopsies. The following technique was finally evolved to perform PFT's in children using a dual-tube technique and stimulating the pancreas with secretin and pancreozymin.

(1). Period of fasting.

After 6 p.m. on the day prior to the test, feeds of ½ strength Darrow's and 2½% dextrose solution only were given. These were continued until midnight from which time tap water only was given to the child. After 6 a.m. the patient was given nothing at all by mouth except for the sedation given orally at 7 a.m.

(2). Sedation.

Chloral hydrate was used for its hypnotic effect in the standard dosage of 45 mg. per kilogram body weight. The first dose was given at 10.30 p.m. on the night prior to the test; i.e. about 45 minutes before the first tube was passed. If the child was particularly restless, a second dose was given at 2.30 a.m.; this was rarely necessary. In all cases a further dose was given at 7 a.m. on the day of the test; this usually ensured that the patient slept during the period of aspiration of the juice. By maintaining adequate sedation vagal stimulation from higher centres was minimised.

(3). Duodenal intubation.

This was commenced at about 11.15 p.m. on the night prior to the

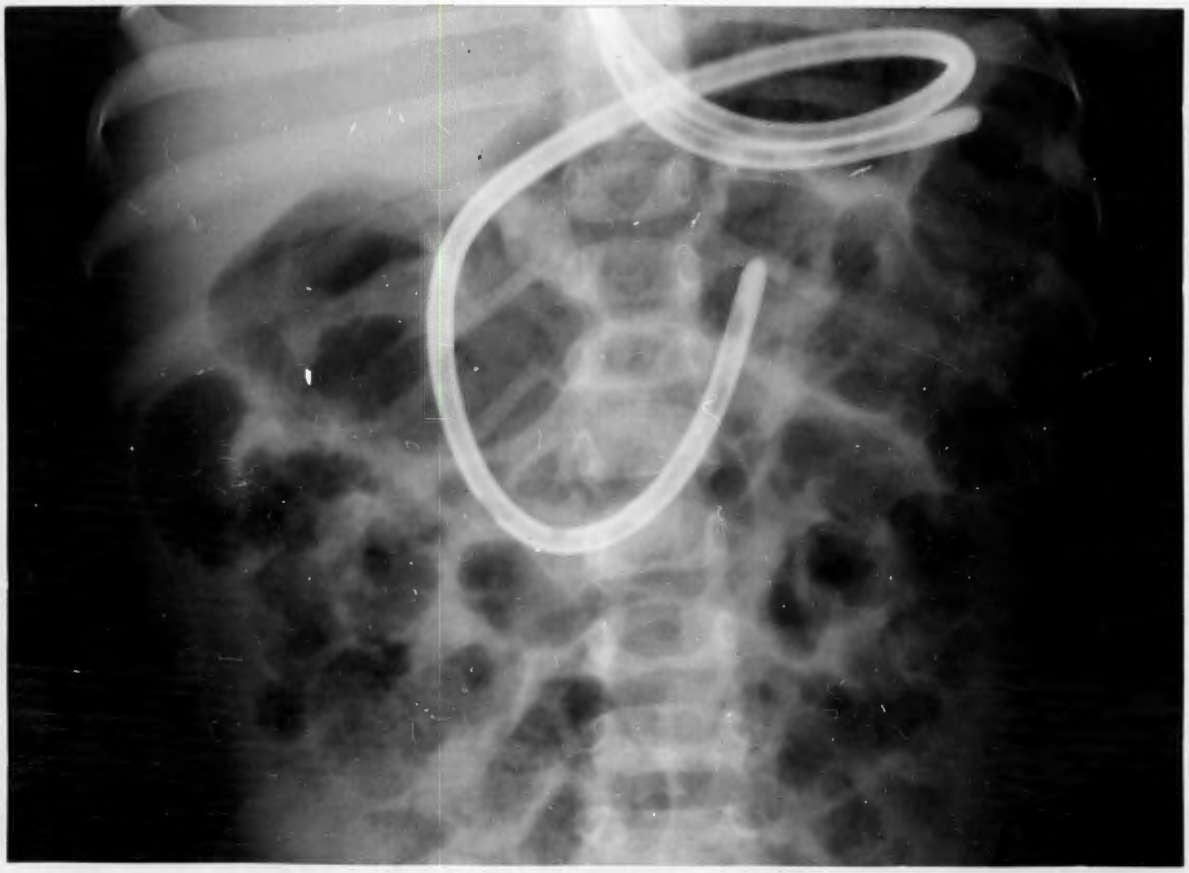


Plate 2.

X-ray of abdomen with one tube in the duodenum and the other in the stomach.

actual test. The patient was restrained with hands and feet loosely held with "stockinette". A Rusch x-ray tube (size 14) was used for the intubation. The length of tube first introduced was assessed using the rough formula of 2 x the length of the sternum plus 1 inch. The tube was lubricated using KY jelly or xylocaine anaesthetic jelly; the local anaesthetic minimised the discomfort of advancing the tube. The naso-gastric route was used to introduce the tube. Once the tube was in the stomach it was strapped in position to the upper lip. The patient was then turned on his right side where he remained until the x-ray was taken the following morning. The tube was marked at intervals of approximately 1 to 1½ inches commencing at the nose, one mark for each hour of the night. (Plate 1). The night nurse then advanced the tube one mark per hour. The following morning at 8 a.m. bile was aspirated from the tube by a syringe indicating that the tube had probably entered the duodenum.

(4). Gastric intubation.

A second Rusch tube was introduced into the stomach via the free nostril.

(5). X-Ray.

One straight x-ray of the abdomen was taken in the supine position. (Plate 2). Care was taken to shield the gonads with lead rubber in every child. One x-ray usually provided the answer as to the position of the tube, but in the rare instances where there was any doubt, a lateral plate was taken or the patient was screened under an image intensifier. Minor adjustments to the positions of the tubes were sometimes necessary to ensure that the tip of the duodenal tube was between the 3rd part of the duodenum and the duodeno-jejunal flexure, and that the gastric tube be in the dependent part of the stomach.

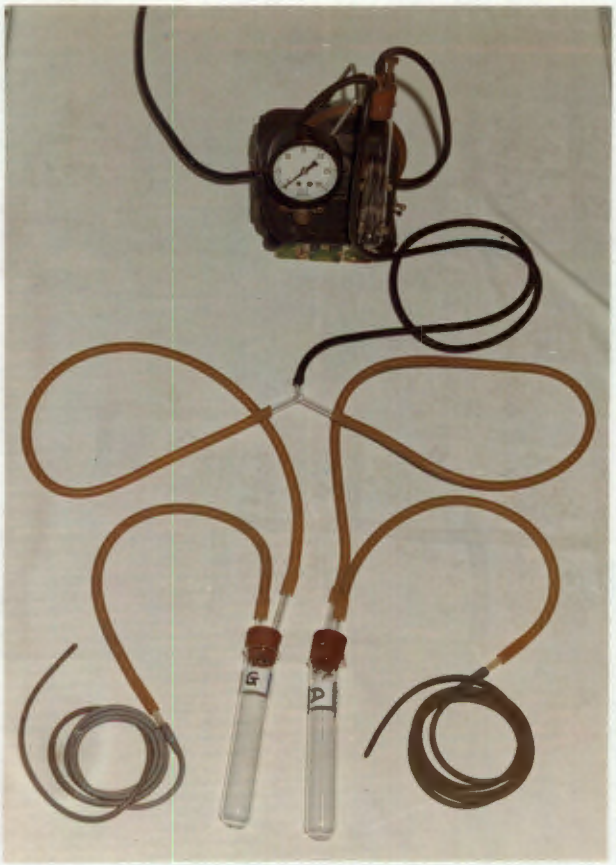


Plate 3.

Apparatus for the aspiration of duodenal juice.

(6). Aspiration of juices.

(a). Apparatus. (Plate 3). The apparatus used was very simple, consisting basically of a Wappler Stedman suction pump and a system of tubes leading to the patient. The tube system was interrupted by 2 test-tubes, the one to collect the duodenal juice and the other the gastric juice. The former was kept surrounded by ice cubes.

(b). Procedure. The patient was put down in his cot lying on his left side and restrained in this position. This was done for 2 reasons:- (i) to keep the pylorus in the highest part of the stomach and thus minimise the entry of acid gastric juice into the duodenum, and (ii) to reduce the chances of the gastric tube entering the duodenum during the aspiration period.

An intravenous infusion was then commenced using a "scalp vein set" and introducing the needle into a scalp vein or a vein in the arm or dorsum of the hand. Half strength Darrow's and 2½% dextrose solution was infused at the rate of 12 to 15 drops per minute - in this way 180 ml. of fluid was used during the whole procedure. The drip served a dual purpose; (i) to maintain adequate hydration, and (ii) as a venous pathway for the injection of secretin and pancreozymin.

Using a syringe, the duodenum and stomach were aspirated clear of all secretions. The patient was then connected to the collection apparatus and the basal aspiration commenced. A careful record of the time and all the relevant clinical observations were kept on a bedside chart. The juice was aspirated at a very low vacuum; it was attempted to keep the needle on the gauge between the first and second marks on the vacuum dial which correspond to approximately 33 and 66 cms. of water respectively. (Plate 4). Any leak in the system was immediately revealed by a drop in the

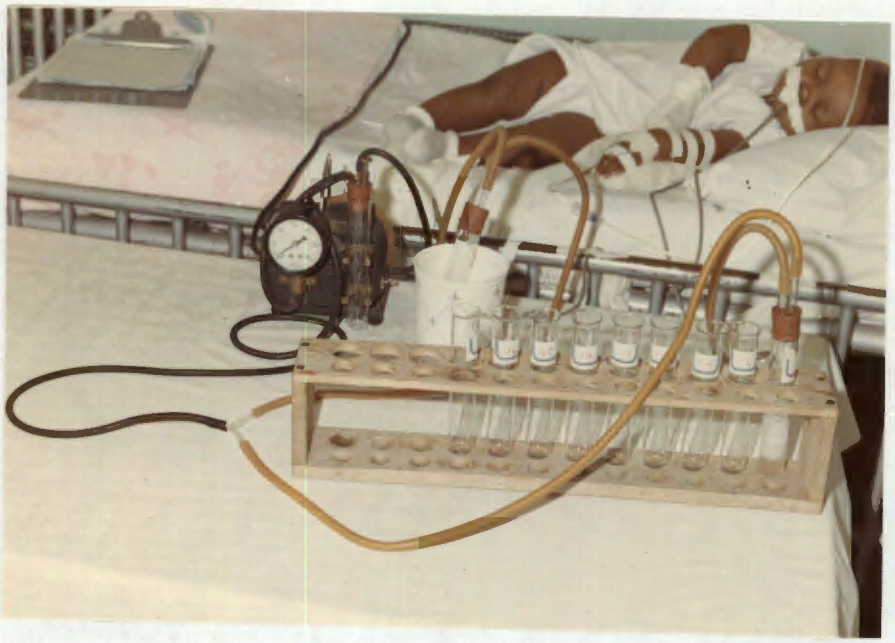


Plate 4.

A pancreatic function test in progress.

Note:- (a). The patient asleep on his left side.

(b). The drip infusion in a vein on the dorsum of the left hand.

(c). The low negative pressure on the dial of the vacuum pump.

(d). The clear gastric juice in the collecting tube at the end of the test-tube rack.

(e). The duodenal juice is being collected in a test-tube immersed in ice-chips in a separate carton.

vacuum on the gauge. If the basal aspiration was macroscopically contaminated with gastric juice, it was discarded and the collection recommenced. After sufficient basal juice has been aspirated (about 4 ml. or more), the secretin was prepared for injection.

The secretin powder (Boots Pure Drug Company) was dissolved in normal saline and the dose calculated proportional to the body weight of the patient (1 unit per kilogram body weight). This was again well diluted in normal saline. The test-tube receiving the duodenal aspirate was then changed and the secretin injected very slowly through the intravenous drip. The time of the commencement of the injection was noted. The response to secretin was usually dramatic, with an increase in the volume of the aspirated juice while the injection was still being given. A careful watch was kept for any reactions to secretin. The collecting test-tube, on ice, was changed every 10 minutes.

The investigator always remained at the bedside; if the patient became restless he was pacified quietly. The collecting tubes were repeatedly tested for patency by blowing about 5 ml. of air through them with a syringe. The collecting test-tubes were also closely watched to detect any contamination of the gastric juice with bile or vice versa. When any contamination occurred, it was corrected immediately by adjusting the faulty tube. A correction for any loss in volume was recorded on the bedside chart.

Every 10 minute-sample was measured for volume and pH immediately it was completed. Each two consecutive 10-minute samples were then pooled to give one 20-minute sample. One ml. of this juice was diluted 1 in 1 with glycerol and thoroughly mixed. The remainder of the juice was placed in a separate tube, and all the tubes placed in a beaker of chipped ice. Six 10-minute samples were collected in this way.

Pancreozymin (Boots) was prepared by diluting the crystalline

hormone in normal saline. A dose of 1.5 units per kilogram body weight was used, the hormone being very well diluted in saline. This was injected very slowly through the drip exactly 1 hour after the secretin injection; 5 to 10 minutes were taken over the injection. Constant watch was kept on the patient to detect any signs of a reaction. While the pancreozymin was being injected, the duodenal aspirate turned from a light yellow to a dark greeny-black colour. Four samples, each aspirated over a 10-minute period, were collected from the time of the commencement of the pancreozymin injection. These samples were treated in an identical manner to those collected after secretin stimulation as described previously.

Both tubes were then withdrawn. Mucus from the tip of the duodenal tube was placed on two glass slides; to the one a drop of duodenal juice was added, and to the other a drop of iodine. Cover slips were placed on the specimens and they were examined immediately under the microscope.

The intravenous drip was discontinued and the patient became free to do whatever his original clinical state would permit. The patients usually showed very little, if any adverse reactions to the test or the investigator. As soon as the discomfort of a tube in each nostril was over, and especially after being given some food, they returned to their "normal selves" with surprising rapidity.

(7). Difficulties encountered in performing PFT's in children.

(a). The clinical condition of the patient. Patients whose condition deteriorated during their first 24 hours in hospital sometimes made them unsuitable to undergo a PFT. Dehydration as a result of severe gastro-enteritis was an important factor in this respect. Intravenous fluids were administered when indicated to try to overcome this difficulty. Hypothermia is known to depress pancreatic function, so patients were kept suitably warm.

(b). Sedation. Too heavy sedation increased the failure rate of duodenal intubation. However, adequate sedation is necessary to keep the patient quiet and to dampen down the impulses from higher cerebral centres affecting nervous tone, particularly that of the vagus. Chloral hydrate proved to be a satisfactory hypnotic in most of the children and was used in all cases.

(c). Duodenal intubation. This was successfully achieved in 96% of cases using the technique described in this chapter. (124 intubations were performed). The reasons for the failure of some duodenal intubations were:- the tube coiling in the stomach (3%); and the tube coiling back on itself half in the duodenum and half in the stomach (1%). The tubes used were large in diameter and passing them through the nostrils was sometimes tricky; sodium bicarbonate solution was helpful in clearing mucus. Large tubes are unfortunately necessary for accurate volume collections. The slow gastric emptying time in some children with P-C.M. occasionally led to their vomiting their last feed when the tube was introduced. In 2 children the test had to be abandoned because of food in the duodenum on the morning of the test. One patient vomited his duodenal tube while the gastric tube was being introduced; this test was also abandoned.

(d). X-Ray. There was sometimes some doubt as to the exact position of the duodenal tube as judged by one straight x-ray of the abdomen. A lateral plate or screening the patient under an image intensifier was very helpful in this respect.

(e). Aspiration of juice. During the aspiration, particularly after the injection of pancreozymin, there was a tendency for the gastric tube to slip through the pylorus into the duodenum. By carefully watching the collecting tubes this could be immediately detected and corrected by withdrawing the gastric tube a short distance. This was prevented in most

cases by not permitting any slack tube to remain in the stomach at the commencement of the aspiration. In smaller children, the percentage of fluid lost through incomplete aspiration must of necessity have been greater than in larger children owing to the very small volume of juice produced. It was attempted to collect as much fluid as possible by aspirating at a low vacuum (about 35 to 50 cms. water) through large tubes, and by repeatedly testing the patency of the tubes with a syringe. An interesting phenomenon was seen in certain children who were aroused during the test by various stimuli; this sometimes caused an increase in the volume flow of juice and was often associated with an elevated enzyme output during the corresponding period. Nervous mechanisms may have been responsible for this, and adequate sedation would minimise this effect. On two occasions roundworms (Ascaris lumbricoides) found their way into the lumen of the duodenal tube.

(f). Stimulation. The first hurdle was the cost of secretin and pancreozymin; particularly in children where small doses are used there is much wastage, as the ampoule may only be opened once. The next problem was to inject the hormones intravenously. This was overcome by using an intravenous drip infusion technique, although this was not always easy in oedematous children with extensive skin lesions. Once the hormones were injected, there was the danger of reactions. No reactions were observed with secretin, but 32% of the children receiving pancreozymin showed some reaction. These manifested themselves as non-specific discomfort (20%), abdominal pain or cramps (8%), headache (2%) and nausea (2%). These effects were minimised by injecting the hormones very slowly and well diluted with normal saline. No reactions were of a sufficient severity to necessitate abandoning the procedure.

This list of difficulties appears to be a long and depressing

one, but with experience and a little patience they are by no means insurmountable.

B. Volume.

This was simply measured at the bedside immediately a 10-minute collection had been completed; a 1 cm. diameter graduated measuring cylinder was used for this purpose.

C. pH.

Owing to the small quantities of juice available in most cases, the pH was measured using BDH narrow gauge indicator paper. To minimise error, this was done by the same observer using the same make of paper in the same way. This estimation was performed at the bedside at the termination of the collection of each 10-minute sample.

D. Enzyme Assays.

(1). General.

All specimens were collected under ice and kept in test-tubes immersed in a beaker containing ice chips until the time of actual enzyme assay.(251). The amylase, lipase, trypsin and chymotrypsin assays were always commenced on the same day the juice was collected, usually within 2 hours of the completion of the PFT. Ribonuclease, being a stable enzyme (234), was assayed 1 to 3 days later, usually on the day following the PFT; the juice was kept at 4°C. (not frozen) during the period of storage. The colour index was estimated from 1 to 3 days after the PFT. The precaution was taken of introducing a pledget of cotton wool into the top of pipettes before use. Juice diluted 1 in 1 with glycerol was used for the amylase and lipase assays whereas pure juice was used for the other assays.

It was attempted to separate the enzyme protein from the bile-stained juice by precipitating the protein with ice cold ethanol and then resuspending it in normal saline as suggested by Lundh.(251). This procedure led to a significant decrease in enzyme activity, as was found by Bang(25), and was thus abandoned.

Existing methods were modified and established principles were applied in developing methods applicable to the determination of the main enzymes in the pancreatic juice of children, some of whom were anticipated to have a low enzyme output. The modifications to the established enzyme assay methods will be discussed in this chapter; the full detailed methodology of each test as used in this study may be found in the appendix (page 169).

(2). Amylase.

The method used was that of Pimstone (301), using a substrate of buffered starch and measuring the residual colour produced with iodine after a standard incubation period.

(a). Apparatus. A Klett-Summerson photoelectric colorimeter was employed for the final readings, using a red (No. 66) filter.

(b). Modifications. In order to adapt the method for small volumes of juice, the dilution was carried out in a different way. The juice-glycerol mixture, thoroughly mixed, was diluted 1 in 500 with 1.5% sodium chloride solution (0.1 ml. juice made up to 50 ml. with 1.5% Na Cl). If the enzyme concentration was expected to be low, 1.0 ml. of this solution was added to the starch substrate, and only 0.5 ml were added if the enzyme concentration was expected to be high. (This corresponded to the 1 in 50 and 1 in 100 dilutions respectively of the original method). This dilution was performed immediately before the enzyme assay. An indication of which dilution to use was obtained from the result of the chymotrypsin assay which

was commenced first. If the dilution used proved to be the wrong one, the test was repeated using the alternative dilution. It was found that better reproducibility of the assay was possible when a larger volume of diluted juice was added to the substrate; with the addition of only 0.1 ml. diluted juice to the substrate, it was difficult to ensure that all the enzyme reached the substrate and did not remain on the side of the flask. Fifty ml. volumetric flasks were used for the incubation. The remainder of the method was followed as suggested in the original description.

(c). Reproducibility. This was tested by performing 10 assays on the same juice under standard conditions. The mean result of these assays was 4,539 units, with a range of 4,510 to 4,560 units and a standard deviation (S.D.) of 20 units. The mean deviation from the mean was 0.4%. The accuracy of these results is exaggerated by the use of the same juice and reagents at the same time and using the same pipettes under the same environmental conditions. Under the conditions of the routine assay from day to day there would, therefore, be some greater degree of variation.

(d). Difficulties. This method was simple to perform with relatively few difficulties. However, while experience was being gained with the method, it was found that the presence of any mucus in the juice led to a wider variability in the results. Storage of the juice, particularly at room temperature, led to a fall in enzyme activity; enzyme assays were thus always performed on the day of collection and kept under ice until the time of assay.

(3). Lipase.

The method of Cherry and Crandall (63) was modified after a series of experiments designed to obtain a greater degree of accuracy using

this method. The basic principles of the method, in outline, were an incubation of duodenal juice with an olive oil emulsion, and a titrimetric determination with alkali of the free fatty acids liberated.

(a). Modifications.

(i). Dilution. The glycerol-pancreatic juice mixture was used in a dilution of 1 in 10 with distilled water (0.4 ml. made up to 4 ml.). Two ml. of this diluted juice was used for the test and 2 ml. boiled and used in the control incubation. In this way 0.1 ml. of the pure pancreatic juice was used for each part of the test.

(ii). Buffer. The buffer used was 0.05M phosphate buffer of pH 8.0. The pH was raised from 7 to 8 to enable lipase to act at its optimum pH. The quantity of buffer was increased from 0.5 ml. to 1.0 ml. to make allowance for the increased liberation of fatty acids at the optimum pH.

(iii). Substrate. The quantity of olive oil emulsion was increased from 2.0 to 2.5 ml. This ensured that there was sufficient substrate when high enzyme concentrations were being determined. Continuing the incubation for longer than 24 hours led to a slow but definite continuation of hydrolysis. Adding a greater quantity of juice resulted in more fatty acids being liberated in 24 hours.

(iv). Incubation. This was performed for 24 hours in a water bath equipped with a flask shaker - this enabled the substrate and enzyme to be thoroughly mixed during the period of incubation.

(v). Indicator. Thymolphthalein was used as the indicator during titration as suggested by Tietz.(364). The pH range of this indicator is 9.3 to 10.5 whereas that of Phenolphthalein is only 8.2 to 10.0 (272). Using thymolphthalein ensured that all the fatty acids liberated were titrated against alkali.

(vi). Titration. The fatty acids liberated from olive

oil on hydrolysis are soluble in alcohol but not in water. (272). The alkali used for the titration of these fatty acids was therefore made up in an alcoholic solution and not an aqueous one. Potassium hydroxide (0·1.N) was used, the stronger concentration giving a sharper end point to the titration.

(b). Reproducibility. As in the case of amylase this was tested by performing 10 assays on the same juice under standard conditions. The mean result of the titration was 10.10 ml. with a range from 9.60 to 10.35 ml., and an S.D. of 0.24. The mean deviation from the mean result was 1.92%.

(c). Calculation of units. In the past this has been expressed as the volume of alkali (ml.) titrated to reach the end point. An attempt was made to define a lipase unit more accurately in terms of substrate split in relation to a fixed quantity of pancreatic juice:- i.e. 1 lipase unit is present when 1 mg. of olive oil is hydrolysed by 1 ml. of juice under the standard conditions of the assay. The correction factor applied to the volume of alkali titrated was determined in the following way:-

0.1 ml. juice was used in the assay.

By definition, if this juice hydrolyses 0.1 mg. olive oil,

1 unit of lipase activity is present.

The saponification value of olive oil is approximately

193 (i.e. 193 mg. KOH will saponify 1 g. olive oil).

Therefore, 0.0193 mg. KOH will saponify 0.1 mg. olive oil.

1 ml. 0·1 N. KOH contains 5.61 mg. KOH

Therefore, 1 ml. 0·1 N. KOH is equivalent to $\frac{5.61}{0.0193}$ units, i.e. 290 units

Therefore, from the titration: Units = (T-B) X 290

where T = ml. 0·1 N.KOH titrated in test sample

and B = ml. 0·1. N. KOH titrated in blank.

(d). Difficulties. These were relatively few.

(i). Titration. The end-point of the titration was sometimes difficult to determine when large volumes of alkali had to be titrated. A blank was run for each sample and the final colour of the test sample was compared with that of the blank. The titration was performed by the same person, thus enabling a constant interpretation of the end point.

(ii). 24-hour incubation. This was long and inconvenient but was retained for the sake of accuracy. It was found that the most rapid liberation of fatty acids was during the first few hours of incubation, there being a distinct slowing of hydrolysis after 6 hours. It was reasoned that there would be less error in continuing into the time of slow activity.

(4). Ribonuclease (RNase).

The method used for the assay of RNase activity was basically that of Anfinsen et al (16) modified for the enzyme determination in biological fluids. Dialysed yeast nucleic acid was used as the substrate and the products of digestion estimated by ultra-violet light absorption after the precipitation of the residual nucleic acid with uranium acetate and perchloric acid. All the assays, standards and unknowns, were performed in duplicate.

(a). Apparatus. A Beckman model DB spectrophotometer was used for the final readings at 260 mμ.

(b). Modifications.

(i). Dilutions of pancreatic juice. By experimenting with this juice it was found by trial and error that a dilution of 1 in 5 with 0.1.M. acetate buffer of pH5 was most suitable for the assay. Higher dilutions led to significantly decreased sensitivity while lower dilutions

placed too much strain on a buffer of pH5.

(ii). Standard curve. This was determined using RNase of known activity obtained from Seravac Laboratories (Pty.)Ltd., Cape Town. Dilutions of 0.4375, 0.875, 1.75, 3.5 and 7 γ per 1.5 ml. acetate buffer pH5 were prepared and incubated at the same time as the test samples. At dilutions greater than 0.4 γ per 1.5 ml. the variability between duplicates became too great to permit satisfactory interpretation of the results for a standard. A standard curve was determined for every assay. From the standard curve it was a simple task to calculate the enzyme activity of the unknown samples in Kunitz units per ml. juice.

(iii). Temperature of incubation. As mentioned in the original method, high readings were obtained from blank tubes (containing no enzyme), particularly after storage of the substrate. The temperature for the incubation was raised to 30°C. in order to try to separate the readings obtained from the blank and the test samples containing low concentrations of RNase. At temperatures higher than 30° the rate of enzyme activity became too great for the satisfactory interpretation of results from juices of low enzyme concentration.

(iv). Period of incubation. This was raised from 25 to 30 minutes for the same reasons as those explained previously for the temperature . This was timed to the second with a stopwatch.

(c). Reproducibility. From the difference between 295 pairs of duplicate spectrophotometer readings of test samples, the standard deviation of a single determination was calculated. (For the method of calculation see appendix, (page203)). This was found to be 4.09; the mean spectrophotometer reading was 371, making this a percentage standard deviation of 1.10%.

(d). Difficulties.

(i). High blank readings. These readings became progressively higher with storage of the substrate. Fresh substrate was dialysed and prepared at regular intervals.

(ii). Calculation of results. At very low enzyme concentrations it was often difficult to read off exact values from the standard curve. Calculating the final results in Kunitz units gave such low figures that all the results were expressed in terms of milli-Kunitz units.

(iii). Non-specificity. It was found that chymotrypsin and to a lesser extent, trypsin, (at pH's of 7 and 8 respectively) also hydrolysed ribonucleic acid. However, this interference in the RNase assay was largely excluded by performing the assay at pH5 which is at a pH far removed from the optimum for trypsin and chymotrypsin and which, nevertheless, permits adequate RNase activity.

(5). Trypsin.

The principle applied in this method was that propounded by Schwert and Takenaka (325), using N - benzoyl - L - arginine ethyl ester hydrochloride (BAEE) as a synthetic substrate and measuring the esterase activity of trypsin. This method was basically similar to that employed in the Seravac Laboratories.(330). All estimations were performed in duplicate.

(a). Apparatus. A model DB Beckman spectrophotometer with a recording apparatus installed was used for the enzyme determinations. The temperature in the cuvette chamber was kept constant, at 25°C. using a Colora Ultrathermostat apparatus.

(b). Modifications.

(1). Dilution of juice. This was determined by trial and error for each sample of juice until a dilution was obtained which gave a

satisfactory range of readings. The dilutions employed ranged from 1 in 5 to 1 in 20; that of 1 in 5 was the lowest dilution used owing to the interference by biliary pigments and interference with the buffer at lower dilutions.

(ii). Control cuvette. This was set up as in the original method using 3.0 ml. buffered substrate and 0.2 ml. 0.001. N. HCL, but in addition a cuvette containing 3.0 ml. BAEE was placed in the "R" cuvette chamber of the spectrophotometer.

(iii). Readings. These were recorded directly on to the graph of the recording apparatus. At the suitable dilution chosen, a straight line was drawn on the graph indicating a steady enzyme activity. This was continued for a period of 3 minutes once the graph was following a straight course. If the graph showed no deviation at all after 6 minutes, it was assumed that there was no enzyme activity; if during these 6 minutes there was any suggestion of activity, the recorder was allowed to run for a further 3 minutes.

(iv). Standard assay. A freshly prepared trypsin solution (crystalline trypsin was obtained from Seravac Laboraties (Pty.)Ltd.) was assayed every time the test was performed. This provided a standard whereby one test could be compared with another. Comparable activities were obtained with the standard solutions.

(c). Calculation of results. One unit of activity was defined as "that activity which causes a change in optical density at 253mμ of 0.001 per minute" under the given standard conditions. The change in optical density during a 3-minute period was determined from the graph. From this the change in 1 minute was calculated. Multiplying this result by the dilution factor gave the units present in 0.2 ml. juice (0.2 ml. juice was

used for the actual assay). This result multiplied by 5 was expressed as the concentration of enzyme in units per ml. juice.

e.g.:-

Dilution	Readings	Difference	Time	Diff./min.	Units/ 0.2 ml.	Units/ml.
1 in 5	71 - 125	54	3 mins.	18.0	90	450

No attempt was made to correct these results to absolute figures in terms of micro-moles of substrate split; this correction would have involved multiplying all the results by a constant factor and this would not have affected the ultimate conclusions.

(d). Reproducibility. From the difference between 324 pairs of duplicate test sample results in units per ml. juice, the standard deviation of a single determination was found to be 59.17 units. The mean result in units per ml. juice was 1171 units, making the percentage standard deviation 5.05%. In order to test the reliability of performing the test at different dilutions, assays were performed on the same sample but varying the dilution from 1 in 10, to 1 in 11, to 1 in 12 right up to 1 in 20. The average deviation from the mean results in units per ml. juice determined from readings obtained from assays in 11 different dilutions of the same juice was 3.36%.

(e). Difficulties.

(i). Mucus. The presence of any mucus in the specimen of juice led to an irregular graph which was difficult to read. This was avoided by letting any mucus settle at the bottom of the test-tube and pipetting the juice from the middle of the fluid.

(ii). Bile. The presence of varying amounts of bile in the juice led to the readings being obtained over different ranges of optical density; however, as a difference between the initial and final readings during a fixed time period was being measured and not an absolute quantity

per se, this did not significantly affect the results.

(iii). Low enzyme concentrations. With very low concentrations of trypsin, it was sometimes difficult to detect the presence of activity; the graph sometimes had to be run for 9 minutes before any valid conclusions could be drawn.

(6). Chymotrypsin.

The esterase activity of chymotrypsin on the synthetic substrate N - acetyl - L - tyrosine ethyl ester monohydrate (ATEE) was measured as suggested by Schwert and Takenaka.(325). The procedure was basically similar to that performed in the Seravac Laboratories.(330). All estimations were performed in duplicate.

The laboratory procedure of this method was almost identical to that for trypsin. The apparatus used and the difficulties encountered were, therefore, common to both methods.

(a). Modifications.

(i). Dilution of juice. This was determined as for the trypsin assay, but it was usually found that a higher dilution to that used for the trypsin assay was necessary. A dilution varying from 1 in 5 to 1 in 40 was employed.

(ii). Control cuvette. To set the spectrophotometer at 0.200 on the optical density scale, a mixture of 0.75 ml. 0.001. N. HCL and 2.25 ml. buffered ATEE solution was placed in the "R" cuvette chamber in addition to the usual 0.2 ml. 0.001 . N. HCL and 3.0 ml. buffered ATEE in the "S" chamber.

(iii). Readings. These were recorded by the recording apparatus, the optical density decreasing at a rate proportional to the enzyme activity.

(iv). Standard assay. A standard solution of chymotrypsin (crystalline enzyme was obtained from Seravac Laboratories (Pty.)Ltd.) was assayed every time the test was performed.

(b). Calculation of results. One unit of activity was defined as "that activity which causes a decrease in optical density at 237mμ of 0.001 per minute" under the given standard conditions. The calculation of the enzyme concentration in units per ml. from the graph was identical in principle to that for trypsin.

(c). Reproducibility. In estimating the standard deviation of a single determination from all the duplicate results in units per ml. juice, all the zero results were excluded from this calculation as the numerous duplicate zero results would give a falsely high degree of accuracy. From 246 pairs of duplicate results, the S.D. of a single determination was then found to be 99.17 units, and the mean result in units per ml. juice was 3,784 units. The percentage standard deviation of a reading was, therefore, 2.62%. Testing the reliability of performing the test at different dilutions, the average deviation from the mean determined from readings obtained from the assay of the same juice in 11 different dilutions was 5.41%.

(7). Calculation of results of enzyme assays. The enzyme assays yielded results in units per ml. juice, giving the concentration of enzyme in the sample tested. Knowing the volume of juice secreted in a given time, it was possible to calculate the units of enzyme secreted in that time $\left(\frac{\text{Concentration x Volume}}{\text{Time}}\right)$ and to correct this to enzyme output in units per minute. As the bigger children produced a greater volume of pancreatic juice (page101), a correction factor was applied for the body weight in kilograms. The final result was, therefore, expressed as units per minute per kilogram body weight. Figure 1 illustrates the differences between

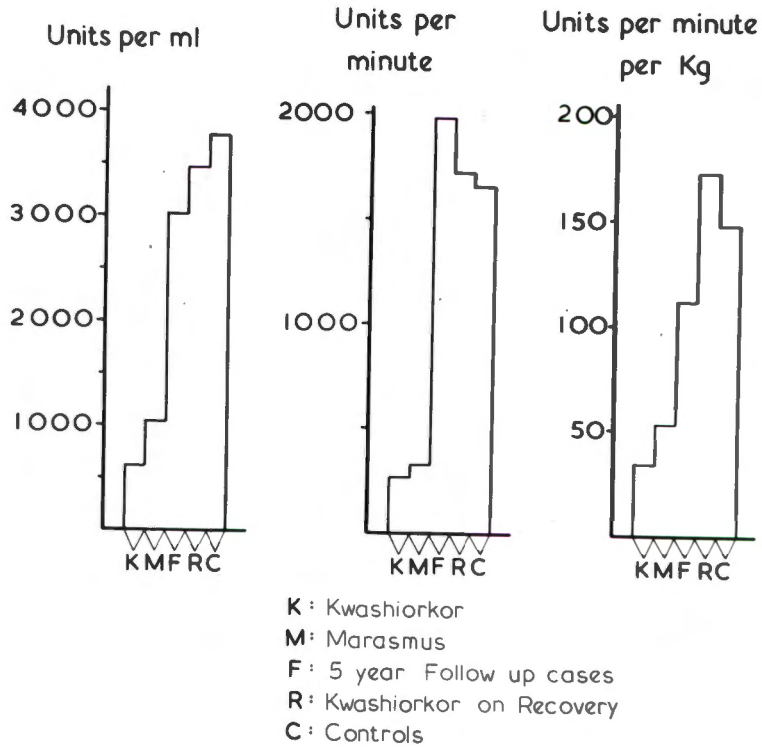


Figure 1.

Comparison of units per ml., units per minute and units per minute per kg. body weight. (Mean values after secretin and pancreozymin stimulation).

units per ml., units per minute and units per minute per kilogram body weight.

During the basal collection the enzyme output was corrected for time and the patient's body weight and expressed in units of enzyme activity per one minute per kilogram body weight.

Similarly, during each of the three 20-minute aspiration periods after secretin stimulation, each sample was corrected to give units of enzyme activity per one minute per kg. The final result of the secretin test was expressed as the mean of these three values in units per one minute per kg. ($\frac{S + S + S}{3}$).

In the same way the final result of the pancreozymin test was expressed as the mean of the two 20-minute periods in units per one minute per kg. ($\frac{P + P}{2}$).

For the combined secretin-pancreozymin test (S + P), the final result was calculated as the mean of the five 20-minute collections after stimulation in units per 1 minute per kg.($\frac{S + S + S + P + P}{5}$).

The peak value was that value during any of the 6 periods of aspiration (1 basal, 3 after secretin, and 2 after pancreozymin stimulation) with the greatest result in units per minute per kg.

E. Colour Index.

The method used was modified from that of Henry et al (176). A comparison was made between the colour of the juice and a standard solution of potassium dichromate.

(a). Apparatus.

A Beckman model DB spectrophotometer was used for the determinations.

(b). Modifications.

(i). Dilution of juice. The original method was described for the determination of the colour index of serum. The dilution used for samples of duodenal juice was thus increased from 1 in 10 to 1 in 50 because of the greater concentration of bile present; an appropriate correction was made to the final calculation.

(ii). Wavelength. This was reduced from 460 mμ to 410 mμ as the latter wavelength corresponded closely to the peak absorption of bile. The reason for the original method having 460 mμ as the recommended wavelength was again that the test was primarily designed for serum; haemoglobin has a maximum absorption at 415 mμ and thus the presence of any haemolysis rendered the readings meaningless. This difficulty did not apply to duodenal juice. All specimens were read at 410 and 460 mμ and there were no significant differences between the final results measured at the different wavelengths. The results derived from readings at 410 mμ only were reported.

F. Microscopy of the duodenal aspirate.

Immediately the duodenal tube was withdrawn at the completion of a PFT mucus was expressed from the tip of the tube and placed on 2 glass slides. To the one a drop of duodenal juice was added and to the other a drop of iodine. The slides were then examined macroscopically and microscopically. No attempt was made to culture the aspirate owing to possible contamination during the tube's passage through the throat and nose.

G. Histology.

Fifty slides were selected as described on page 75 . They were stained with haematoxylin and eosin and examined by light microscopy. The clinical history and pathological findings were examined in each case.

The sections of pancreatic tissue were examined specifically for the following features:- autolysis, acinar structure, zymogen granule depletion, duct structure and distribution, the presence and distribution of connective tissue, the presence and distribution of inflammatory cells (if any), and the state and distribution of the Islets of Langerhans. The degrees of acinar atrophy were defined as follows:-

(a). Mild:

Cloudy smelling and/or decreased cellular cytoplasm with preservation of nuclei.

(b). Moderate:

Crowding together of cells but acinar structure maintained.

(c). Severe:

Complete loss of acinar structure.

The findings were all tabulated under the headings described previously.

In every case a section of the liver was examined to determine the degree of fatty change if this was present at all.

No special stains were used for the more accurate determination of the distribution of connective tissue. Likewise, no special techniques were employed in the examination of the Islets of Langerhans.

H. Serum proteins.

Venous blood was taken from the external jugular vein. The biuret method as employed by Wolfson et al (416) was used for the determination of total protein. This was standardised by Kjeldahl determinations of total protein. Globulins were precipitated with 27% sodium sulphate.(274). The albumin-containing solution was separated by the ether centrifugation method.(225a).

I. Haemoglobin.

This was determined by pipetting 0.02 ml. venous blood into 5 ml. 0.04% ammonium hydroxide solution and reading the haemoglobin by the oxy-haemoglobin method. A Klett-Summerson colorimeter previously calibrated against standard haemin and cyanmethaemoglobin solution was used.

J. Packed cell volume.

A heparinised micro-capillary tube was filled with venous blood and sealed at one end with plasticene. An International Micro-Capillary centrifuge was used to spin the tubes for 5 minutes at 11,000 r.p.m.

K. Weight.

Body weight was recorded in the nude at the time of admission. Expected body weight was theoretically regarded as that of the 50 Boston percentile weight for age. (286). Weighings were repeated daily.

L. Height.

The crown to heel length was measured by means of a measuring board. This was done on admission or, in the case of children with kwashiorkor, after they had lost their oedema. Expected height was theoretically regarded as that of the 50th Boston percentile height for age.(286).

M. X-Rays.

The x-rays were first examined by the investigator, both in the case of the chest x-rays before admission and the straight x-rays of the abdomen taken during the PFT. They were then submitted to the hospital radiologists for reporting. All screenings under the image intensifier were performed by a radiologist. The x-ray plates were re-examined by the investigator at a

later date when a specific search for evidence of pancreatic calcification was made.

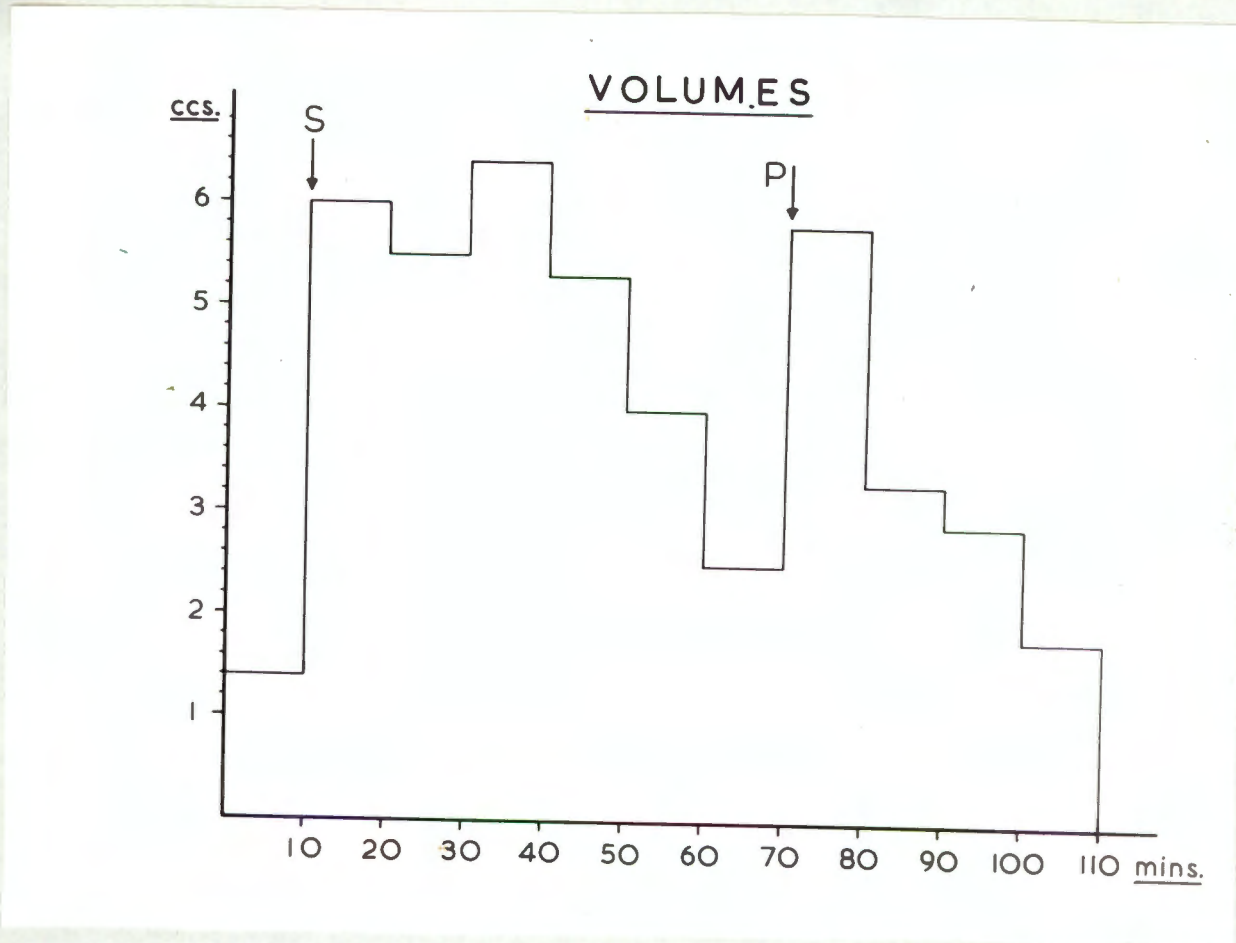


Figure 2.

Volumes - response to secretin and pancreozymin.

CHAPTER 7.

Results.

A. Pancreatic Function Tests.

1. Volume.

Tables giving the details of the volume output in individual children may be found in the appendix. (Tables A to E, pages 178 to 182).

Tables of the results of all the statistical analyses performed may also be found in the appendix. (Tables V and X, pages 205 and 207). Comparisons of interest will be discussed in the text.

(a). Basal secretion. In all the groups tested the volume of juice collected during the period of basal aspiration varied greatly from patient to patient (1.1 to 20.5 ml. per 30 minutes). There were no constant differences between the malnourished and "control" groups of children. No correlation could be found between the basal volume output and body weight during the period of basal aspiration. (correlation coefficient (r) = 0.039).

(b). Response to stimulation.

(i). General. The volume output was dramatically increased after the injection of secretin, and the high volume output was generally sustained for about 40 minutes from the time of injection. A similar increase in volume followed the intravenous injection of pancreozymin; however, this increase in volume was largely due to the cholecystokinetic effect of pancreozymin which resulted in an outpouring of gall-bladder bile. After the first 10 minutes following the pancreozymin injection the volume output decreased gradually to drop within 40 minutes to levels little exceeding those obtained during the basal period. Figure 2 illustrates the typical volume response; values are the means of 8 tests in the "control" group of children.

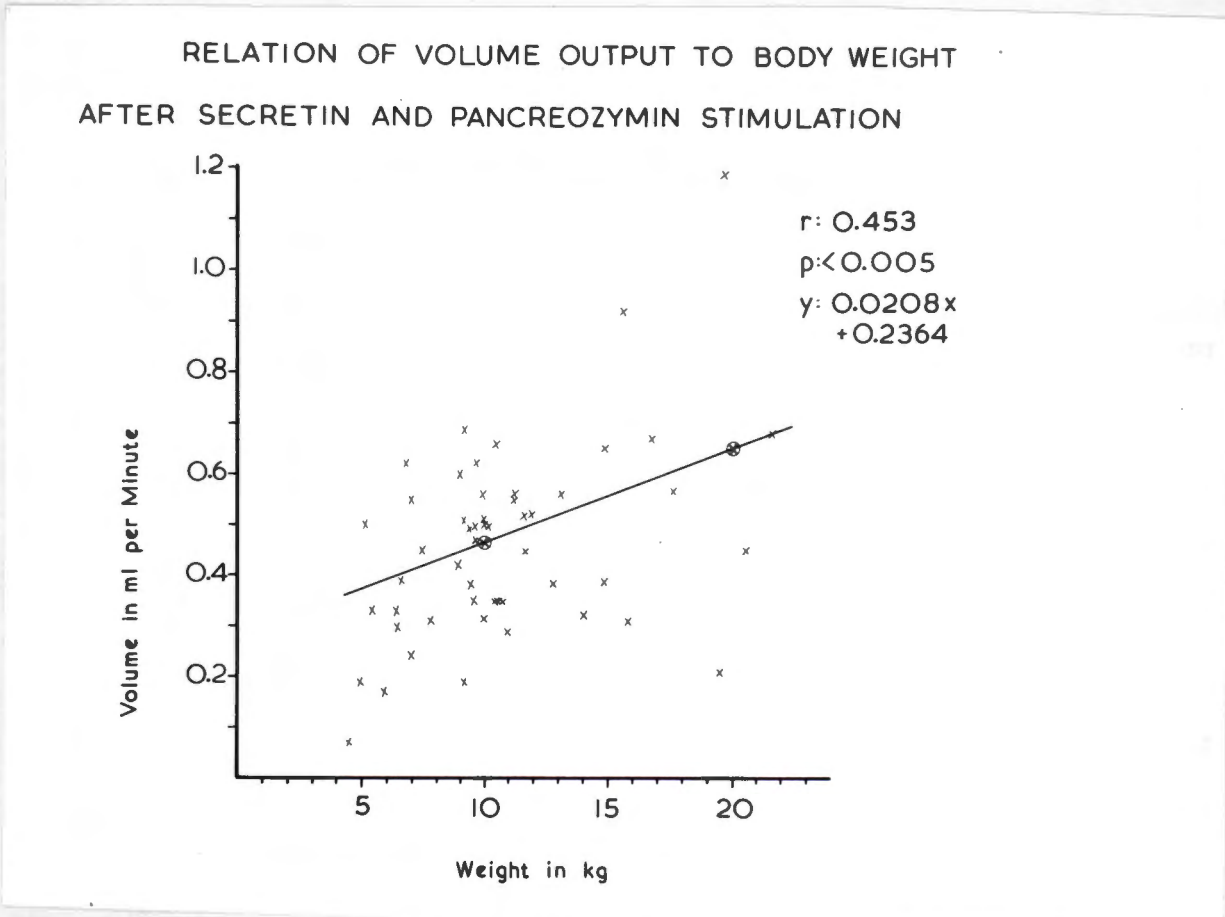

RELATION OF VOLUME OUTPUT TO BODY WEIGHT
AFTER SECRETIN AND PANCREOZYMIN STIMULATION
r: 0.453
p:<0.005
y: 0.0208x
+0.2364
1.2
1.0
0.8
0.6
0.4
0.2
Volume in ml per Minute
5
10
15
20
Weight in kg

Figure 3.

(ii). Relation to body weight. After a number of patients has been studied, it became apparent that smaller children produced less pancreatic juice after secretin and pancreozymin stimulation than larger children. A correlation coefficient was thus calculated, and the relationship between body weight and the volume output from the pancreas in a standard time after hormonal stimulation was found to be statistically significant ($P < 0.005$). (Figure 3). As was anticipated the correlation coefficient between body weight and volume output was greater during the period of secretin stimulation (because of secretin's primarily hydrelatic effect) than during the period of pancreozymin stimulation.(Table 7). It will be noticed from this table that the degree of significance was not altered whether the patients with P-C.M. (kwashiorkor, marasmus and the "follow-up" (i.e. chronic P-C.M.) groups) were considered separately or as part of the overall number of children tested including the "controls" and children who had recovered from their illness.

Making a correction for body weight enables a comparison of the volume output of children of differing weights. Figures 4 and 5 illustrate how the volume output was modified by applying this correction; the groups most affected by the correction were those with the lowest mean weight (the marasmus group) and those with the highest mean weight (the "follow-up" group).

For the purpose of comparing the volume output before and after stimulation, the mean output during one minute per kg. body weight was calculated for the collection under basal conditions and following each of the hormonal stimulations. These mean values were also employed in comparing one group with the other.

(iii). The response to stimulation in the different clinical groups.

Kwashiorkor. The volume of juice secreted by the pancreas

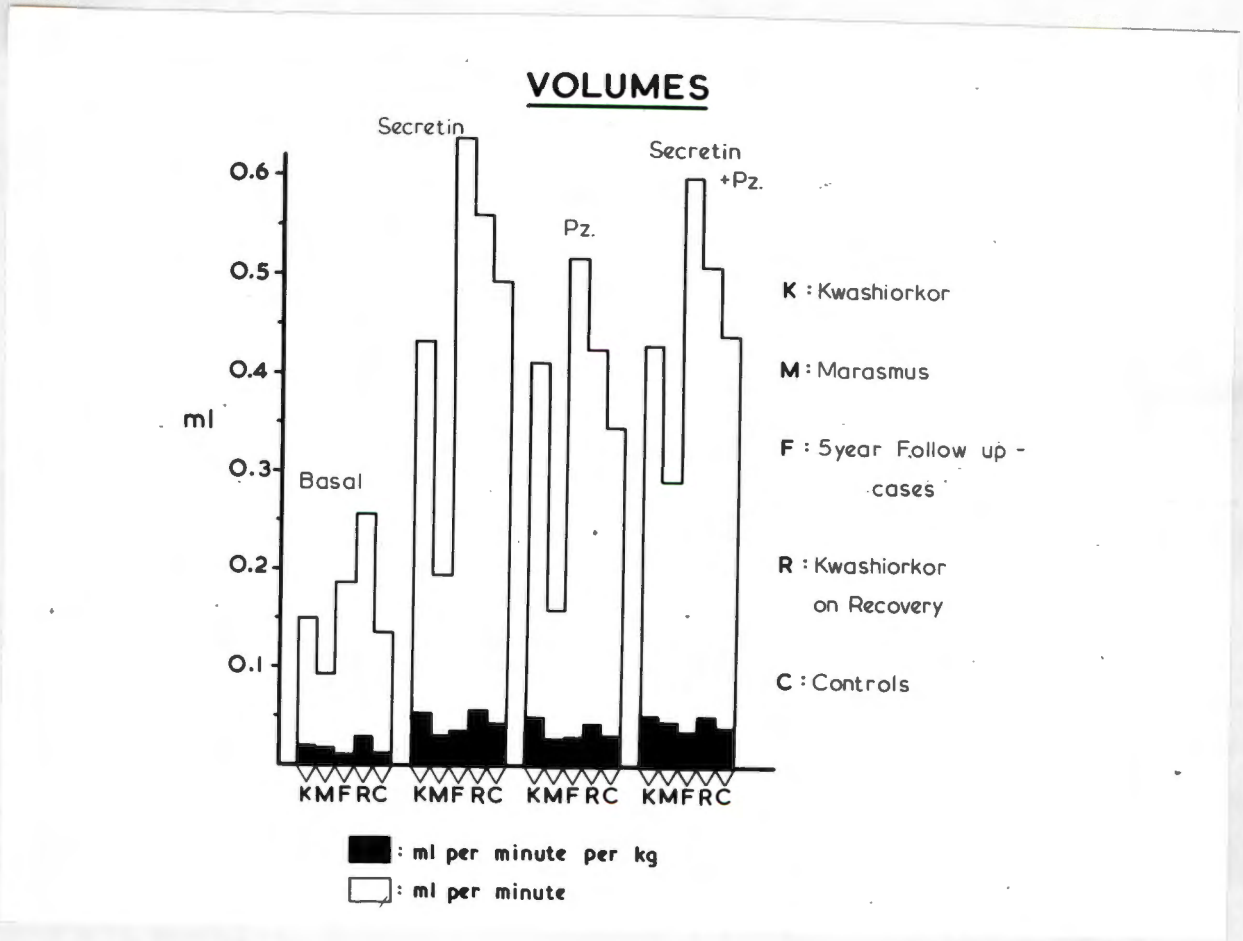


Figure 4.

Volumes - comparison between groups. (ml/min).

TABLE 7.

Relation between Pancreatic Volume Output in ml. per minute and Body Weight in kg.

Test	Clinical Group	Number Tested N	Correlation Coefficient r	Regression Line Formula y = mx + c *	Significance P
Basal (B)	P-C.M.	31	0.192		N.S.
	all groups	50	0.039	y = 0.0013x + 0.1622	N.S.
Secretin (S)	P-C.M.	31	0.529		< 0.005
	all groups	50	0.443	y = 0.0225x + 0.2519	< 0.005
Pancreozymin (P)	P-C.M.	31	0.356		< 0.05
	all groups	50	0.317	y = 0.0044x + 0.2308	< 0.05
Mean S and P (S + P)	P-C.M.	31	0.519		< 0.005
	all groups	50	0.453	y = 0.0208x + 0.2364	< 0.005

* x and y axes as in Figure 3.

N.S. = Not statistically significant.

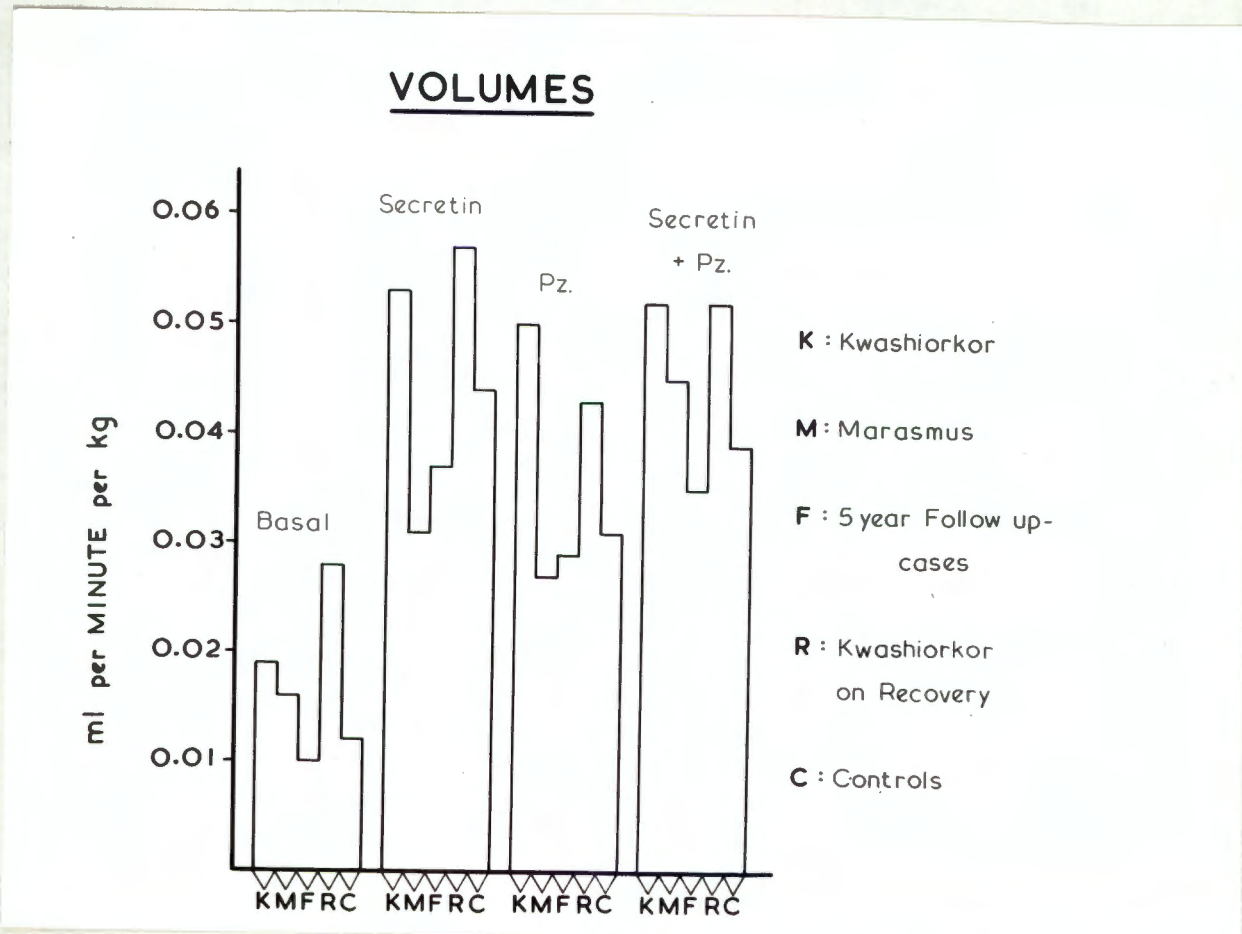


Figure 5.

Volumes - comparison between groups (ml/min/kg.).

under basal conditions was significantly increased by the injection of secretin and pancreozymin.($P < 0.01$). There was, however, no difference in volume output when the two hormonal stimulations were compared with each other.

Marasmus. All the children in this group showed a marked rise in volume output between the basal secretion and the secretion after pancreozymin stimulation; similarly 6 of the 7 children demonstrated a marked increase in volume output after secretin stimulation. (See Table B, page 179). However, because of the large range in volume output found among the children in this group and the small number of patients tested, the rise after stimulation was not statistically significant.

"Follow-up". There was a significant rise in volume output from basal levels after secretin stimulation in these children ($P < 0.01$) and the mean output after secretin and pancreozymin stimulation was also significantly elevated above the basal output.($P < 0.01$).

Recovered Kwashiorkor and "Controls". The difference between the volume output under basal conditions and after stimulation with secretin and pancreozymin was a highly significant one.($P < 0.01$). However, after pancreozymin stimulation significantly less juice was produced than after secretin stimulation.($P < 0.05$).

(c). Comparison between groups.

(i). Basal Test. The children with kwashiorkor and marasmus did not show any difference from the "controls" with regard to basal volume output. The patients in the "follow-up" group tended to have a lower output per kg. body weight than those in the other groups ($P < 0.05$); as these "follow-up" children were of a greater body weight than the others this difference was of doubtful significance. The production of more juice by

the children who had recovered from kwashiorkor than the "controls" was probably modified by a similar factor in the basal aspiration period.

(ii). Secretin Test. After secretin stimulation, the children in the "follow-up" group produced significantly less juice than those who had recovered from kwashiorkor.($P < 0.02$). The other groups did not show any difference among themselves.

(iii). Pancreozymin Test. There was no significant difference in volume output between any of the groups after pancreozymin stimulation.

(iv). Mean of Secretin and Pancreozymin tests. The "follow-up" children again produced significantly less juice than the children with kwashiorkor before and after treatment.($P < 0.05$). The small differences between the other groups were not significant.

(v). Response to Stimulation. In assessing the ability of the patients to raise their volume output after each hormonal stimulation, the parameters secretin minus basal (S-B) and pancreozymin minus basal (P-B) were calculated. There were no statistical differences between the groups in these parameters.

(d). Summary - Volume.

No consistent difference could be found between the groups during the period of basal aspiration. All children showed a good response to hormonal stimulation. The volume output from the pancreas may be correlated with body weight after hormonal stimulation. Comparing the different groups, the children in the "follow-up" group tended to produce less juice after hormonal stimulation than the children in other groups. Acute P-C.M. did not impair the patients' ability to exceed their basal volume output after hormonal stimulation.

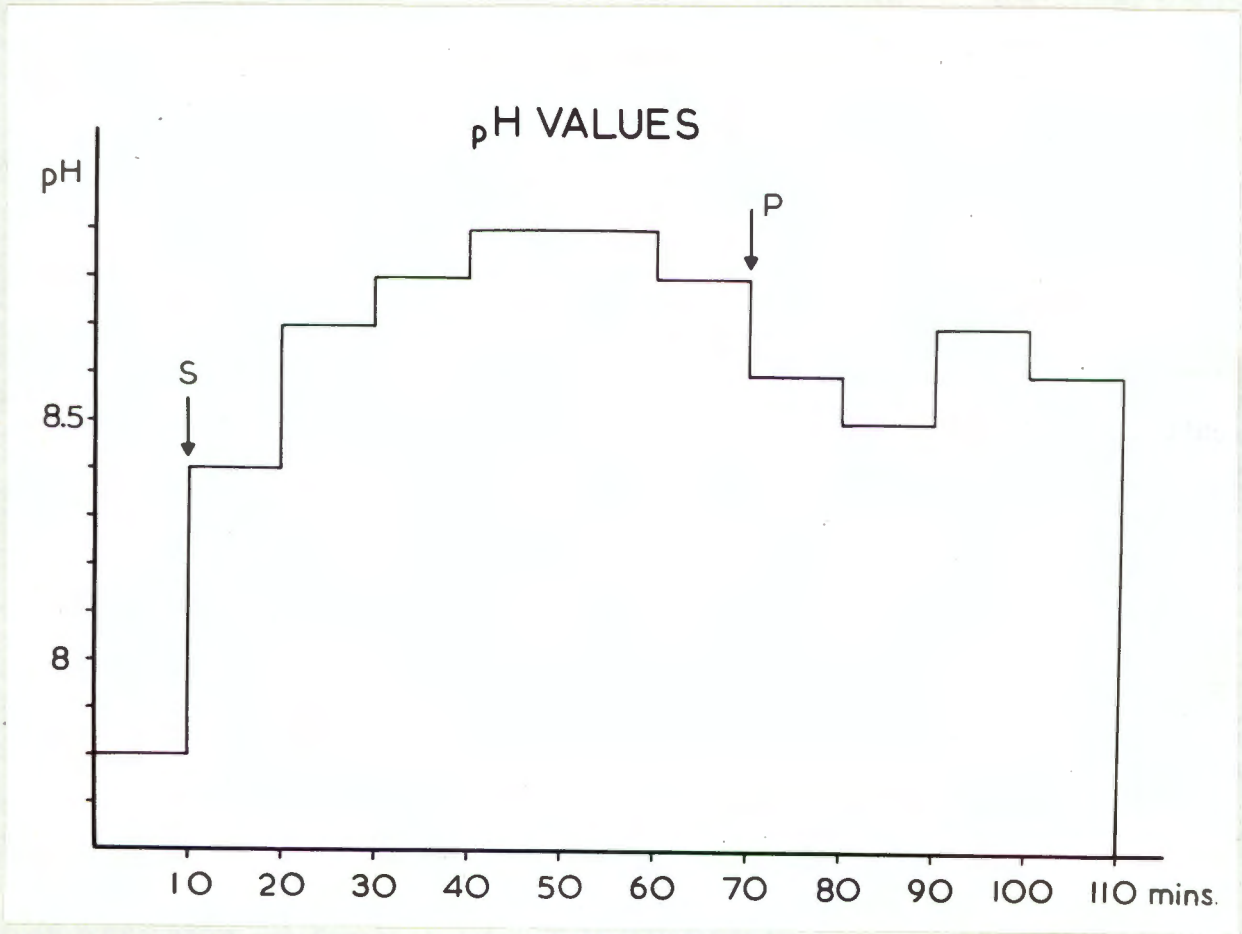


Figure 6.

pH Values - response to secretin and pancreozymin.

2. pH.

The pH values obtained in each individual patient in the different groups may be found tabulated in the appendix (Tables F to K, pages 183 to 187) and the detailed statistical comparisons in Tables V and X,(pages 205 and 207).

(a). Basal secretion. A wide variation in pH was found during the period of basal aspiration. This ranged from mildly acid, (pH 6.0), to moderately alkaline.(pH 8.6). In one child the pH was 4.0 at the onset of the test; this must be ascribed to some degree of gastric contamination. The mean pH among the different groups varied from 7.4 to 8.4. None of the children in the "follow-up" group had a basal duodenal pH less than 8.0.

(b). Response to stimulation. In every child studied the pH of the duodenal aspirate showed a steady rise after the injection of secretin. The pH usually reached its peak 30 to 40 minutes after the injection. Figure 6 illustrates the typical pH response; the values are means from 8 tests in the "control" group of children. The injection of pancreozymin invariably led to a drop in pH, largely owing to the admixture of bile, but the post-pancreozymin pH remained higher than the basal pH.

All the groups tested showed a highly significant rise in pH between the basal juice and that after secretin stimulation.($P < 0.01$). A significant rise in pH was also found between the basal juice and that after pancreozymin stimulation ($P < 0.02$ to $P < 0.01$) except in the "follow-up" group where this difference was not significant. The "follow-up" children and those who had recovered from kwashiorkor had a pH after pancreozymin stimulation which was significantly lower than the pH after secretin stimulation.($P < 0.01$). The peak pH (i.e. the highest pH during any 10-minute collection) was significantly higher than the basal pH in all the groups. ($P < 0.01$).

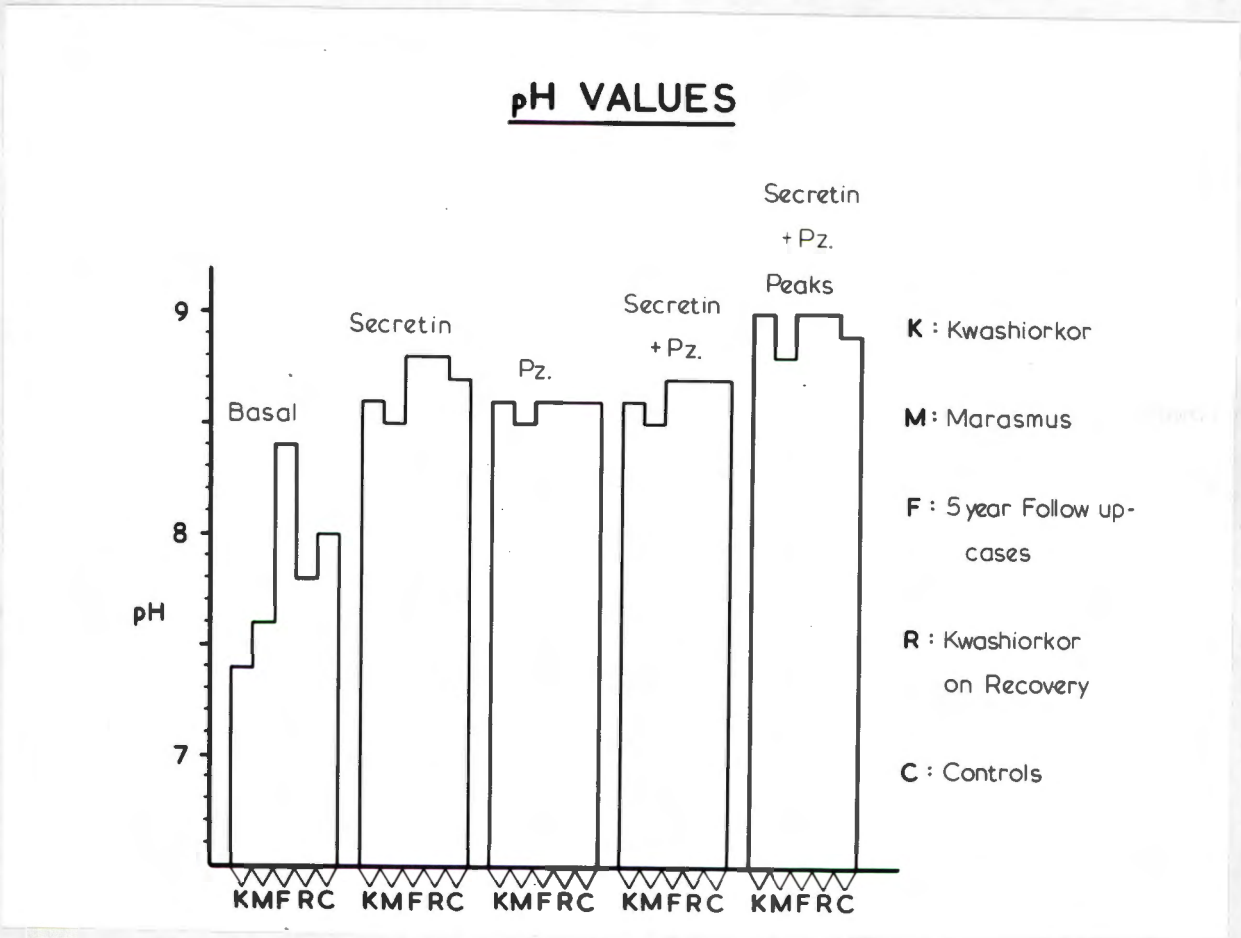


Figure 7.

pH Values - comparison between groups.

(c). Comparison between groups. (Figure 7).

(i). Basal test. The "follow-up" children had a higher duodenal pH than those in the marasmus group and the children before and after the treatment of kwashiorkor. ($P < 0.01$). There was no difference between the remainder of the groups.

(ii). Secretin test. The marasmic children had a lower pH than those in the "follow-up" group ($P < 0.01$) and those who had recovered from kwashiorkor.($P < 0.02$). The values among the kwashiorkor children were also lower before treatment than after treatment, and lower than in the "follow-up" group. ($P < 0.02$). There were no significant differences between any group and the "controls".

(iii). Pancreozymin Test. There was no difference between any of the groups after pancreozymin stimulation.

(iv). Mean of Secretin and Pancreozymin tests. The children who had recovered from kwashiorkor had a juice of higher mean pH than the untreated kwashiorkor patients.($P < 0.05$).

(v). Peak. There was no difference in peak pH between any of the groups.

(vi). Response to stimulation. The rise in pH after secretin stimulation (S-B) was less among the "follow-up" children than those with marasmus ($P < 0.02$), kwashiorkor ($P < 0.01$) and those who had recovered from kwashiorkor.($P < 0.01$). The "controls" also had a lower increment than the children with kwashiorkor.($P < 0.02$). After pancreozymin stimulation (P-B) the "follow-up" children again did not show as great a rise as the children with kwashiorkor before and after treatment and the marasmic patients. ($P < 0.01$). The peak increment (Peak B) showed identical results to the secretin response (S-B). That these parameters were lower in the "follow-up"

group may be explained by their high basal pH; the final pH attained was no different from that of the other groups.

(d). Summary - pH. There was a wide variation in basal duodenal pH among the groups. The patients in all the groups showed a significant rise in duodenal pH after hormonal stimulation. The few differences between the groups did not show any definite pattern; no group had a poorer response than the "controls". Duodenal pH was not constantly affected in the children with P-C.M.

3. Enzymes.

All enzyme units are expressed in terms of units per 1 minute per kilogram body weight (as defined on page 94) unless otherwise specified. A detailed table of all the results of individual patients in each group may be found in the appendix (Tables L to P, pages 188 to 192).
The results of the detailed statistical analysis are also in the appendix (Tables V and W, pages 205 and 206).

I. Amylase.

(a) Basal secretion. There was great variation in the amylase output under basal conditions. The output in the kwashiorkor and marasmus groups was lower than in the other groups, but it was only in the kwashiorkor group that no amylase could be detected on 2 occasions.

(b). Response to stimulation. Among the "control" children and those who had recovered from kwashiorkor, there was a significant rise in amylase output from the basal secretion after secretin and after pancreozymin stimulation. The peak enzyme output (as defined on page 95) in these groups was significantly greater than the basal, the post-secretin and the post-pancreozymin output.(P from < 0.05 to < 0.01).

In the kwashiorkor group there was a significant increase in

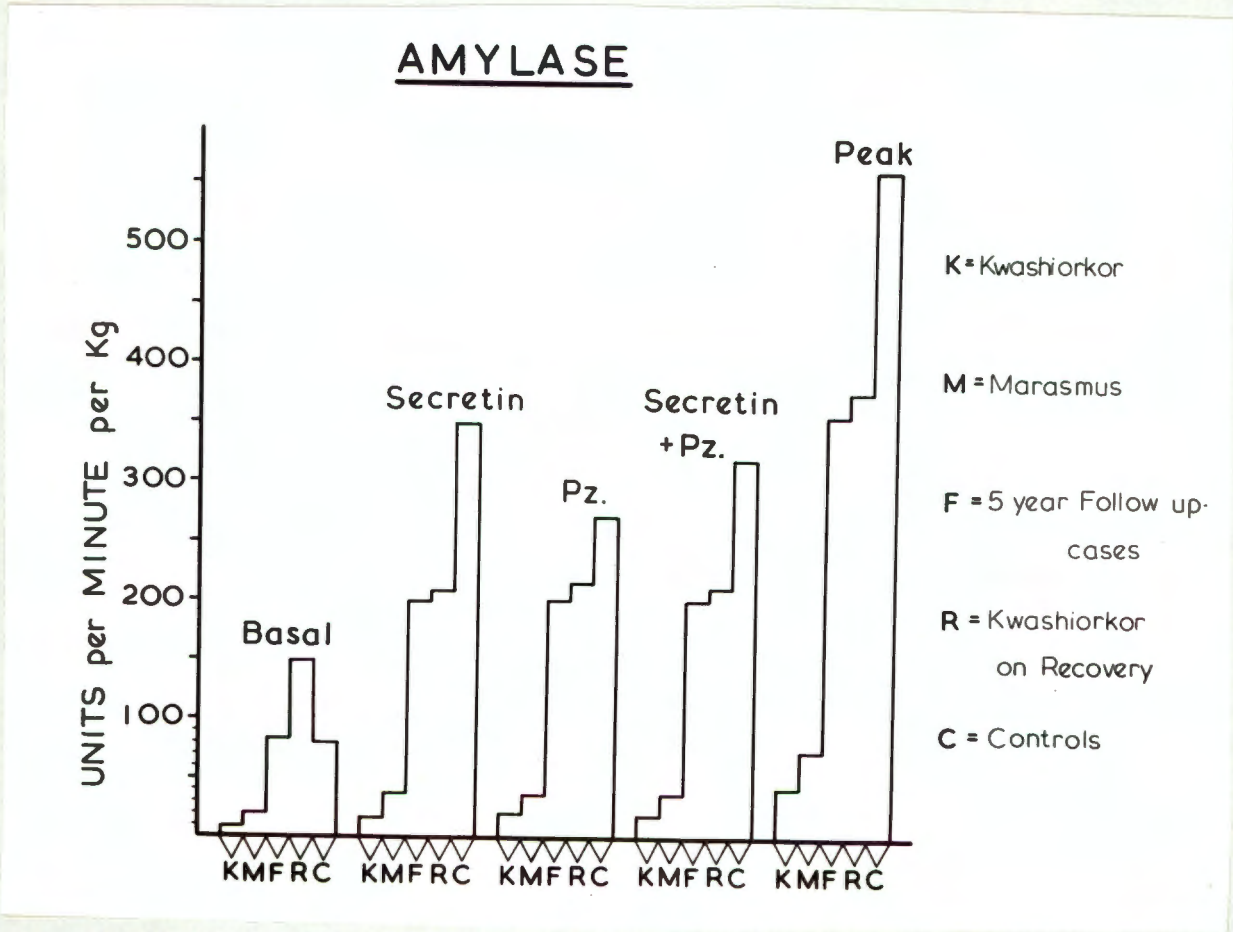


Figure 8.

Amylase - comparison between groups.

amylase between the basal output and that after pancreozymin stimulation and also between the basal and the mean secretin and pancreozymin output.($P < 0.05$). The peak output was significantly higher than the basal output ($P < 0.01$), the post-secretin output ($P < 0.02$) and the post-pancreozymin output.($P < 0.05$). This indicated that these children could respond to hormonal stimulation.

All the marasmic children but 2 showed a rise in amylase output after secretin stimulation, and in all but 1 there was a rise from basal levels after pancreozymin stimulation.(See Table M, page 189). However, because of the wide range of these results, they were not statistically significant. The rise between the basal output and the peak output was a significant one in these children.($P < 0.05$).

The "follow-up" children showed a rise between the basal and pancreozymin values ($P < 0.05$) and between the basal and peak values.($P < 0.02$).

(c). Comparison between groups.(Figure 8).

(i). Basal Test. The patients with kwashiorkor produced significantly less amylase than the "follow-up" children, the "controls", and the treated kwashiorkor patients.($P < 0.01$). The marasmic children also produced less amylase than the "controls" and the patients who had recovered from kwashiorkor.($P < 0.01$).

(ii). Secretin Test. Stimulation with secretin yielded results which demonstrated the same statistical differences between the groups as the basal test except that after stimulation the "control" children in addition produced significantly more amylase than the children who had recovered from kwashiorkor.($P < 0.01$).

(iii). Pancreozymin Test. Using pancreozymin to stimulate the pancreas did not alter the differences between groups shown by the basal

test except that the "follow-up" children produced significantly more amylase than the marasmic children.($P < 0.01$).

(iv). Mean of secretin and pancreozymin tests. Finding the mean value for the secretin and pancreozymin tests did not demonstrate any differences not shown by the pancreozymin test alone.

(v). Peak values. Comparing the peak values of amylase output between each group gave similar results to those of the pancreozymin test.

(vi). Response to stimulation. The increment in amylase output between the basal output and the secretin-stimulated output (S-B) was lower in the kwashiorkor children than in those who had recovered from kwashiorkor ($P < 0.05$), the "follow-up" children ($P < 0.02$) and the controls.($P < 0.01$). The marasmic children also responded more poorly than the "controls" ($P < 0.01$); the "control" group of children demonstrated a significantly greater increment than those in any other group.(P from < 0.05 to < 0.01).

The rise in amylase output after pancreozymin stimulation (P-B) demonstrated the same pattern of difference between the groups as the rise after secretin stimulation except that there was no difference between the "control" and the "follow-up" patients.

The maximum rise in amylase output (Peak-B) was less pronounced in the kwashiorkor patients and in those with marasmus than in the children in all the other groups.(P from < 0.05 to < 0.01). The "control" children showed a higher increment than those in all the other groups. (P from < 0.05 to < 0.01).

(d). Summary - Amylase. There was a marked variation in amylase output among the different groups under basal conditions; the basal

output was, however, generally lower among the children with kwashiorkor and marasmus.

All the groups showed a significant increase in amylase output after hormonal stimulation; in the kwashiorkor and "follow-up" groups this was only demonstrated after pancreozymin stimulation, and in the marasmus group only when the peak output was considered.

The "control"children produced more amylase than any other group, including those patients who had recovered from kwashiorkor. The children with kwashiorkor and marasmus had a poor amylase output. The patients with kwashiorkor showed a dramatic rise in enzyme output after treatment.

The different forms of hormonal stimulation produced a similar pattern of results.

II. Lipase.

(a). Basal secretion. The range in all the groups was great during the period of basal aspiration and there was some degree of overlap between all the groups. The children with kwashiorkor tended to have lower values than the others; lipase was completely absent in only one child in this group.

(b). Response to stimulation. The "controls", the "follow-up" children, and those who had recovered from kwashiorkor showed a highly significant rise from the basal output of lipase after secretin and pancreozymin stimulation, whether the hormonal stimulations were considered separately or together. ($P < 0.01$). In addition, the peak lipase output was much greater than the basal output and that after each hormonal stimulation. ($P < 0.01$).

The kwashiorkor children also showed a rise from basal levels

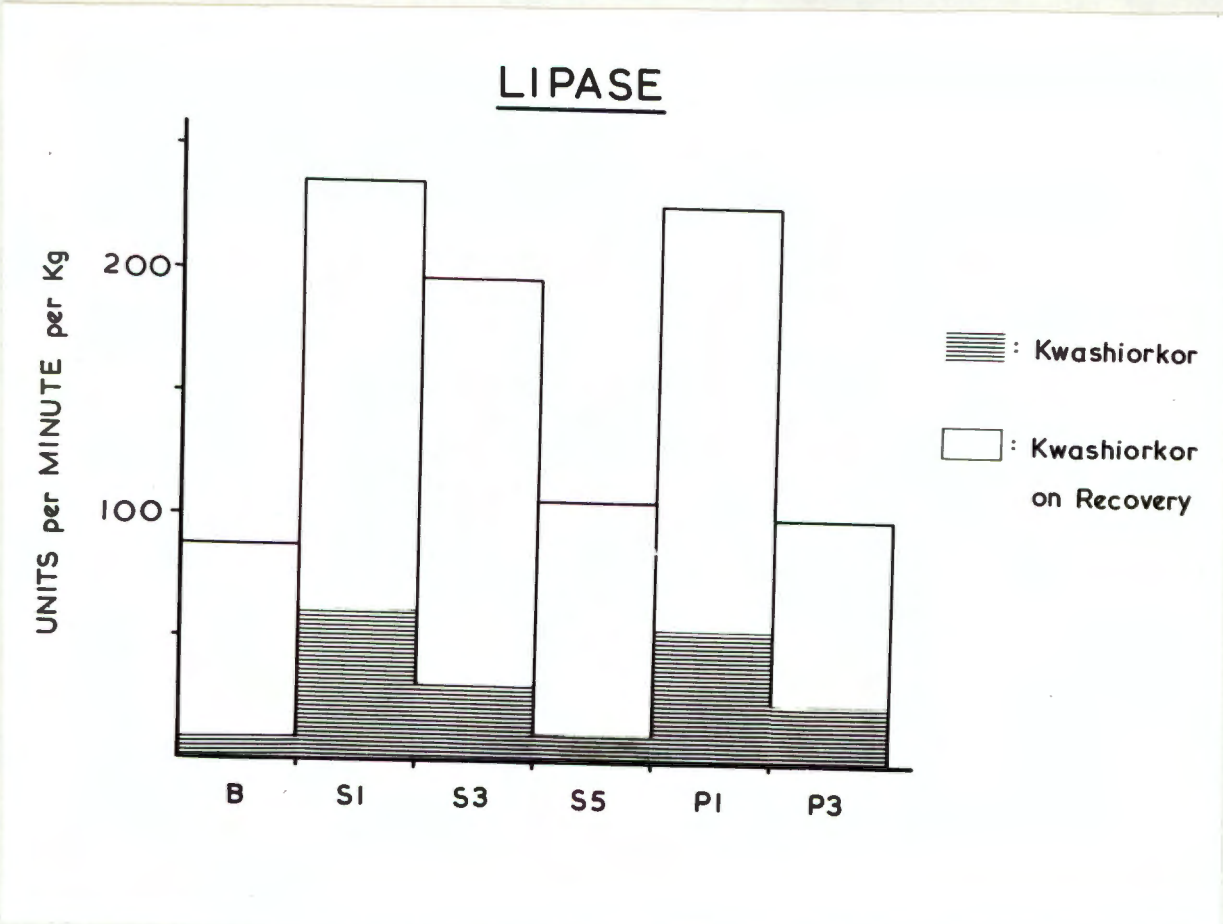


Figure 9.

Lipase - output before and after the treatment of kwashiorkor.

B = Basal.

S1, S3 and S5 = successive 20 minute periods after the injection of secretin.

P1 and P3 = successive 20 minute periods after the injection of pancreozymin.

after secretin and pancreozymin stimulation ($P < 0.01$), but this rise was at a much lower level than in the children who had recovered from their illness. (Figure 9). The peak value in these patients was greater than the basal value ($P < 0.01$) and the secretin stimulated value.($P < 0.05$).

Among the marasmic children, the difference between the basal output and the peak output was significant.($P < 0.05$). Although every child in this group showed a rise in lipase output after secretin and pancreozymin stimulation (Table M, page 189), this rise was not statistically significant owing to the large range and the small number of patients in this group.

Hormonal stimulation produced a significant increase in lipase output in every group.

(c). Comparison between groups.

(i). Basal Test. The patients with kwashiorkor had a significantly lower lipase output than those who had recovered from their illness ($P < 0.01$) (Figure 9), the "controls" ($P < 0.01$) and the "follow-up" children.($P < 0.02$). The children with marasmus and the "follow-up" children also had a significantly lower output than those in the recovered kwashiorkor group, ($P < 0.01$ and < 0.02 respectively).

(ii). Secretin Test. This showed a similar pattern to the basal test except that the marasmic children produced significantly less lipase than the "controls" after stimulation. ($P < 0.01$).

(iii). Pancreozymin Test. The results after pancreozymin stimulation showed little difference from those of the secretin test, except that there was no difference between the children in the "follow-up" and the recovered kwashiorkor groups.

(iv). Mean of secretin and pancreozymin tests. Using this parameter did not alter the information provided by the basal test except that

the marasmic children were found to produce less lipase than the "follow-up" children. ($P < 0.05$).

(v). Peak values. The peak values demonstrated the same differences between groups as the secretin test.

(vi). Response to stimulation. The rise in lipase output between the basal and the secretin-stimulated collections (S-B) was significantly less in the kwashiorkor and marasmus groups than in the "control" and recovered kwashiorkor groups.(P from < 0.02 to < 0.01).

A similar difference was found in assessing the rise after pancreozymin stimulation (P-B) but, in addition, the kwashiorkor children were found to respond more poorly than the "follow-up" children.($P < 0.05$). The greatest rise in lipase (Peak-B) did not alter the pattern shown by the parameter P-B.

(d). Summary - Lipase.

Under basal conditions, only the kwashiorkor patients demonstrated a significantly lower lipase output than the "controls".

All the groups showed a significant rise in lipase output after stimulation with secretin and pancreozymin. In the kwashiorkor and marasmic groups this rise was at a lower level and not as marked as in the normal children.

The children who had recovered from kwashiorkor had lipase levels no different from those of the "controls".

III. Ribonuclease.

(a). Basal secretion. The overlap between groups was great, but the kwashiorkor children had a very poor RNase output. Under basal conditions there was at least 1 patient in each group (except the "controls") with no detectable RNase in the pancreatic juice.

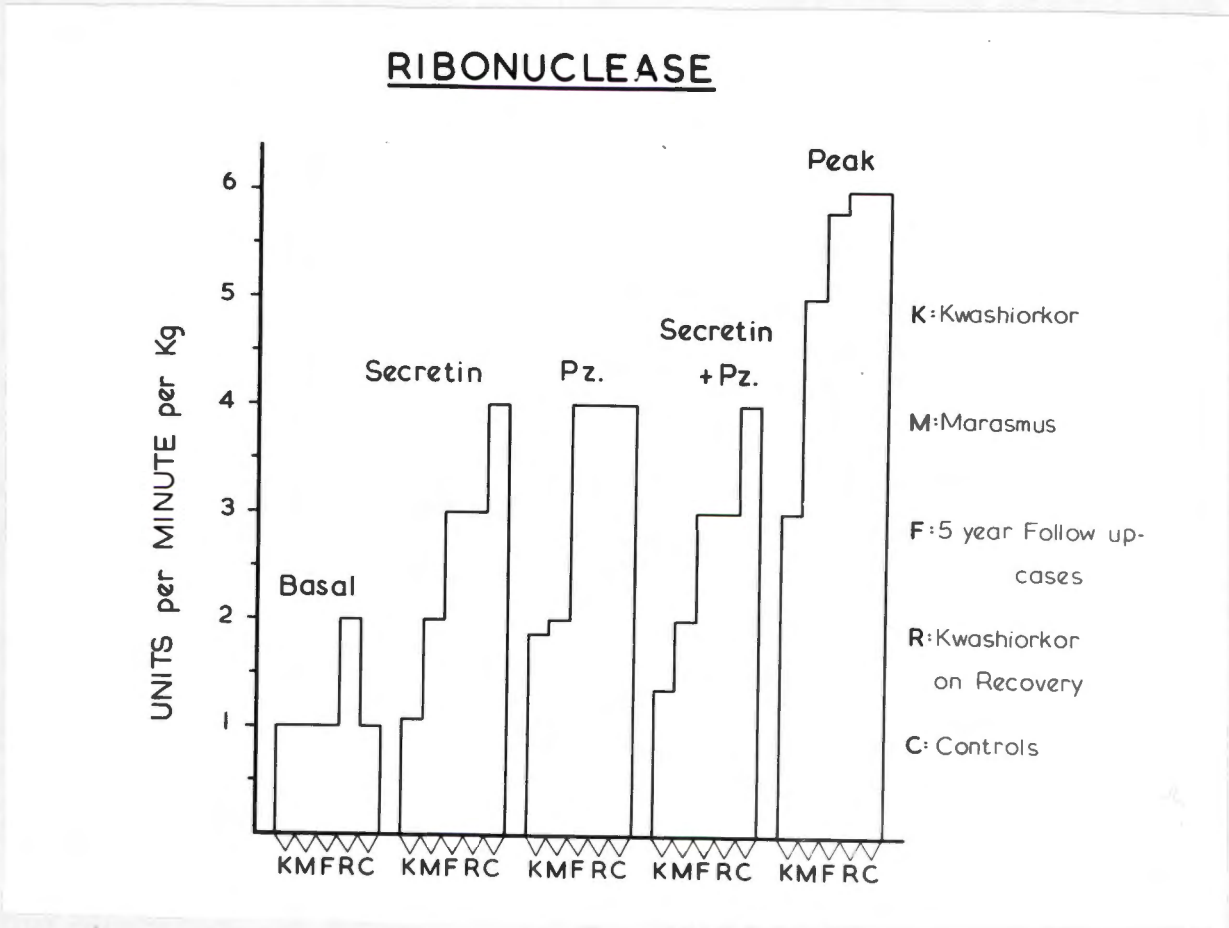


Figure 10.

Ribonuclease - comparison between groups.

(b). Response to stimulation. The "follow-up" children and the "controls" showed a significant increase in RNase output from basal levels after stimulation with secretin and with pancreozymin.(P from < 0.05 to < 0.01). Among the children with kwashiorkor there was also an increase above basal levels with pancreozymin stimulation ($P < 0.02$) but not with secretin stimulation alone. In the marasmic children the peak output was significantly greater than the basal output.($P < 0.02$). All the children thus showed a significant response to hormonal stimulation.

(c). Comparison between groups. (Figure 10).

(i). Basal Test. The children with kwashiorkor had a significantly lower RNase output than the "controls".($P < 0.05$). The children who had recovered from kwashiorkor produced the greatest RNase output, this being significantly greater than those with kwashiorkor ($P < 0.01$), the "controls" and the "follow-up" group. ($P < 0.05$).

(ii). Secretin Test. After secretin stimulation the children with kwashiorkor were the only ones to have a RNase output significantly lower than any other group; they were lower than the "controls", the recovered kwashiorkor group ($P < 0.01$) and the "follow-up" group ($P < 0.05$), but no different from those in the marasmus group.

(iii). Pancreozymin Test. After pancreozymin stimulation the kwashiorkor children still had a lower RNase output than the "controls", the recovered kwashiorkor group and the "follow-up" group.($P < 0.01$). In addition the marasmic children also had a lower output than the "follow-up" children and those who had recovered from kwashiorkor. ($P < 0.05$).

(iv). Mean of secretin and pancreozymin tests. The kwashiorkor children were the only ones who showed an output less than the "controls", the recovered kwashiorkor patients and the "follow-up" children. ($P < 0.01$). All the other differences were not significant.

(v). Peak values. The differences between the groups were the same as with the combined secretin and pancreozymin test.

(vi). Response to stimulation. After secretin stimulation (S-B) the kwashiorkor children produced a smaller response than those of the "follow-up" group ($P < 0.02$) and the "controls" ($P < 0.05$).

After pancreozymin stimulation (P-B) both the kwashiorkor and the marasmic children had a lower increment than the "follow-up" group. ($P < 0.02$ and < 0.05 respectively).

In assessing the maximum rise (Peak-B), the kwashiorkor children were again lower than the "controls" ($P < 0.05$) and the "follow-up" group.($P < 0.02$).

(d). Summary - Ribonuclease. There was a considerable overlap in the basal RNase output between the groups; the children with kwashiorkor demonstrated the most constant depression in the output of this enzyme under basal conditions.

All the groups were capable of increasing their RNase output after hormonal stimulation; the children with kwashiorkor and marasmus showed the poorest response.

RNase output was most significantly depressed among the children with kwashiorkor. After pancreozymin stimulation the marasmic children also produced less RNase than the other groups.

After treatment the patients who had had kwashiorkor showed no difference from the "controls".

IV. Trypsin.

(a). Basal Secretion. The variation in the basal output between different children was great, and there was some overlap between the groups. The children with kwashiorkor generally had a depressed basal trypsin output.

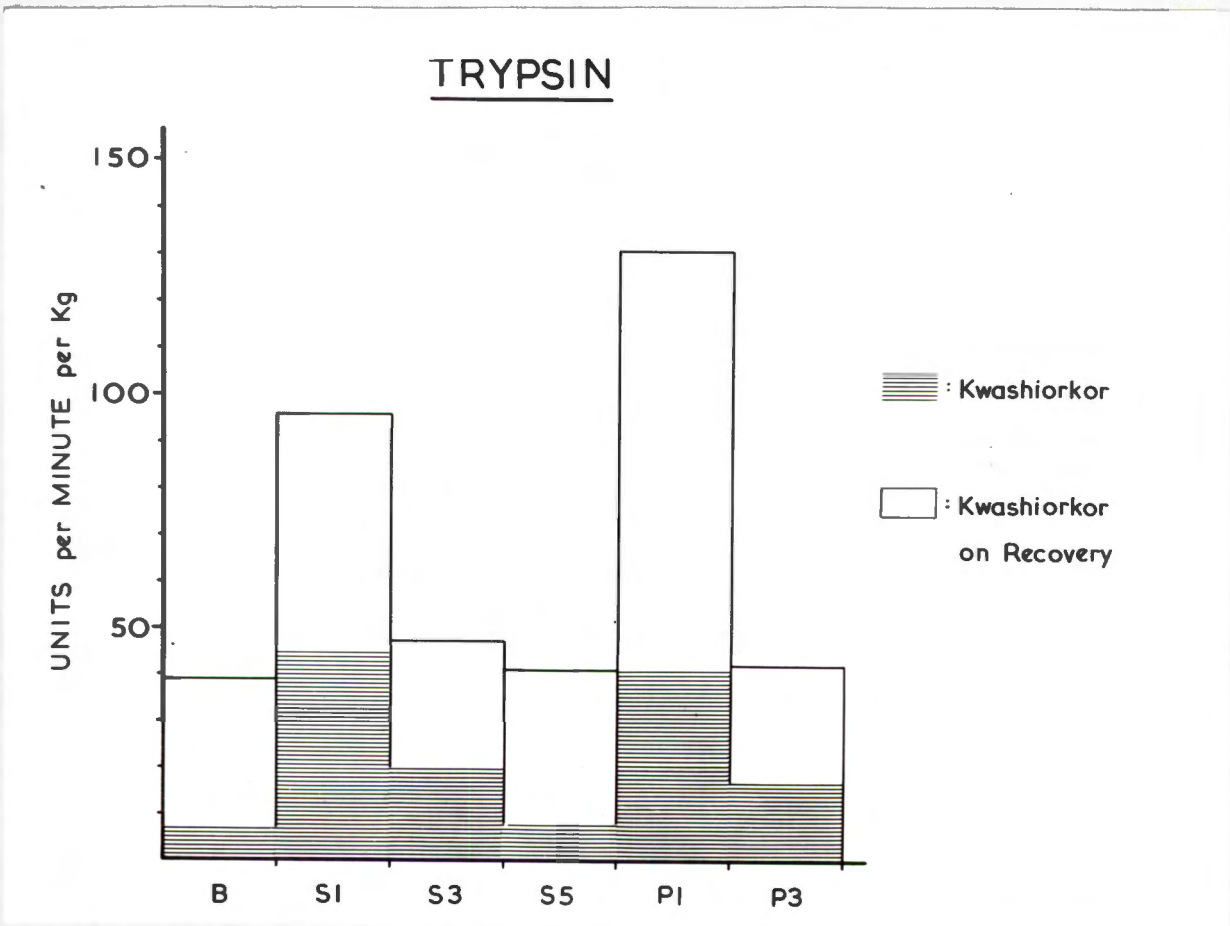


Figure 11.

Trypsin - output before and after the treatment of kwashiorkor.

B = Basal.

S1, S3 and S5 = successive 20 minute periods after the injection of secretin.

P1 and P3 = Successive 20 minute periods after the injection of pancreozymin.

(b). Response to stimulation. In the kwashiorkor patients, the "follow-up" children and the "controls", there was significant rise above basal levels after secretin and after pancreozymin stimulation (P from < 0.02 to < 0.01); in the recovered kwashiorkor group there was a significant rise after pancreozymin stimulation ($P < 0.01$) and if the mean of the secretin and pancreozymin-stimulated outputs were considered together. ($P < 0.02$).

The peak value was significantly greater than all the other parameters in the "control" and the recovered kwashiorkor groups. ($P < 0.05$ to < 0.01). In the kwashiorkor and "follow-up" groups the peak value was higher than all the other parameters except that after pancreozymin stimulation. ($P < 0.05$ to < 0.01).

The children in the "control", recovered kwashiorkor and "follow-up" groups all showed a significant rise between the secretin-stimulated output and the pancreozymin-stimulated output. ($P < 0.05$ to < 0.01).

Although all the children with marasmus showed an increase in enzyme output after hormonal stimulation (Table M, page 189), this increase was not statistically significant owing to the large range among the results and to the small number of patients studied.

All the children increased their trypsin output after hormonal stimulation.

(c). Comparison between groups.

(i). Basal Test. The children with kwashiorkor produced less trypsin than those who had recovered from their illness ($P < 0.01$) (Figure 11) and the "controls". ($P < 0.02$). The children in the "follow-up" group also produced less than those in the recovered kwashiorkor group. ($P < 0.05$).

(ii). Secretin Test. After secretin stimulation the kwashiorkor children still had a lower trypsin output than the "controls" ($P < 0.02$)

and the children who had recovered from their illness.($P < 0.01$).

(iii). Pancreozymin Test. The patients with kwashiorkor had a lower trypsin output than those who had recovered from kwashiorkor, the "controls" and the "follow-up" children.($P < 0.01$). This stimulation also demonstrated that the marasmic children had a lower trypsin output than the "controls" ($P < 0.01$) and the children who had recovered from kwashiorkor.($P < 0.05$).

(iv). Mean of secretin and pancreozymin Tests. This did not show any changes which were not demonstrated by the pancreozymin test alone.

(v). Peak values. Statistical comparisons were similar to those of the pancreozymin test except that the differences were all highly significant.($P < 0.01$).

(vi). Response to stimulation. Comparing the response to secretin stimulation between the groups (S-B), no statistical significance could be demonstrated. Figure 11, however, shows that the rise in the kwashiorkor children was at a much lower level than that of children who had recovered from their illness.

After pancreozymin stimulation (P-B) the children with kwashiorkor and marasmus had a lower increment in trypsin output than the "follow-up" children ($P < 0.02$ and < 0.05 respectively) and the "controls". ($P < 0.01$).

The peak response to stimulation (Peak-B) showed a similar picture to the response to pancreozymin (P-B); the children with kwashiorkor produced less of an increment than the "follow-up" children ($P < 0.01$), the "controls" ($P < 0.01$) and those who had recovered from kwashiorkor.($P < 0.05$). The marasmic children also produced a lower increment

than those of the "follow-up" group ($P < 0.02$) and the "controls".($P < 0.01$).

(d). Summary - Trypsin. The basal trypsin output demonstrated a considerable degree of overlap between the groups; the children with kwashiorkor had the lowest output.

All the groups demonstrated a rise in trypsin output after hormonal stimulation.

The children with kwashiorkor and marasmus demonstrated a poorer response to stimulation than those in the other groups, and a lower total output of trypsin. It required pancreozymin stimulation to show up this deficit in the marasmic patients. No child had a complete lack of trypsin after stimulation.

There was no difference between any group with regard to the increment of trypsin output after secretin stimulation.

After the treatment of kwashiorkor there was no deficit in trypsin output.

V. Chymotrypsin.

(a). Basal secretion. This was severely depressed in the children with kwashiorkor; 72% of these children had an unrecordable chymotrypsin output. Four of the 7 marasmic children also had no chymotrypsin detectable in their basal aspirate. The "follow-up" children with the poorest enzyme output in general also had very reduced chymotrypsin levels.

(b). Response to stimulation. The children in all the groups (except the marasmus group) demonstrated a significant rise from basal to peak values.(P from < 0.05 to < 0.01). In the "follow-up" children and those who had recovered from kwashiorkor, the peak output was greater than the secretin-stimulated output.($P < 0.05$).

The "follow-up" children, the "controls" and the children who had recovered from kwashiorkor showed a significant rise from the basal

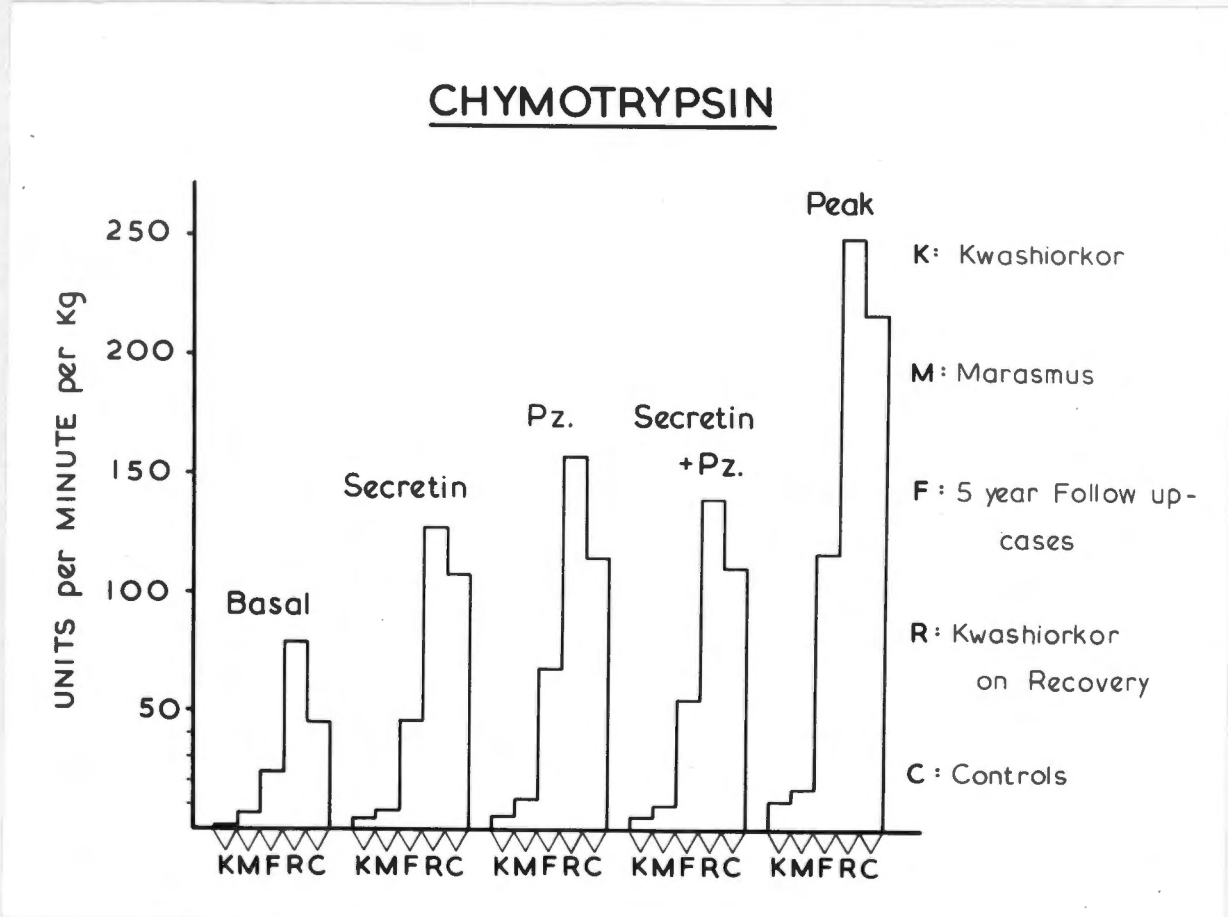


Figure 12.

Chymotrypsin - comparison between groups.

secretion after pancreozymin stimulation.($P < 0.05$ to < 0.02).

Four of the 7 marasmic children studied demonstrated a response to hormonal stimulation; the remainder had a complete absence of chymotrypsin. Owing to the small number of patients this was not statistically significant. (See Table M, page 189).

Of the 14 children in the kwashiorkor and marasmus groups who demonstrated a complete absence of chymotrypsin in their basal aspirate, 8 failed to produce any of this enzyme after hormonal stimulation.

(c). Comparison between groups. (Figure 12).

(i). Basal Test. The children with kwashiorkor produced significantly less chymotrypsin than the children who had recovered from their illness (Figure 13), the "controls" and the "follow-up" children. ($P < 0.01$). The marasmic children also had a chymotrypsin output which was lower than the "controls" and the patients who had recovered from kwashiorkor.($P < 0.01$). The "follow-up" children had a lower output than those who had recovered from kwashiorkor.($P < 0.02$).

(ii). Secretin Test. The differences between the groups were the same as under basal conditions except that the marasmic children had lower values than the "follow-up" children.($P < 0.01$).

(iii). Pancreozymin Test. After pancreozymin stimulation the differences between the groups were similar to those obtained with the secretin tests.

(iv). Mean of secretin and pancreozymin Tests. The differences shown using this parameter were similar to those of the secretin test.

(v). Peak. The children with kwashiorkor and marasmus had a significantly lower output than those in all the other groups.($P < 0.01$).

(vi). Response to stimulation. The increment from the

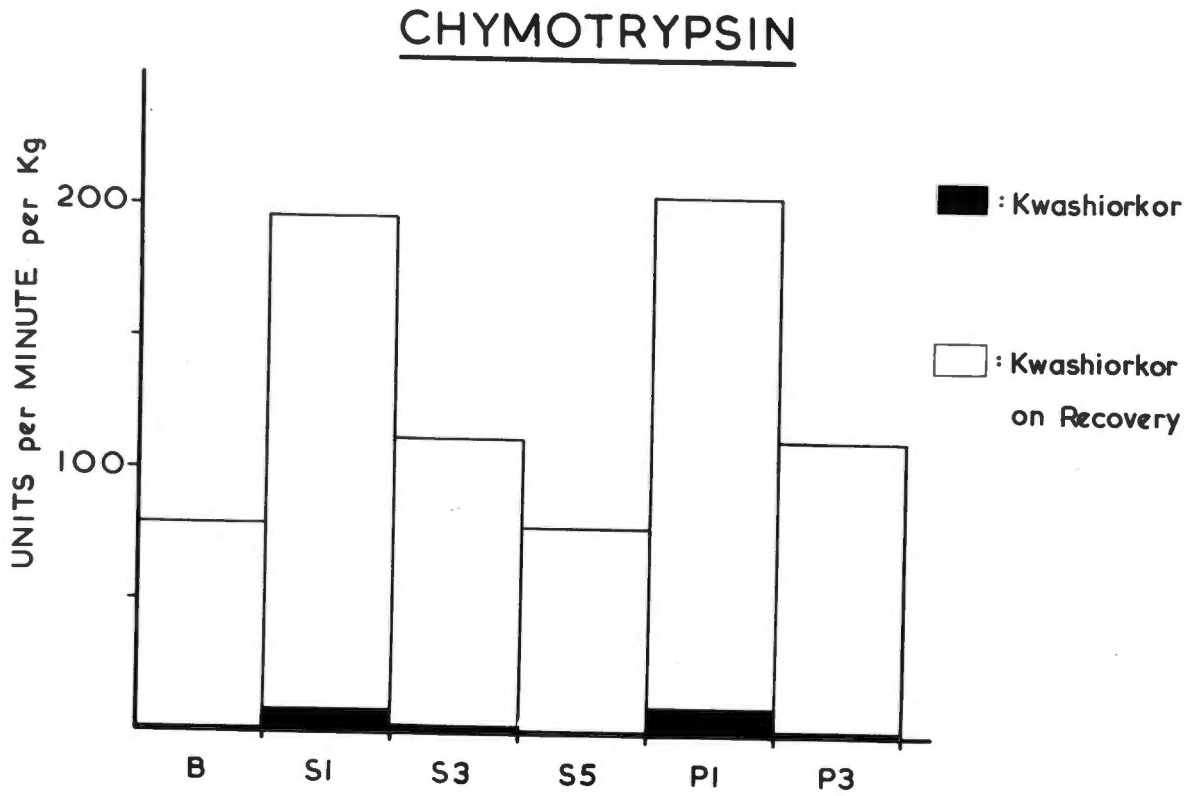


Figure 13.

Chymotrypsin - output before and after the treatment of kwashiorkor.

B = Basal.

S1, S3 and S5 = successive 20 minute periods after the injection of secretin.

P1 and P3 = successive 20 minute periods after the injection of pancreozymin.

basal to the secretin-stimulated output (S-B) was significantly lower in the kwashiorkor and marasmus groups than in the "follow-up" patients ($P < 0.02$), the recovered kwashiorkor patients ($P < 0.02$) and the "controls".($P < 0.01$). The "follow-up" children were also less responsive than the "controls"($P < 0.05$).

After pancreozymin stimulation, the increment in chymotrypsin output (P-B) was lower among the kwashiorkor and marasmus groups than in all the other groups. (P from < 0.05 to < 0.01).

The increment from the basal to the peak output (Peak-B) was also lower in the kwashiorkor and marasmus groups than in all the other groups.($P < 0.01$).

(d). Summary - Chymotrypsin. In the basal secretion, chymotrypsin was frequently absent in children suffering with P-C.M. Among these children there was often no response to stimulation.

All the children who had some chymotrypsin in their basal aspirate (and some in whom this enzyme could not be detected under basal conditions) showed a rise in output after hormonal stimulation, but this was not as great as with the other enzymes.

This was the most severely affected enzyme in children with P-C.M. The malnourished children demonstrated a poorer response to hormonal stimulation than the normal or recovered children. The "follow-up" children also demonstrated a depression in chymotrypsin output.

The differences between the groups remained fairly constant irrespective of whether the basal juice or the juices after hormonal stimulation were compared one group with the other.

After the treatment of kwashiorkor, the chymotrypsin output returned to normal levels.

VI. Pancreatic function after protein repletion in marasmic children.

Two children from the marasmus group (numbers 2 and 8, Table 3, page 65) were submitted to repeat PFT's.

The first child was discharged prematurely from the ward as he had come into contact with measles. He had received 20 days treatment during which time his serum albumin concentration had risen from 1.88 g. to 2.46 g. per 100 ml. and his general condition had improved considerably. The results of his test were as follows:-

Test	Volume ml/min/kg	pH units	Amylase u/min/kg	Lipase u/min/kg	RNase u/min/kg	Trypsin u/min/kg	Chymotrypsin u/min/kg
Basal	0.031	8.3	160	34	10	75	31
Secretin	0.082	8.6	243	59	15	144	33
Pancreo-zymin	0.037	8.5	153	53	7	86	21
Mean S+P	0.064	8.6	207	56	11	121	28
Peak	-	8.8	321	78	16	174	48

These results are within the lower range of normal and show a great improvement in enzyme output on the original test.(Test 2, Table M, page 189).

The second child was a gross example of nutritional marasmus. After 77 days of treatment his percentage expected weight had risen from 55 to 80% and his serum albumin concentration from 2.71 to 3.40 g. per 100 ml. He made good clinical progress. A repeat PFT showed the following results:-

Test	Volume ml/min/kg	pH units	Amylase u/min/kg	Lipase u/min/kg	RNase u/min/kg	Trypsin u/min/kg	Chymotrypsin u/min/kg
Basal	0.056	8.2	192	133	4	54	127
Secretin	0.079	8.7	281	268	5	119	197
Pancreo-zymin	0.064	8.7	186	238	5	207	161
Mean S+P	0.073	8.7	243	247	5	154	182
Peak	-	9.0	363	370	7	380	255

These results are well within the range of normal.

The results demonstrated in these 2 children suggest that in nutritional marasmus as in acute kwashiorkor, the pancreas recovers its exocrine function with protein repletion. It was interesting to note that chymotrypsin was the enzyme showing the slowest return to normal in the first child. The RNase in this child was the highest ever recorded in this series.

VII. Recovery of pancreatic function in P-C.M.

(a). Acute kwashiorkor. From the work thus far it has been shown that children with acute kwashiorkor recover their exocrine pancreatic function after protein repletion. An attempt was made to determine how soon this recovery begins after the onset of protein repletion and whether an improvement precedes or follows a rise in serum albumin concentration.

Two children with severe kwashiorkor (C.N. and E.M.) were selected and PFT's performed on admission and after 4 days on a protein-rich diet. In the one patient the test was repeated a third time after a further 4 days.

The enzyme results are shown in Table 8. The volume output and pH were within the normal range and did not vary sufficiently between each test to warrant any comment.

The enzyme output was found to increase by the 4th day of dietary therapy. This increase in enzymes occurred before a rise in serum albumin concentration could be detected. After 8 days in the one child (C.N.) the serum albumin concentration had begun to rise accompanied by a further rise in enzyme output.

(b). Chronic P-C.M. ("Five-year follow-up"). After approximately 1 month's dietary therapy all the children with kwashiorkor showed a dramatic return to normal of their exocrine pancreatic function. It was decided to reinvestigate 2 children in the "follow-up" group

TABLE 8.

RATE OF RECOVERY - ACUTE KWASHIORKOR.

Patient	C. N.			E. M.	
Test Number	55	56	58	57	59
Treatment (Days)	0	4	8	0	4
Serum Albumin Concentration (g. per 100 ml.)	1.16	1.12	1.34	1.62	1.49
	Units per min. per kg.				
Amylase:					
B	0	6	14	0	21
S	0	10	31	0	35
P	0	19	23	0	35
S+P	0	14	28	0	35
Peak	0	22	48	0	65
Lipase:					
B	0	3	11	3	44
S	0	9	44	34	112
P	0	39	70	18	64
S+P	0	21	54	28	93
Peak	0	59	108	48	215
RNase:					
B	0	0	1	2	1
S	1	0	1	2	2
P	1	1	4	2	1
S+P	1	0	2	2	2
Peak	2	1	6	2	5
Trypsin:					
B	0	2	8	6	19
S	1	7	14	16	54
P	3	18	31	10	24
S+P	2	11	21	14	42
Peak	3	27	50	20	119
Chymotrypsin:					
B	0	0	0	0	9
S	0	0	3	0	20
P	0	0	9	0	11
S+P	0	0	5	0	17
Peak	0	0	15	0	47
Total Enzymes:					
S+P	3	46	110	44	189

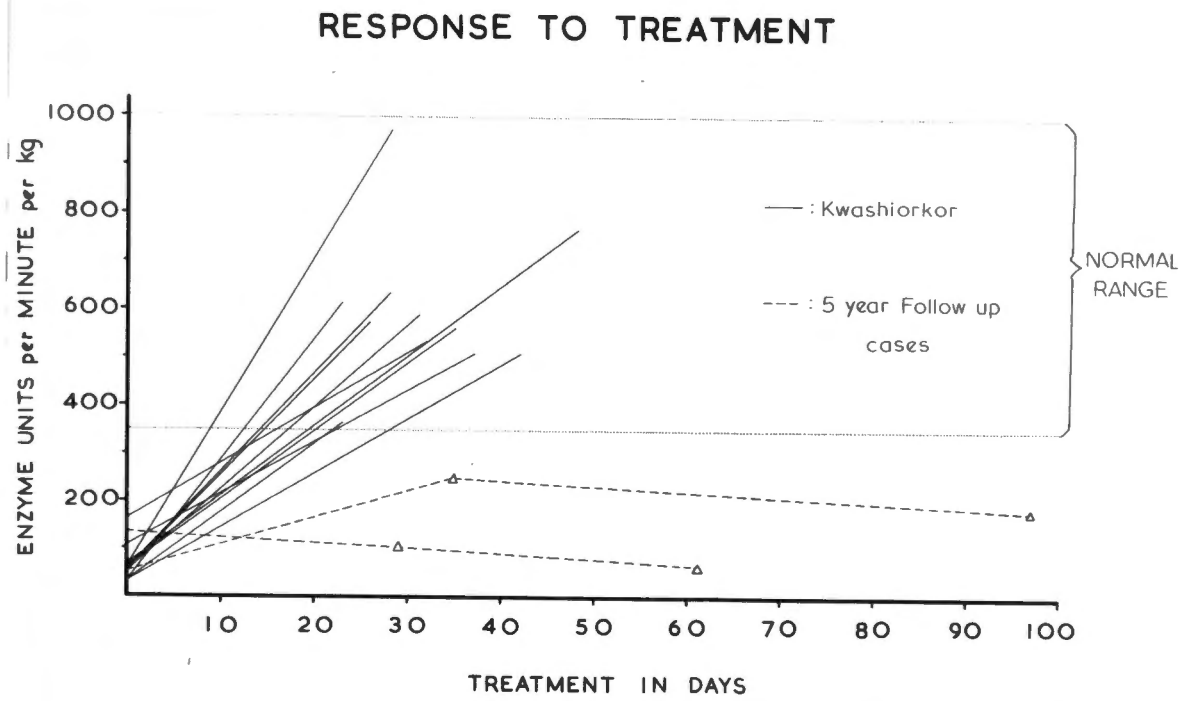


Figure 14.

Response to treatment - total enzyme output before and after protein repletion. (Related to time).

(Patients number 42 and 50, Table 5, page 69) after 1 month in hospital on a nutritious diet. This was done to determine whether they would also recover their exocrine pancreatic function; their initial PFT's were grossly abnormal.

The enzyme results of the serial PFT's are recorded in Table 9. The difference in the volume output between the successive tests did not materially affect the results. The pH readings were normal.

After 1 month's dietary therapy, the one child (G.M.)showed no improvement and the other (W. v.d. B.) a minimal increase in enzyme output. In the former the serum albumin concentration had returned to normal at the time of retesting but in the latter the rise in albumin concentration was negligible.

After a further month's therapy in the one child and 2 months in the other, there was still no improvement in pancreatic function despite general clinical improvement and a rise in serum albumin concentration to levels greater than 3.5 g. per 100 ml. (Figures 14 and 15). Unlike the children in all the other clinical groups, these patients had an abnormal pancreatic function despite a normal serum albumin concentration. (See page127).

These results suggest that in acute P-C.M. the exocrine pancreatic dysfunction at an acinar level could be corrected by protein repletion. However, if the nutritional insult to the pancreas was prolonged, as in the case of the "5-year follow-up" children, irreversible damage may result.

One patient (W. v.d. B.) was investigated more fully to try to gain a more complete picture of his physical and biochemical state and to perhaps find a primary cause for his pancreatic dysfunction.

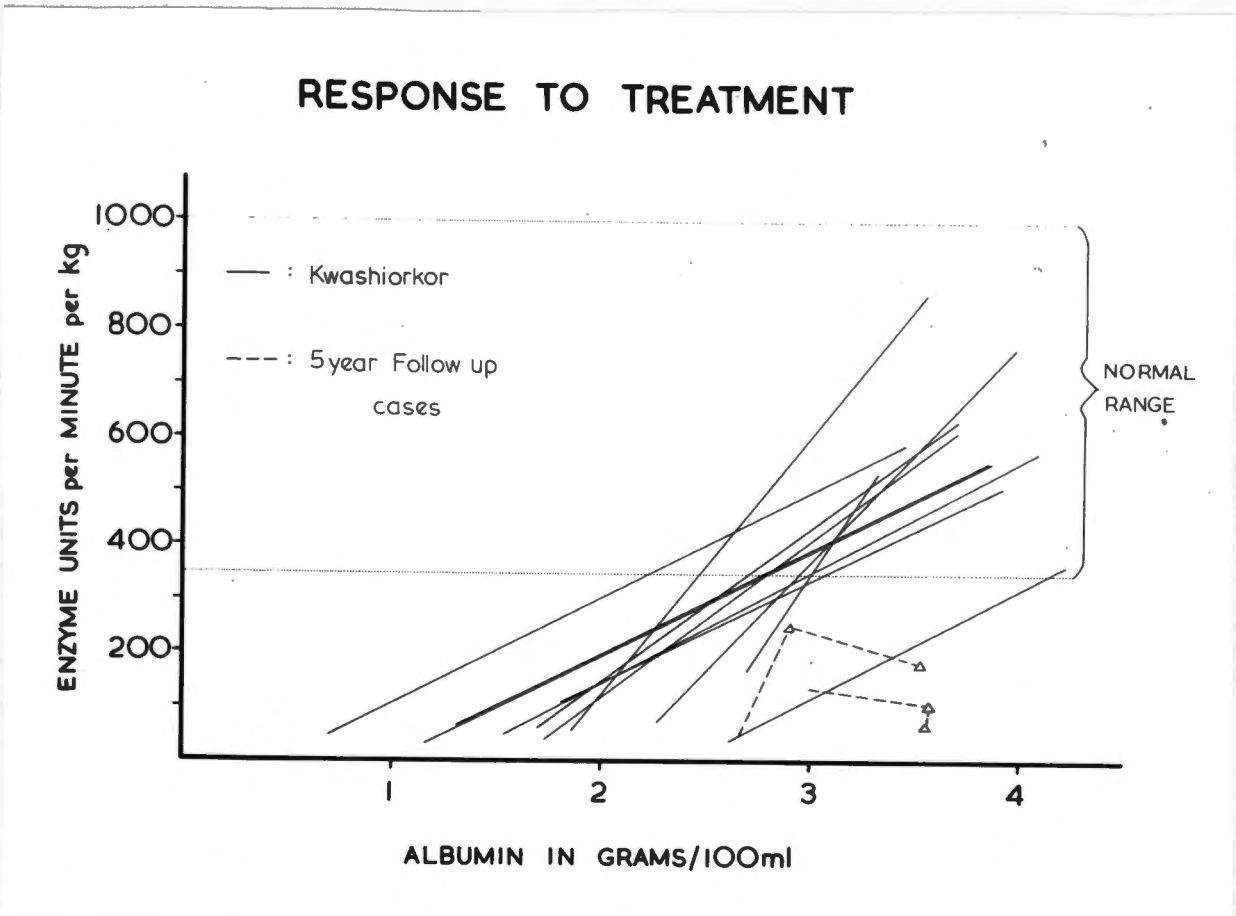


Figure 15.

Response to treatment - total enzyme output before and after protein repletion. (Related to serum albumin concentration).

TABLE 9.

Repeated P.F.T's in "5- year follow-up" Children.

Patient	W. v.d. B.			G. M.		
Test Number	42	60	63	50	61	62
Treatment (Days)	0	35	97	0	29	61
Serum Albumin (g. per 100 ml.)	2.66	2.90	3.52	2.99	3.57	3.55
	Units per min. per kg.					
Amylase:						
B	9	29	167	3	2	1
S	23	142	91	13	15	15
P	27	140	138	12	15	17
S+P	24	141	110	12	15	16
Peak	44	217	230	26	29	17
Lipase:						
B	5	15	78	5	6	1
S	12	54	37	50	57	21
P	15	88	45	115	76	42
S+P	13	67	40	76	65	32
Peak	27	138	78	201	151	42
RNase:						
B	1	1	2	0	1	0
S	2	3	1	1	2	1
P	2	5	2	4	14	4
S+P	2	4	1	2	5	3
Peak	3	7	2	5	14	4
Trypsin:						
B	1	2	34	2	1	0
S	5	18	7	17	6	3
P	10	29	25	34	14	8
S+P	7	22	14	24	9	6
Peak	17	41	41	55	25	8
Chymotrypsin:						
B	0	2	38	2	0	0
S	1	15	10	14	5	4
P	3	18	24	33	18	12
S+P	2	16	16	22	10	8
Peak	5	26	40	56	30	12
Total Enzymes:						
S+P	48	250	181	136	104	65

The following tests were performed:-

Blood chemistry - Urea 24 mg/100 ml.

Sodium 134 m. equiv./litre

Potassium 4.7

Chloride 107

Bicarbonate 24.1

Calcium 10.3 mg/100 ml.

Inorganic phosphorus 6.5 mg/100 ml.

Liver function tests - Thymol turbidity 5 units

Thymol flocculation +

Zinc turbidity 18 units

Alkaline phosphatase 6.5 units

Serum glutamic-pyruvic transaminase (SGPT) 20 units

Serum amylase - 75 Somogyi units

Sweat electrolytes - Sodium 17.7 m.equiv./L

Chloride 19.7

Serum proteins in g/100 ml. - (and body weight in grams).

	22/12/66	27/1/66	2/3/66	30/3/66	14/4/66	4/5/66*	7/7/66
Albumin	2.66	2.74	2.90	3.01	2.96	3.52	3.39
Globulin	3.34	3.97	3.67	3.84	4.39	4.29	4.05
Body wt.	19,012	20,250	20,586	-	22,724	22,950	-

* (Discharged from hospital 5/5/66).

Wasserman Reaction - Negative.

Haematology - Haemoglobin 12.75 g./100 ml.

Packed cell volume 35.0%

Mean corpuscular haemoglobin content 36.43

Leucocyte count 17,400/mm^3

Differential leucocyte count:-

Polymorphs 61%

Lymphocytes 23%

Monocytes 4%

Eosinophils 12%

Basophils 0%

Platelets 435,000/mm^3

Microscopy of duodenal juice -

	22/12/66	3/3/66	3/5/66
Giardia lamblia	+	-	-
Ascaris lumbricoides	+	+	-
Scanty pus cells	+	+	+

Glucose tolerance tests - (True blood glucose in mg/100 ml. was measured by a glucose oxidase method).

	27/4/66	29/4/66	2/5/66	4/5/66
Fasting	100.1	99.9	110.0	109.7
30 mins.	165.5		152.2	
60 mins.	173.8		116.5	
90 mins.	160.5		117.3	
120 mins.	124.1		99.9	
150 mins.			66.6	
180 mins.			90.3	

(Glucose was given in a dose of 2 g. per kg. body weight).

Urinalysis - No abnormality detected. There was never any glycosuria even during a glucose tolerance test.

Urinary amino acids - There was no significant aminoaciduria.

Xylose absorption - 5 hour urine - 70.3% absorption

24 " " - 70.9% "

Nitrogen balance - (Diet of maize meal, milk, cream and eggs).

Absorption 81%

Retention 29%

Fat balance - Absorption 91%

Stool examination - Microscopy: Initially ova of *Ascaris lumbricoides* and *Giardia lamblia.* These disappeared with appropriate therapy.

Culture:- No pathogenic organisms were cultured.

A summary of the positive findings in this investigation revealed the following:- Moderately abnormal liver function tests associated with a high serum globulin concentration; a leucocytosis and eosinophilia associated with recent helminthic infestation; hypoalbuminaemia which showed a slow return to normal levels on a high protein diet; a persistently raised fasting blood sugar with one abnormal glucose tolerance test; a borderline normal absorption of nitrogen and a minimally abnormal absorption of fat. No specific cause for his pancreatic deficiency could be demonstrated.

VIII. Enzyme output in relation to serum albumin concentration.

The clinical improvement in the children suffering with P-C.M. ran parallel with a rise in their serum albumin concentration. This being an indication of their protein repletion, it was thought likely that this might also reflect the recovery of the pancreas, an organ with a very rapid protein turnover.

An attempt was thus made to correlate the enzyme output in

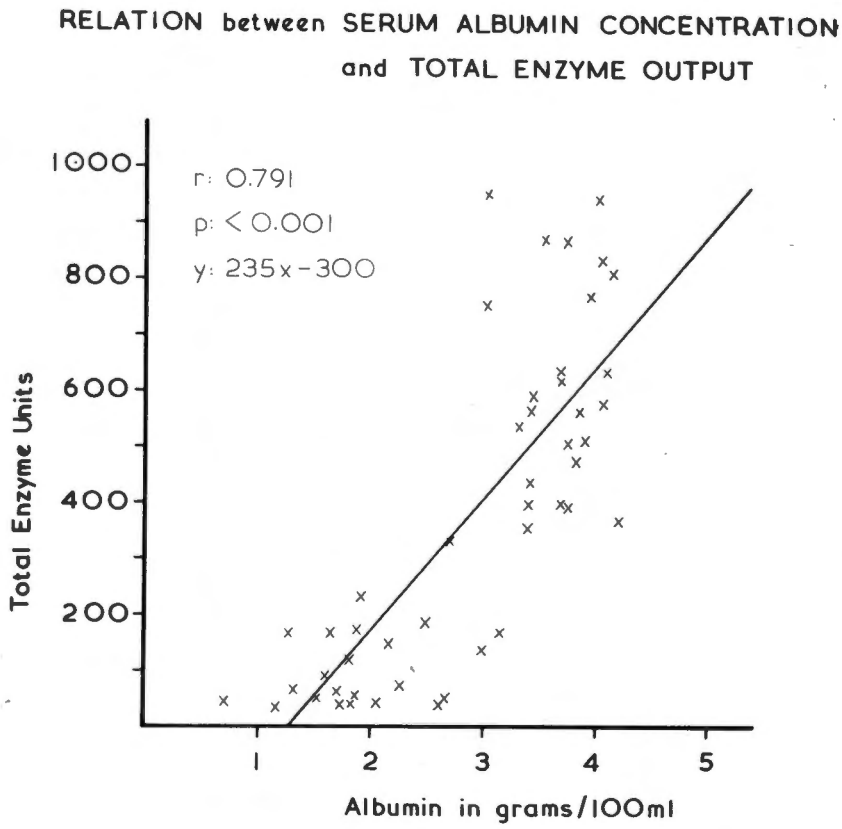

RELATION between SERUM ALBUMIN CONCENTRATION
and TOTAL ENZYME OUTPUT
r: 0.791
p: < 0.001
y: 235x - 300
1000
800
600
400
200
Total Enzyme Units
1
2
3
4
5
Albumin in grams/100ml

Figure 16.

units per minute per kilogram body weight for each enzyme with the serum albumin concentration in grams per 100 ml. This was calculated for the children in the protein-calorie malnourished groups as a whole(kwashiorkor, marasmus and "follow-up" groups) and for all the children tested including "controls" and those who had recovered from kwashiorkor.

As may be seen in Table 10, the correlation between the serum albumin concentration and the basal enzyme output was statistically significant except in the case of trypsin and ribonuclease among the children who were suffering from P-C.M.; these enzymes were proportionally not as depressed as the others. However, after stimulation with secretin and pancreozymin the correlation between serum albumin concentration and enzyme output became highly significant for all enzymes, whether the children with P-C.M. were considered separately or as part of all the children tested. Once again the correlation coefficient was lower for trypsin and ribonuclease than for the other enzymes.

Figure 16 demonstrates the relationship between the total enzyme output (the sum of the mean of the combined secretin and pancreozymin tests for each enzyme) and the serum albumin concentration. This parameter may seem to be derived from a very heterogeneous mixture of enzymes, but its use is justified for the following reasons:- (a) all the enzymes (except RNase) have a similar normal range in pancreatic juice; (b) little is known of the function of RNase in pancreatic juice, and its function is to some extent duplicated by the proteolytic enzymes in the gut (see page 90) - it, therefore, contributes little to this parameter; and (c) it gives an indication of the total output of enzyme activity from the pancreas in a given time under standard conditions.

The relation between enzyme output and serum albumin

TABLE 10.

The Relation between Serum Albumin Concentration in grams per 100 ml. and Enzyme Output in units per minute per kg. body weight.

Enzyme	P.C.M. 30 Tests Correlation Coefficient r	P.C.M. 30 Tests Significance P	All groups 49 Tests Correlation Coefficient r	All groups 49 Tests Significance P
Amylase : B	0.495	0.01	0.444	< 0.01
S + P	0.763	0.001	0.771	< 0.001
Lipase : B	0.452	0.02	0.477	< 0.001
S + P	0.645	0.001	0.762	< 0.001
RNase : B	0.106	N.S.	0.392	< 0.01
S + P	0.482	0.01	0.483	< 0.01
Trypsin : B	0.318	N.S.	0.440	< 0.01
S +P	0.561	0.01	0.596	< 0.001
Chymotrypsin: B	0.579	0.001	0.559	< 0.001
S + P	0.757	0.001	0.639	<0.001
Total: S + P	0.746	0.001	0.791	< 0.001

B = Basal, S + P = Mean Secretin + Pancreozymin

N.S. = Not significant.

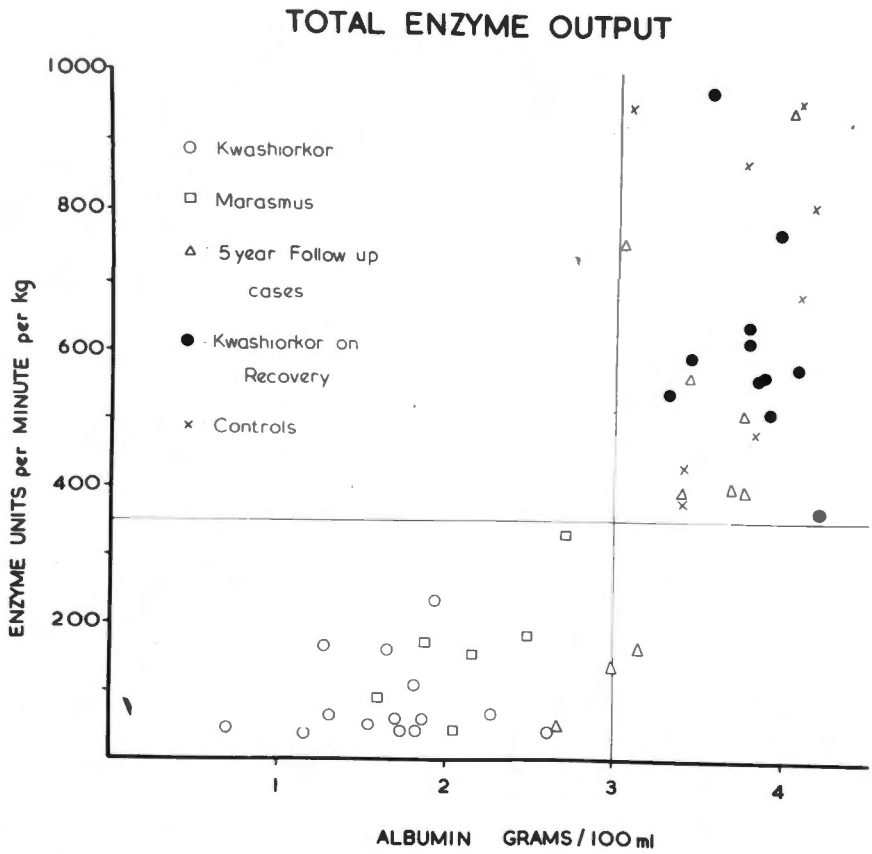


Figure 17.

Relation between serum albumin concentration and total enzyme output in the different clinical groups.

concentration brought to light some significant facts when each patient was considered as part of a clinical group.(Figure 17). The children with kwashiorkor and marasmus who had a low serum albumin concentration had a low enzyme output; the children with marasmus who tended to have a higher serum albumin concentration than the patients with kwashiorkor also had a slightly better enzyme output. All the patients who had a serum albumin concentration of less than 3g. per 100 ml. had an abnormal enzyme output. All the patients who had a serum albumin concentration greater than 3 g. per 100 ml. had an enzyme output within the normal range with 1 exception; this exception was a child in the "five-year follow-up" group. (Patient 51, Table 5, page 69).

It appeared that if protein depletion was of such severity that the serum albumin concentration dropped below 3 g. per 100 ml., the pancreas was unable to produce its optimum quantity of enzymes.

IX. Enzyme output in relation to height and weight.

It was not possible to correlate enzyme output with the percentage expected height or percentage expected weight of the patients; the improvement in pancreatic function with dietary therapy in these patients was not accompanied by a proportional improvement in height and/or weight, but paralleled their improvement in general condition and serum albumin concentration.

X. The effect of sex on pancreatic enzyme deficiency in P-C.M.

Among the patients with kwashiorkor, there were 7 children of each sex. There were no statistically significant differences between the sexes in any parameter of any of the 5 enzymes studied.

There were 2 females and 1 male among the "follow-up" children who had a low enzyme output despite a serum albumin concentration greater than 3 g. per 100 ml.

There is thus no evidence that sex protects the pancreas or renders it more vulnerable to a dietary insult.

XI. Diet and Enzyme Output.

Among the children who had recovered from kwashiorkor, it was observed that the 4 children who had received a diet of chicken, rice and milk had a better chymotrypsin output than the 7 children who had received milk and a standard diet. (Table 11). Despite the small number of children tested, the difference in chymotrypsin output after hormonal stimulation was statistically significant between the groups who had and those who had not received the chicken and rice diet.($P < 0.02$). The basal trypsin output was also significantly greater in the children receiving the chicken and rice diet. ($P < 0.02$).

The only significant differences between the "controls" and the children who had recovered from kwashiorkor were those concerning their amylase output in response to hormonal stimulation; the "control" children demonstrated a more pronounced response.(page 108). It may have been significant that the "control" children were drawn from a section of the population where, despite an adequate protein intake, carbohydrates still formed the bulk of their dietary intake. On the other hand the children who had recovered from kwashiorkor had been receiving a high protein diet during their stay in hospital. This may have contributed towards the difference in amylase response between the 2 groups.

These observations suggest that the enzymes secreted by the pancreas may be qualitatively and quantitatively affected by dietary intake. The time necessary for the enzyme content to be modified is not known.

XII. Giardiasis and the recovery of pancreatic function.

Of the 11 patients who underwent repeat testing of pancreatic function after the cure of kwashiorkor had been initiated, 6 were free of

TABLE 11.

Diet and Enzyme Output.

Enzyme	Chicken and Rice Diet	Standard Diet
Amylase:		
B	198	121
S+P	165	237
Peak	323	406
Lipase:		
B	129	65
S+P	197	159
Peak	339	286
RNase:		
B	2.5	2.1
S+P	3.5	2.6
Peak	6.5	6.0
Trypsin:		
B	66	24
S+P	77	68
Peak	172	156
Chymotrypsin:		
B	134	48
S+P	234	87
Peak	390	170
Albumin (Serum)	3.59	3.88

All figures are mean values for each group.

Enzymes: Units per min. per kg.

Albumin: g. per 100 ml.

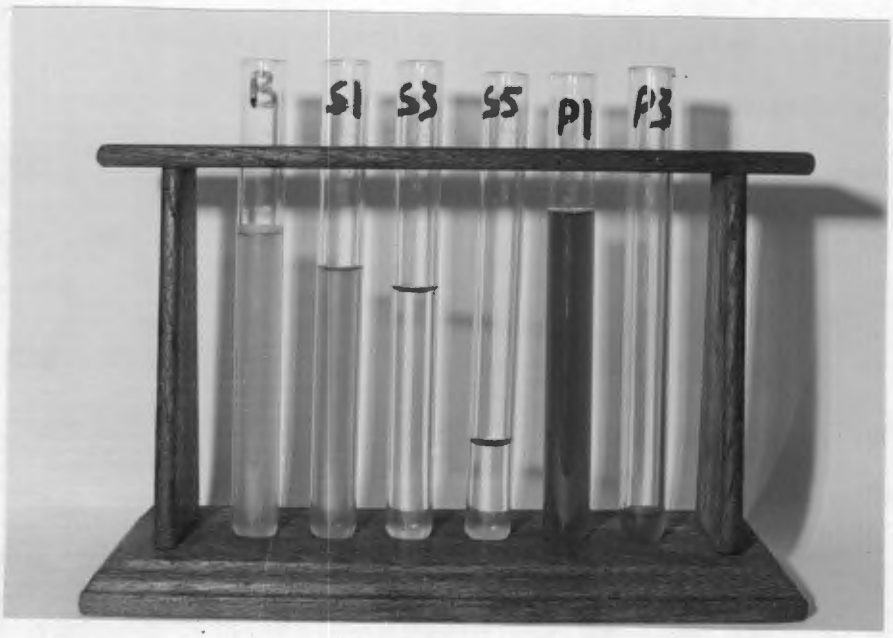


Plate 5.

Juice obtained during a pancreatic function test.

B = Basal.

S1, S3 and S5 = Successive 20 minute periods after the injection of secretin.

P1 and P3 = Successive 20 minute periods after the injection of pancreozymin.

Giardia lamblia at the time of re-testing and 5 were left untreated to determine whether this persistent infestation retarded the recovery of pancreatic function.

All the children, irrespective of whether they were infested with Giardia lamblia or not, showed a return to normal pancreatic function after the initiation of cure of kwashiorkor. However, the peak lipase output, the basal trypsin output and the secretin-stimulated RNase output were all significantly greater in the children free of giardiasis. ($P < 0.05, < 0.02$, and < 0.01 respectively). It was noted that a dietary factor may have played a part in these differences (page 131) (and vice versa) as all the children who had received a chicken and rice diet were among those who were treated for giardiasis.

It can, nevertheless, be said that pancreatic function can return to normal in the presence of giardiasis.

4. Colour Index.

Details of individual tests may be found in the appendix (Tables Q to U, Pages 193 to 197). The details of the statistical analysis may also be found in Tables V and X, pages 205 and 207.

(a). Basal secretion. During the period of basal secretion the degree of bile staining of the aspirated juice varied greatly from child to child but was generally not marked.

(b). Response to stimulation. Immediately after the injection of secretin there was often a transient increase in the amount of pigment in the juice owing to a "flushing out" of the duodenum and possibly the terminal part of the bile duct. The juice then became progressively clearer until it reached a water-like clearness in some children; this clear juice was pure pancreatic juice. During the injection of pancreozymin (con-

/taining

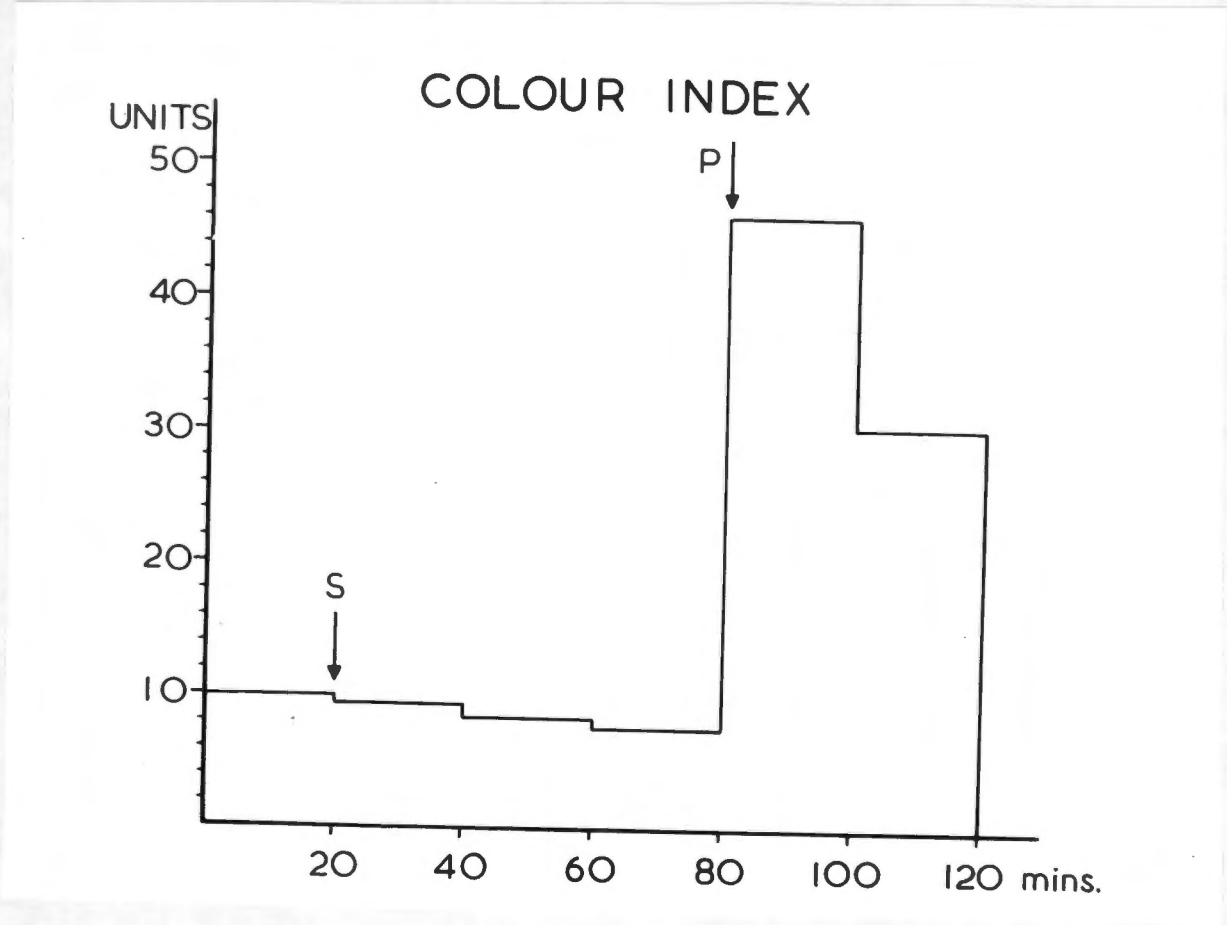


Figure 18.

Colour Index - response to secretin and pancreozymin.

taining cholecystokinin as an impurity) there was a dramatic increase in the degree of biliary pigmentation of the aspirate. Plate 5 illustrates the variation in pigmentation during a typical test. (B = Basal; S1, S3 and S5 each represent successive 20-minute collections after secretin stimulation and P1 and P3 successive 20-minute collections after pancreozymin stimulation). In Figure 18 the mean values in the "control" children are shown graphically, indicating at which points the secretin (S) and pancreozymin (P) were injected.

All the clinical groups showed a significant rise in colour index between the secretin-stimulated juice and the pancreozymin-stimulated juice. (P from < 0.05 to < 0.01).

(c). Comparison between groups. (Figure 19). The colour index did not show much variation between the groups. The "follow-up" children had a higher index than the kwashiorkor children after pancreozymin stimulation ($P < 0.05$) and the peak colour index tended also to be higher among the "follow-up" children. No group showed a significant difference from the "Controls".

(d). Summary. There was usually a mild degree of biliary staining of the basal aspirate; this varied from child to child. The colour index was low in all groups during the period of secretin stimulation.

After the injection of pancreozymin there was a dramatic rise in colour index in all the groups. This rise after pancreozymin reached the highest levels in patients of the "follow-up" group.

Patients with P-C.M. were capable of secreting bile in good quantities.

5. X-Ray of the abdomen.

All the patients on whom pancreatic function tests were performed

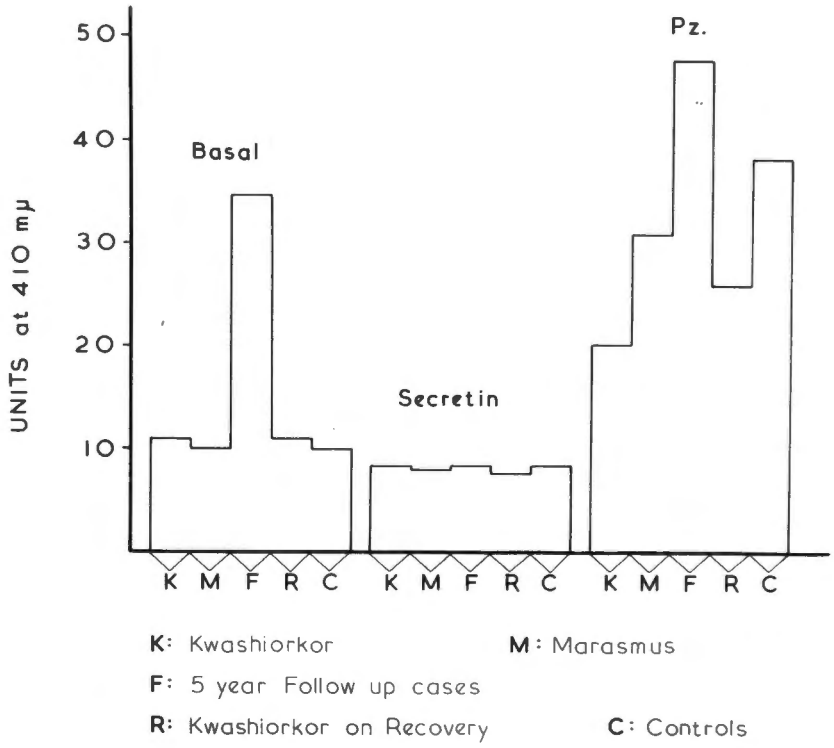


Figure 19.

Colour Index - comparison between groups.

had an x-ray taken to determine the position of the duodenal and gastric tubes. No patients were found to have any suggestion of pancreatic calcification.

In a further series of nearly 50 patients suffering with P.C.M. who were submitted to intestinal biopsies, pancreatic calcification was never encountered on abdominal x-ray.

6. PFT as a diagnostic aid.

(a). Fibrocystic disease of the pancreas.

A child aged 22 months with proven fibrocystic disease of the pancreas (as judged by the history, chronic diarrhoea and malabsorption relieved by oral pancreatin, chronic pulmonary disease confirmed on x-ray, and an abnormally high sweat electrolyte concentration) was submitted to a full PFT.

During the course of the aspiration of the juice, it soon became evident that this child did not respond to pancreatic stimulation in a normal way. The basal aspiration yielded a very small quantity of juice (0.002 ml./min./kg.). After stimulation with secretin and with pancreozymin there was virtually no flow of pancreatic juice; it was calculated that a mean of 0.0002 ml./min./kg. were collected during the 100 minutes after stimulation. There was no doubt about the patency of the tubes or their position. When the tubes were withdrawn the juice and mucus adhering to the duodenal tube were extremely thick and viscid. There was no lack of gastric juice; 65 ml. of watery juice (pH3) were collected during the 60 minutes of basal aspiration. After the injection of secretin and pancreozymin only 2.5 ml. of juice was aspirated from the stomach. It was possible that some of the 1.3 ml. of juice collected from the duodenum during the basal aspiration period represented some residual gastric juice in the duodenum.

Owing to the small volume of juice aspirated, the enzyme assay was limited. The concentration of chymotrypsin in the basal juice was greatly reduced (29 units per ml.) while trypsin was completely absent. In no child with kwashiorkor was the trypsin completely absent, although an absence of chymotrypsin was frequently encountered. There was insufficient juice to permit any enzyme assay after stimulation.

The duodenal juice was alkaline, having a pH of 8.5 under basal conditions and 8.6 after stimulation. With such small volumes of juice it was not possible to comment on the capacity of the ductule cells to produce bicarbonate.

The findings in this child confirm those of Gibbs (131) and Maddock et al (255) who described a failure to respond to pancreatic stimulation with secretin among children with fibrocystic disease of the pancreas.

(b). Diarrhoea of uncertain origin. A female child aged 4 years 4 months and weighing 23 pounds, who had chronic diarrhoea from the age of 10 months was referred to the investigator for an opinion on pancreatic function. Previous investigation in another centre had shown a raised sweat electrolyte concentration as determined by the screening test using an agar plate impregnated with silver nitrate. The quantitative estimation of the sweat electrolyte concentration was repeatedly normal. There were repeated infections, mostly viral (herpes stomatitis), but no radiological signs of chronic pulmonary disease. The xylose tolerance test was normal as was the haematological picture. Her serum albumin concentration was normal, but serum electrophoresis revealed her to have a hypogammaglobulinaemia.

A full PFT was performed as described previously (page 76)

in an attempt to determine the cause of her diarrhoea. The results were as follows:-

Test	Volume	pH	Amylase	Lipase	RNase	Trypsin	Chymo-trypsin
	ml/min/kg	units	ml/min/kg	ml/min/kg	ml/min/kg	ml/min/kg	ml/min/kg
Basal	0.036	7.0	75	90	5	60	28
Secretin	0.042	8.9	93	104	2	40	22
Pancreo-zymin	0.044	8.6	203	158	7	112	58
S+P	0.043	8.7	137	125	4	69	36
Peak	-	9.5	336	263	12	36	97

From these results it could be concluded that the diarrhoea and failure to thrive in this child were not of pancreatic origin. The fact that they were in the lower range of normal could be adequately explained by general debility.

Microscopy of the duodenal aspirate showed nil of note.

B. Microscopy of the duodenal aspirate.

1. Kwashiorkor.

Of the 17 children studied in this group, 12 (71%) had *Giardia lamblia* in their duodenal aspirate. In some children this infestation was so heavy that the flagellates could be seen in their hundreds in each different plane of the same field viewed under the microscope. Boiling the tubes for 3 minutes before each intubation prevented transferring the infestation from one child to the next. Five children (29%) showed the presence of a fungus with hyphae and spores morphologically resembling those of *Candida albicans*. It was noted that the 5 children who had a fungus in their duodenal aspirate were the same 5 children who did not have giardiasis.

The presence of polymorphonuclear lymphocytes in the duodenal juice was commonly observed (12 children - 71%); however, in only 2 of these (12%) was the exudate a very striking one, the remainder having only a few scattered cells visible. It was not possible to correlate the severity of the cellular exudate with the severity of the parasitic infestations. In one child the ova of Trichuris trichuria were seen.

2. Marasmus.

Among the 7 children with marasmus 4 (57%) were shown to have Giardia lamblia in their duodenal aspirate. One of these children with giardiasis and one other (a total of 29%) had spores and hyphae suggestive of monilia in their juice. In 2 children the ova of Ascaris lumbricoides were seen. The presence of pus cells was noted in 6 (86%) of these patients, but in only 1 child was this of any severity.

It is interesting to note that of the kwashiorkor and marasmus patients Giardia lamblia, in the vegetative form or cysts, was detected in the stools in only 11% of specimens by the routine hospital laboratory. In 1 of these children the duodenal aspirate had failed to reveal the parasites.

3. "Five-year follow-up" group.

Seven of the 10 children in this group showed infestation with Giardia lamblia. None of these children showed the presence of a fungus; these children were significantly older than those with kwashiorkor and marasmus. One child showed a very heavy infestation with Ascaris lumbricoides; up to 23 ova were seen on 1 low-power microscope field. Nine of the 10 children had pus cells in their duodenal juice, but these were significantly numerous on only 2 occasions. In 1 child there was a large number of epithelial cells, and in 1 other numerous cholesterol crystals were seen.

4. Recovered kwashiorkor.

All the children who were given mepacrine for the treatment of their giardiasis were free of this infestation on repeat testing. The remaining 6 who were untreated showed the persistent presence of these flagellates despite their nutritional improvement. The 3 children given mycostatin for their fungal infection were fungus-free at the time of re-testing. Eight of the 11 children submitted to repeat testing (83%) had pus cells in their duodenal aspirate and of these, 2 showed a heavy cellular exudate. One child had the ova of Ascaris lumbricoides in his duodenal juice. In 2 children the typical "fern" formation produced by the crystallization of sodium* was seen; this was a reflection of the high sodium* content of the juice. *(Chloride salt)

5. "Controls".

Eight tests were performed on 7 children in this group. In 4 of the tests, the examination was positive for giardiasis. The one child was positive twice despite treatment with mepacrine between the 2 tests; it was probable that re-infection occurred during the 2 months between the tests as he was at home during this period. All the children in this group showed the presence of some pus cells in their duodenal aspirate, but in none of them did this exceed a few scattered cells. None of these children had fungus or ova in their aspirate. In 1 child numerous epithelial cells were seen.

Conclusion:- (Table 12). The incidence of infestation with Giardia lamblia among children with P-C.M. is high (68%). Among the younger children the presence of a fungus in the duodenum was commonly found. The finding of a few scattered pus cells in the duodenal aspirate was so common that it was regarded of doubtful pathological significance (possibly a reaction to the duodenal tube). Helminthic ova were not uncommonly found in the duodenum.

TABLE 12.

DUODENAL JUICE MICROSCOPY - SUMMARY.

	Kwashiorkor	Marasmus	Five-year follow-up	Recovered Kwashiorkor	Recovered Marasmus	Controls	Kwashiorkor + Marasmus + Five-year follow-up
Number studied	17	7	10	11	2	8	34
Giardiasis	12	4	7	6*	-	4	23
Moniliasis	5	2	-	-	-	-	7
Ova:-							
Ascaris	-	2	1	1	-	-	3
Tr. trichuria	1	-	-	-	-	-	1
Cellular Exudate:-							
0	5	1	1	3	-	-	7
+	10	5	7	5	1	8	22
++	2	1	2	3	1	-	5
Epithelial Cells	-	-	1	-	-	1	1
Crystals:-							
Sodium Chloride	-	-	-	2	-	-	-
Cholesterol	-	-	1	-	-	-	1

* Untreated for Giardiasis.

C. Histology.

The post-mortem histology of 50 patients was reviewed with particular reference to the pancreas and the liver. Thirty-eight of these patients had died with a diagnosis of kwashiorkor and 12 with a diagnosis of marasmus. The mean age of the children in the kwashiorkor group was 18 months (S.D. ± 7 months) and of those in the marasmus group 19 months (S.D. ± 10 months).

1. Pancreas.

(a). Autolysis. (Table 13). This was absent or minimal in most of the sections examined. Moderate autolysis was present in 12 slides (31%) in the kwashiorkor group, 2 of which were of a patchy distribution around ruptured ducts. In the marasmus group moderate autolysis was found in 1 section, and severe autolysis of a patchy distribution in 1 other.

TABLE 13.

Autolysis	Kwashiorkor		Marasmus	
	No.	%	No.	%
0 to +	26	68	10	83
++	12	31	1	8
+++	0	0	1	8
Patchy	2	5	1	8

(b). Acinar structure. (Table 14). Structural appearance is reported here irrespective of the presence or absence of zymogen granules; the latter feature is discussed later. Only 1 (3%) of the children in the

kwashiorkor group had a normal acinar structure; 6 (16%) had mild acinar atrophy, 10 (26%) had moderate atrophy, and the majority (55%) showed evidence of severe atrophy with complete disruption of acinar structure. In contrast, 50% of the children from the marasmus group had a normal acinar structure, 33% had mildly atrophic acini, and only 17% had severely affected acini. Seven from the kwashiorkor group (18%) and 1 from the marasmus group (8%) demonstrated the patchy atrophy suggestive of a regenerating pancreas. This was probably related to the onset of protein repletion.

TABLE 14.

Acinar structure	Kwashiorkor		Marasmus	
	No.	%	No.	%
Normal	1	3	6	50
Mild Atrophy	6	16	4	33
Moderate "	10	26	0	0
Severe "	21	55	2	17
Patchy "	7	18	1	8

(c). Zymogen granules. (Table 15). A normal complement of granules could not be detected in any of the sections in the kwashiorkor group. In a few of these children (16%) a few granules could be detected in patches of acini in the section; this suggested that this was due to therapy. The remainder (84%) had no detectable zymogen granules. Among the children of the marasmus group, granules were seen in normal quantities in 1 section (8%); they were present but reduced in quantity in 6 sections (50%) and entirely absent in the remainder (42%). It was noted that although 1 child

with kwashiorkor had a normal acinar structure, none had a normal zymogen granule content.

TABLE 15.

Zymogen Granules	Kwashiorkor		Marasmus	
	No.	%	No.	%
Normal	0	0	1	8
Decreased	6	16	6	50
Absent	32	84	5	42

(d). Ducts. (Table 16). In all but 1 of the kwashiorkor group the ducts were normal in architecture, but in 17 (45%) of the sections there was a relative excess of normal ducts owing to the marked acinar atrophy. The 1 slide which showed the presence of abnormal ducts also showed evidence of acute haemorrhagic pancreatitis; this adequately explained the disruption of the duct system. The duct architecture was normal in all the sections in the marasmus group; the 2 sections demonstrating severe acinar atrophy also showed a relative excess of the number of ducts. No cysts were seen in any of the sections.

TABLE 16.

Ducts	Kwashiorkor		Marasmus	
	No.	%	No.	%
Normal	20	53	10	83
Relative excess	17	45	2	17
Abnormal	1	3	0	0
Cysts	0	0	0	0

(e). Connective Tissue. (Table 17). In the kwashiorkor group the majority of sections (68%) showed a normal distribution of connective tissue. In 21% of the slides there was a relative excess of connective tissue which could be explained by the severe degree of acinar atrophy. The connective tissue was very oedematous in 9 sections (24%) but the fibrous tissue was not excessive in quantity or abnormal in distribution in these sections. However, in 4 slides (11%) there was an absolute excess of connective tissue representing a true fibrosis; of these 4 sections, 3 had definite evidence of an inflammatory process with the exudation of polymorphonuclear and mononuclear cells and some erythrocytes into the connective tissue spaces. Thus the fibrosis under these circumstances was secondary to a pancreatitis. Although a septicaemia was suspected clinically in these children with pancreatitis, this was not proven by a positive blood culture. One section remained in which fibrosis was demonstrated without a primary cause being found. In all the sections from the marasmus group the connective tissue was normal in quantity and distribution.

TABLE 17.

Connective Tissue	Kwashiorkor		Marasmus	
	No.	%	No.	%
Normal	26	68	12	100
Relative Excess	8	21	0	0
Excess and Pancreatitis	3	8	0	0
Fibrosis	1	3	0	0
Oedema	9	24	0	0

(f). Inflammatory Cells. (Table 18). The presence of these cells in any significant quantities was not a regular feature of either the

kwashiorkor group (14%) or the marasmus group (17%). Where they were present in the marasmus group (17%) they were only scanty in number. In the kwashiorkor group, 1 section showed the presence of a moderate and 1 a severe inflammatory cell infiltration both accompanied by extravasated red cells; a further 3 slides showed a mild inflammatory reaction.

TABLE 18.

Inflammatory Cells	Kwashiorkor		Marasmus	
	No.	%	No.	%
Absent	33	87	10	83
Present +	3	8	2	17
++	1	3	0	0
+++	1	3	0	0
Red Blood Cells	2	5	0	0

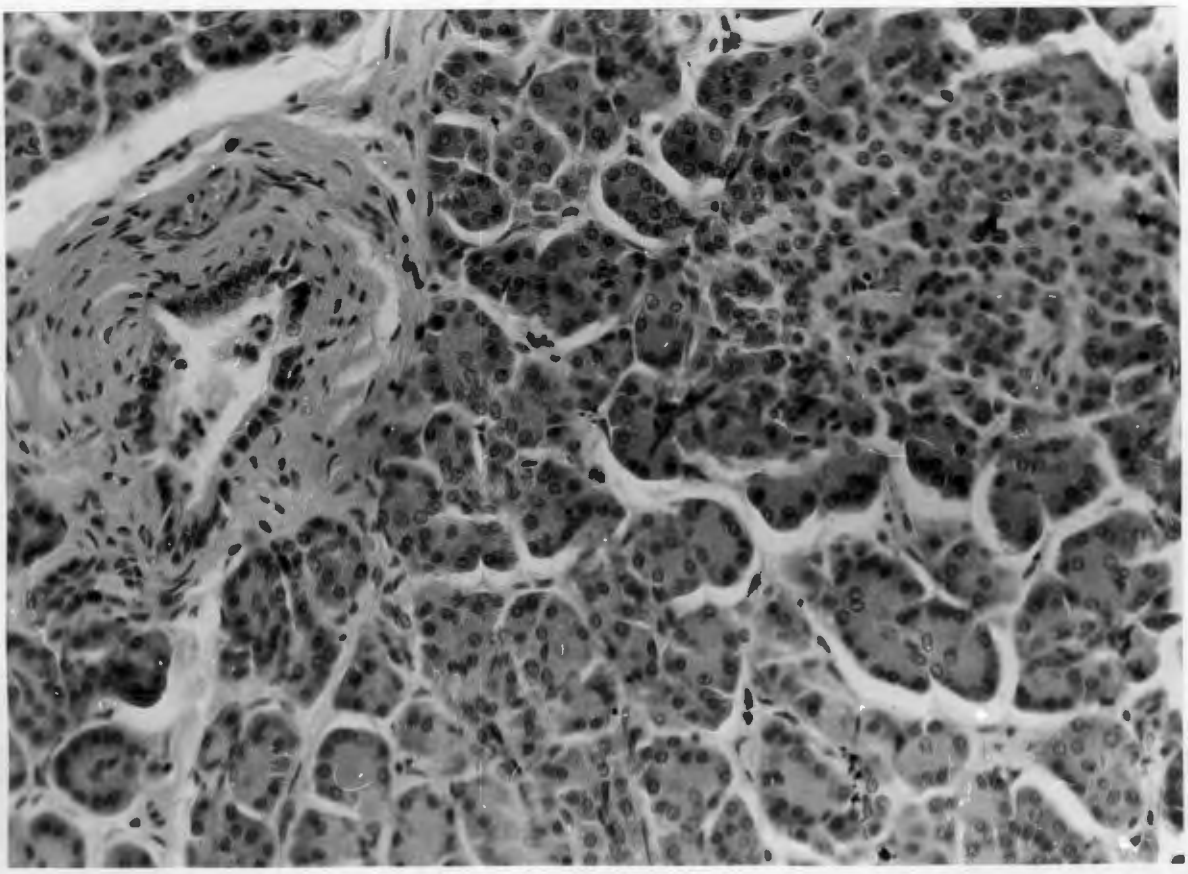
(g). Islets of Langerhans. (Table 19). On routine microscopic examination these structures were not affected by P-C.M. In some sections among the kwashiorkor group (24%) there appeared to be a relative excess of Islets secondary to the severe degree of acinar atrophy.

TABLE 19.

Islets of Langerhans	Kwashiorkor		Marasmus	
	No.	%	No.	%
Normal	38	100	12	100
Abnormal	0	0	0	0
Relative Excess	9	24	0	0

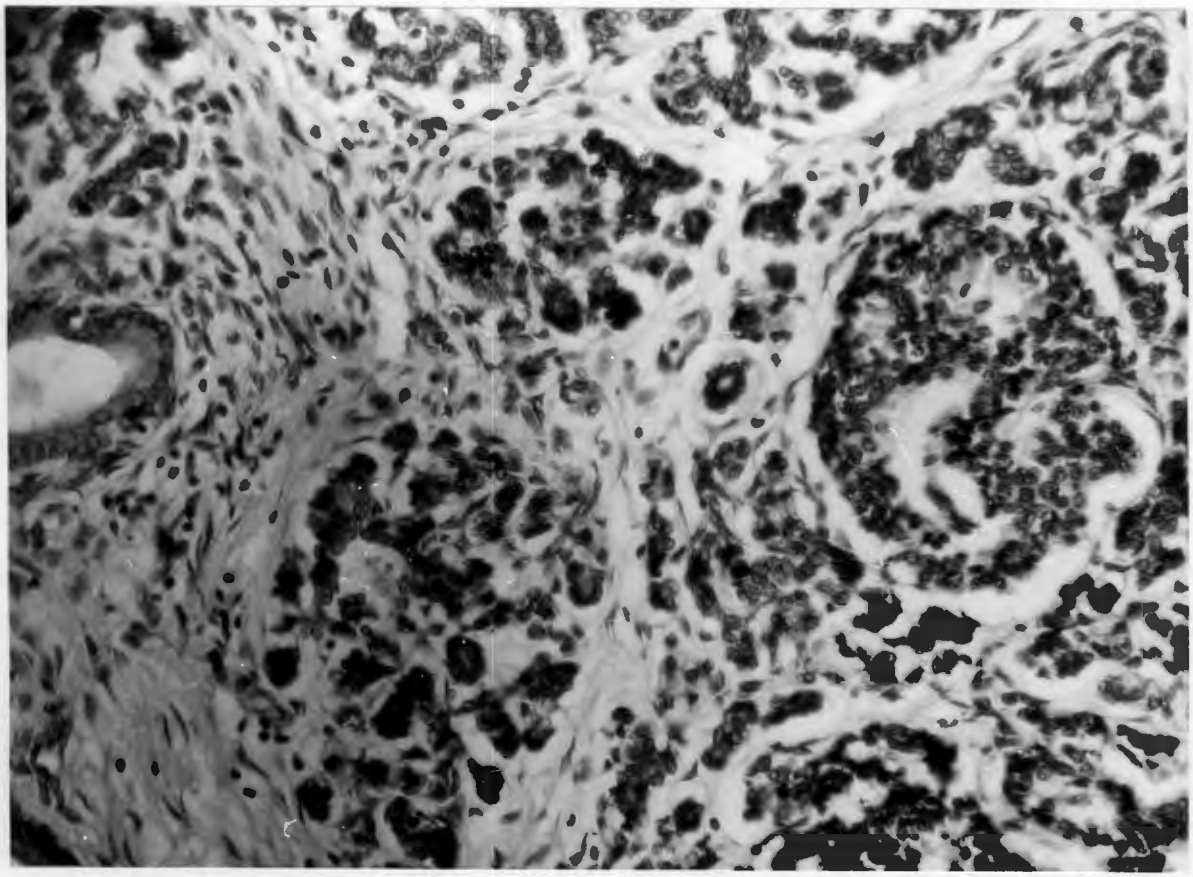
(h). Illustrative plates.

PLATE 6.



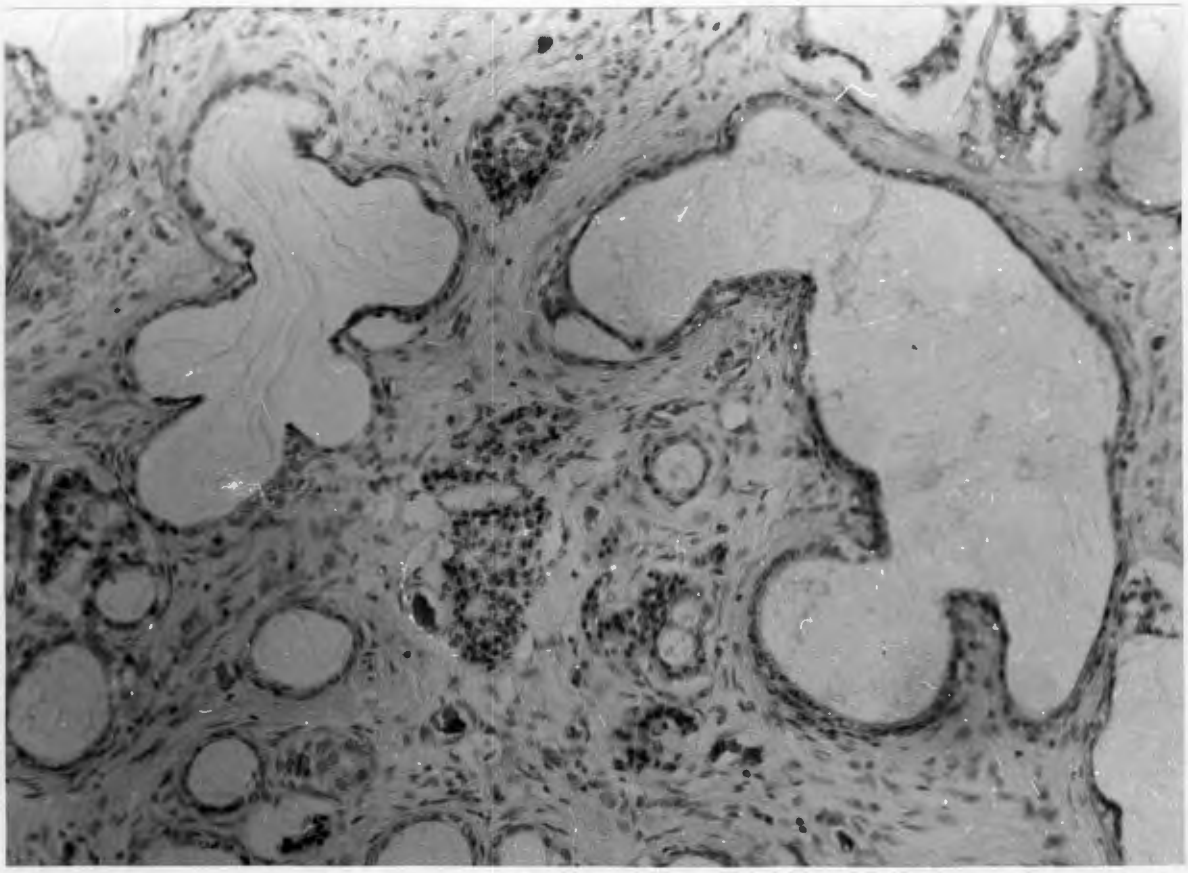
Magnification X 128. The histological picture of normal pancreatic tissue taken from a child who died with marasmus. Note the well formed acini with abundant cytoplasm and numerous zymogen granules. The duct on the upper left side of the photomicrograph demonstrates a normal columnar epithelial lining and is surrounded by connective tissue. There is a normal Islet of Langerhans in the upper right side of the plate.

PLATE 7.



Magnification X 128. This photomicrograph demonstrates the typical findings of severe acinar atrophy; the section is one taken from a child who died with kwashiorkor. Some acini are represented by a ring of nuclei while others are completely fragmented. On first appearance the section appears rather fibrous, but the fibrous tissue present is dissipated and unduly prominent because of the severe degree of acinar atrophy. The duct on the left side shows a normal layer of columnar epithelium and is surrounded by diffusely spread interlobular connective tissue.

PLATE 8.



Magnification X 80. The typical histological picture of fibrocystic disease of the pancreas is demonstrated in this photomicrograph. The pathology of this condition is completely different from that of kwashiorkor. The epithelium of the cystically dilated ducts is cuboidal and the bulk of the pancreas is replaced by fibrous tissue. A few Islets are visible and some remnants of a few acini may be discerned with difficulty. (This photomicrograph was taken at a lower magnification than the other 2 so that a better morphological picture could be obtained).

2. Liver. (Table 20).

None of the children dying with kwashiorkor had a normal liver on histological examination. One slide (3%) in this group showed a mild fatty change, 10 (26%) a moderate degree of fatty change and 27 (71%) demonstrated livers almost entirely replaced by fat. In contrast, 3 (25%) of the sections from the marasmus group had a normal liver, 4 (33%) showed a mild degree of fatty change and a similar number a moderate degree of fatty change. Only 1 section showed the picture of severe fatty change.

In 15 sections in the kwashiorkor group (40%) there was a cellular infiltrate of lymphocytes and polymorphs in the portal tracts. This was seen in only 1 slide from the marasmus group.(8%).

The 3 sections from the marasmus group who had a normal liver histology also had a normal pancreatic acinar structure, although in 2 of these 3 sections there was some degree of zymogen granule depletion. Some sections were found which showed a mild degree of pancreatic acinar involvement with the corresponding slide of the liver showing a marked degree of fatty change. It was impossible to say whether the pancreas or the liver was affected first.

TABLE 20.

Liver	Kwashiorkor		Marasmus	
	No.	%	No.	%
Normal	0	0	3	25
Fatty Change +	1	3	4	33
++	10	26	4	33
+++	27	71	1	8
Cellular Infiltrate	15	40	1	8

Summary of histological study. There was an overlap between the histological features of the pancreas in children dying with kwashiorkor and those dying with marasmus. There was, however, a distinct tendency for the pancreas to be more severely affected in the kwashiorkor group as judged by acinar atrophy and zymogen granule depletion. The pancreatic ducts and Islets of Langerhans were not affected by P-C.M. On the 4 occasions in which a true fibrosis of the pancreas could be demonstrated, all but 1 were related to the presence of an acute inflammatory process. The presence of a fatty liver was found in both the marasmus and kwashiorkor groups, but whereas the bulk of the livers in the marasmus group were mildly affected the majority of the kwashiorkor group showed severe fatty change. One quarter of the marasmus children had no evidence of a fatty liver.

SUMMARY OF RESULTS.

A. Pancreatic Function Tests. (PFT).

1. Acute protein-calorie malnutrition. (P-C.M.).

(a). Volume output. This was not affected in acute P-C.M. All the children demonstrated the ability to increase their volume output after hormonal stimulation.

(b). pH. All the children demonstrated a satisfactory increase in duodenal pH after hormonal stimulation.

(c). Enzymes. The patients with severe P-C.M. had a decreased output of all enzymes; this was more marked in kwashiorkor than in marasmus. Chymotrypsin was the most severely affected enzyme and trypsin was generally the least affected enzyme. Lipase was often relatively spared among the marasmic children. RNase and amylase output was decreased in these children.

All the children demonstrated the ability to increase their enzyme output after hormonal stimulation. This was less marked and at a lower level among the patients with P-C.M.

Children with kwashiorkor and marasmus who were treated with a nutritious diet for from 23 to 48 days demonstrated a recovery of their pancreatic enzyme output. Their response to dietary therapy commenced within 4 days of the onset of treatment.

The presence of Giardia lamblia in the duodenum did not prevent the recovery of pancreatic function.

(d). Colour Index. The concentration of bile in the duodenal aspirate showed no consistent differences from that in normal and recovered children. All the children, with a few isolated exceptions, demonstrated good gall-bladder contractability after hormonal stimulation.

2. Chronic P-C.M.

Some children who were chronically malnourished (the "follow-up" group) demonstrated a tendency to a lower volume output and a poorer pH response than children in the acutely malnourished groups. There was a wide range in the output of enzymes and the response to stimulation ranging from that found in kwashiorkor to normality. The output of chymotrypsin was significantly depressed. Patients in this group who had an abnormal enzyme activity were not improved in this respect when fed a nutritious diet for from 61 to 97 days. The colour index tended to be higher in these children than in those of the other groups.

3. General observations.

There was a significant correlation between volume output and body weight after hormonal stimulation.

There was a highly significant positive correlation between serum albumin concentration and the pancreatic enzyme output.

No sex difference was noted among the children with nutritional pancreatic dysfunction.

The diets taken by the patients showed some evidence of qualitatively affecting the enzyme output; provided there was an adequate protein intake, the children on a predominantly carbohydrate diet produced more amylase than those on a high protein diet.

The PFT as used in this study may be used as a diagnostic aid in paediatric patients.

No child showed any evidence of radiological pancreatic calcification.

B. Microscopy of Duodenal Juice.

This demonstrated a high incidence of infestation with *Giardia lamblia*

among malnourished children. Moniliasis was found among numerous younger patients, and the ova of Ascaris lumbricoides were not infrequently seen.

C. Histology.

Pancreatic acinar atrophy was regularly found in children dying with kwashiorkor and to a lesser extent in marasmic children. The ducts and Islets of Langerhans were spared. Three of the 4 sections in which a fibrosis could be demonstrated had a primary inflammatory process.

CHAPTER 8.

Discussion.

A. The assessment of exocrine pancreatic function in children with protein-calorie malnutrition. (P-C.M.).

The use of a dual tube technique and stimulation of the pancreas with secretin and pancreozymin have made possible a more accurate assessment of pancreatic function in young patients. With experience the technique of performing the tests became relatively straightforward and is not beyond the capability of any patient clinician. With due care it may be recommended as a safe procedure in the investigation of gastrointestinal disorders (for example chronic diarrhoea and malabsorption) in paediatric patients. The passing of the tubes into the stomach and duodenum did not present unusual difficulties as shown by the low failure rate. The patients tolerate the tests extremely well with adequate sedation and the continued attention of the investigator. However, in small children (less than 4.5 kg. in weight) the qualitative estimation of pancreatic output becomes more inaccurate than with larger children. It is assumed that there is always an unavoidable loss of juice during the aspiration (107); this constitutes a greater proportion of the total secretion in small children. The side effects which sometimes accompanied the stimulation were always transient and did not lead to any complications or undue discomfort.

Taken as a mean result for a particular group of patients the basal enzyme output generally reflected the findings found after hormonal stimulation but at a much lower level. However, in the individual patient the enzyme concentration in the basal juice yields results which are difficult to interpret. The range of results is great, the lower limit of normal cannot be defined (107),

and pancreatic reserve cannot be determined as it is not possible to know whether the pancreas is actively secreting or "resting" during the time of aspiration. These unsatisfactory parameters have been used in previous pancreatic studies in P-C.M.

Stimulation with hormones enables the pancreatic reserve to be measured, and provides a way of investigating both the hydrelatic and ecbolic functions of the pancreas under standard conditions. It is also clear that drainage of gastric juice through a separate tube will result in greater specificity of a pancreatic function test. An accurate estimation of volume output becomes possible, and the gastric acid does not interfere with enzyme activity or the pH of the duodenal aspirate. The actual output of enzymes in a given time may thus be calculated. (107). In elaborating certain physiological principles, as was done in this study, stimulation with secretin and pancreozymin formed an essential part of the investigation.

The use of both these hormones is indicated for an accurate assessment of pancreatic and biliary function. The administration of secretin resulted in a marked and sustained rise in volume output and in pH whereas this was not achieved with pancreozymin. The increase in volume after pancreozymin was largely due to the outpouring of bile from the gall-bladder and was not sustained. Individual enzyme output after pancreozymin was often raised to levels not achieved by secretin (e.g. chymotrypsin). For a complete test, it will be seen that the action of these 2 hormones is complementary.

The near normal volume output found among children with acute P-C.M. suggests that the pancreatic pathology does not affect the ductules which produce the fluid secretion of this organ. (198, 155). There is no record of this in previous studies on P-C.M. (395). This observation is supported by

the fact that the patients were able to raise the pH of their duodenal juice after hormonal stimulation; the bicarbonate is also produced by the ductule cells. This contrasts dramatically with patients who have fibrocystic disease of the pancreas in whom the ducts are grossly affected. Previous workers have noted that children with this disease failed to respond to secretin stimulation (131, 255); this has been confirmed in the present study. The "Five-year follow-up" patients demonstrated a tendency to a lower volume output which may indicate the presence of more severe pancreatic pathology than in acutely malnourished patients. This may eventually proceed to fibrosis and calcification with all the clinical accompaniments of gross pancreatic disease. (27).

All the children irrespective of their clinical diagnosis, demonstrated the capability of increasing their enzyme output after hormonal stimulation. This indicated that some pancreatic reserve was present although at a depressed level. It is known that the essential amino-acids are reduced in the plasma of children with kwashiorkor.(189a, 321). This deficiency limits the number of "building-bricks" available for enzyme synthesis as pancreatic enzymes are synthesised from amino-acids.(212, 284). The acinar atrophy seen in these patients may also result in an impaired synthesis of enzyme; it is the tissues with the most rapid protein turnover which bear the brunt of protein deficiency.(408). After initiation of cure in the acutely malnourished patients, the same stimulus produced a normal response. The suggestion that it is a lack of stimulation which is the limiting factor in the exocrine pancreatic dysfunction of kwashiorkor(256) is clearly untenable.

The study of a broader enzyme spectrum using specific substrates for

the assay of trypsin and chymotrypsin enables some interesting comparisons to be made. In previous studies using older methods trypsin was equated with "total proteolytic activity" (10, 12, 23, 31, 112, 140, 253, 267); the sensitivity of chymotrypsin and the relative resistance of trypsin to protein depletion in the pancreas was thus missed. Trypsin was never completely absent in the pancreatic juice, even in the most severely affected patients. There was always a good trypsin response to stimulation. It is possible that the presence of trypsin is very important for the recovery of children with P-C.M.; its action on proteins in the gut aids their absorption and enables protein repletion to occur. It is not known why chymotrypsin was the most severely affected enzyme in P-C.M. Its molecular structure, particularly at the active site, is very similar to that of trypsin and its endopeptidase activity is complementary to that of trypsin.(289). It might be postulated that this is a form of enzyme economy, only 1 endopeptidase being regarded as necessary to cope with a low protein intake.

The relatively high concentration of RNase in rat pancreatic juice and its early suppression in states of protein depletion (379) was not found in this study on human subjects. This enzyme plays an important part in the intracellular metabolism of the pancreatic acinar cell (284) but its role in the digestive process in the gut is not clear. The fact that trypsin and chymotrypsin can to some degree hydrolyse ribonucleic acid would suggest that RNase is of minor importance in digestion in the gut. Its concentration in the duodenal aspirate was found to be much less than that of the other enzymes.

A diet which is low in protein and adequate or nearly adequate in carbohydrate leads to the clinical picture of kwashiorkor.(393). Previous workers have demonstrated that pancreatic enzymes can adapt themselves

according to the predominant constituent of the diet. (153, 191). Despite this, the children with kwashiorkor in the present study all had a deficient amylase output. This is a result of their poor protein intake, as this nutrient is essential for enzyme synthesis by the pancreas.(386). In contrast, the "control" children had a very high amylase output. They were drawn from a section of the population where carbohydrates form the bulk of their diet, but in addition they received an adequate protein intake.

There is a distinct and fascinating overlap in the results of the PFT's and the pancreatic histology in patients with kwashiorkor and marasmus. This overlap is understandable if P-C.M. is regarded as a spectrum which includes a number of clinical syndromes. (206, 327). It is unlikely that these conditions are always entirely separate entities as suggested by McCance and Widdowson in a recent publication (266a); their conclusions were drawn from pigs and rats fed under carefully controlled conditions which hardly apply to children seen daily in an outpatient department or in the field. These children eat what they can, but they are all protein deficient. The relatively excessive carbohydrate intake in the children with kwashiorkor may stimulate the pancreas by hormonal and neurogenic mechanisms and be compared with "flogging a tired horse". In some, but not all of the marasmic children with a more balanced dietary deficiency, this factor does not apply. (401). It must be appreciated that marasmus occurs on a low protein, low calorie diet which may be balanced or unbalanced. The protein component may be qualitatively good, as for instance in a child receiving breast or cows' milk in insufficient quantity. However, if the food source is a cereal, then the protein-calorie ratio is unbalanced and the protein of poor quality. It would be interesting to determine whether it is this factor which causes some marasmic children to have poor pancreatic function and/or a fatty liver.

The positive correlation between serum albumin concentration and pancreatic enzyme output has not been previously described. The older methods were not sensitive enough to detect this. (140). The difference between the enzyme output of patients with kwashiorkor and marasmus correlated with the difference in serum albumin concentration in these 2 variants of P-C.M. Some workers have found normal pancreatic function in children with a non-specific nutritional disturbance (as judged by basal tests). (23, 255). These children had presumably not reached a severe enough degree of protein depletion for this to affect their pancreatic enzyme output. It must be emphasised that the children with kwashiorkor and marasmus are those with the most serious forms of P-C.M., while the majority of malnourished children may simply be underweight for age or present with recurrent infections such as gastroenteritis. (413). Protein depletion must be well advanced before the serum proteins are lowered (165) and before pancreatic enzyme output becomes significantly depressed.

Despite the poor pancreatic enzyme output in children with P-C.M., it is surprising that balance studies have revealed a relatively good absorption of nutrients, particularly of nitrogen and fat. (142, 394). The one patient on whom a balance study was performed in this investigation, had grossly abnormal pancreatic function; the absorption of nitrogen and fat, however, was on the lower limits of normality. Analysis of the individual results of balance studies in kwashiorkor recorded in the literature, reveals a very wide range of absorption varying from 48% to 90% (142, 165, 394). The fact that enzymes are catalysts and that very small quantities of these proteins are required for a chemical reaction to occur is an important one. The pancreas also possesses a tremendous reserve (11, 299) and gross dysfunction is necessary before this becomes reflected in abnormal digestion.

Other enzymes such as the carboxypeptidases (289, 406) and the proteolytic enzymes of the intestinal epithelium and gut flora may also play some part in aiding digestion and absorption. Hansen (163) has noted the part played by diarrhoea in aggravating malabsorption. If there is intestinal hurry and poor enzyme function, it is understandable that gross maldigestion and malabsorption will occur; if there is a slow passage of nutrients through the gastrointestinal tract this would allow the small quantities of enzymes present to act and some hydrolysis to take place. (392). Once some protein is absorbed the cycle is commenced where absorbed protein permits the production of more enzymes and also helps to restore the structural integrity of the pancreas. The absorption of nitrogen and fat improves with the recovery of patients with kwashiorkor. (142).

After only 4 days dietary therapy the rapidity of pancreatic recovery in children with acute P-C.M. was very dramatic. A regenerating pancreas is commonly found at autopsy in children dying with kwashiorkor after a few days dietary treatment.(368). Work in this unit has shown that from the first day of protein intake in kwashiorkor there is a significant rise in the concentration of plasma amino-acids in the majority of patients. (321). This phenomenon demonstrates that absorption does take place and this enables more enzymes to be synthesised by the pancreas. (212, 284). In the children with acute P-C.M. this recovery proceeded to complete restoration of normal pancreatic function. Previous workers studying the concentration of enzymes in the basal aspirate also suggested a return to normal function in children with kwashiorkor (140) but no comparison was made with control subjects.

It has been claimed that a failure in the production of digestive enzymes is probably responsible for the diarrhoea associated with malnutrition

(84a). As already discussed, the pancreatic dysfunction may be responsible for a certain amount of malabsorption and diarrhoea, but this must only apply in a significant way to a limited number of severely affected patients. Pancreatic function shows a rapid return to normality with protein repletion in acute P-C.M., but the diarrhoea often persists. The role of lactase deficiency appears to be a very important one in the etiology of diarrhoea among these patients. (44, 45). Our experience here in Cape Town is that the importance of this deficiency eclipses that of the pancreas in this respect. No malnourished child with lactose intolerance has yet been shown to recover lactase activity after dietary therapy which has been continued up to 1 year. The high incidence of enteral infections (414) and infestation with Giardia lamblia must also be of some importance in the production of gastro-intestinal disturbances in malnourished children.

The abnormality of pancreatic function in children with chronic malnutrition raises some interesting problems. It was very significant that the 2 children with the lowest enzyme output did not recover their normal pancreatic function when fed a high protein diet. This may be explained by postulating severe irreversible structural damage to the pancreas as opposed to reversible acinar atrophy. (50). In the one child tested there was already a suspiciously raised fasting blood sugar level which may indicate involvement of the Islets. These findings suggest a distinct possibility that prolonged P-C.M. may result in "silent pancreatitis" (263) or in other gross manifestations of pancreatic deficiency. Young adults and children with this syndrome have been reported from East Africa (27, 28, 332), Ceylon (309) and South Africa. (263, 207). In each of these areas chronic P-C.M. in childhood is common.

The findings of the histological study were compatible with those

anticipated from the study of pancreatic function. Previous studies also described similar changes (47, 305, 392) namely acinar atrophy with a relative sparing of the ducts and Islets . The interlobular fibrosis described by previous workers (85, 259, 377) was, however, not a feature of the sections examined; a relative fibrosis owing to the severe degree of acinar atrophy was frequently encountered. (55). The consequences of a true fibrosis as opposed to a relative fibrosis are very grave; the latter is theoretically reversed with protein repletion while the former is not. One can speculate that this may be the difference between children with acute and chronic malnutrition as studied in this investigation. The true fibrosis that was seen was almost invariably secondary to an inflammatory process; this illustrates how severe pathology may occur in inaccessible organs without the knowledge of the clinician during the acute phase of P-C.M.

B. Duodenal juice microscopy.

There was a remarkably high incidence of intestinal parasites found among malnourished children. Giardia lamblia was the most striking pathogen encountered. The incidence of infestation with this parasite quoted in the literature varies from as low as 3.09% (270) to as high as 32%.(76). Vis et al found the incidence to be 30.8% among children with P-C.M.(382). However, when children are examined after prolonged institutionalisation an incidence as high as 79% has been recorded.(51). This figure is similar to the one found among patients with P-C.M. in this investigation (71%). In previous studies stool microscopy was performed to establish the diagnosis; this is not as reliable as examining the duodenal contents.(61). The symptoms attributed to this parasite vary from steatorrhoea, abdominal pain, anaemia (371, 372) and lactose intolerance (110, 290) to no detectable

symptoms at all.(62). In the present study no symptoms or signs specific to the infestation were detected; most of the children had diarrhoea, but to attribute the cause of this to giardiasis alone would not be justified. Those children who were left untreated until the time of their repeat PFT did not show any slower or less satisfactory clinical recovery. Previous workers among adults have shown a spontaneous diasppearance of these parasites within 5 to 41 days (311); this did not occur in the present study. It is not doubted that giardiasis can lead to symptoms and signs, but a direct relationship could not be established.

The presence of a fungus in the duodenal aspirate was only found among the youngest children; this corresponds with the finding that oral thrush usually occurs in patients less than 1 year of age. Although the pathogenicity of this fungal infestation was uncertain, children in whom this was demonstrated were successfully treated with mycostatin.

The ova of Ascaris lumbricoides were found rather frequently in the duodenum. The history of a child having vomited a roundworm is a common one so it is not surprising that ova should be found in the duodenum.

The regular presence of pus cells in the duodenal aspirate was of doubtful significance as the aspiration tube had been present in the duodenum for many hours. There was no detectable correlation between the degree of cellular exudation and the presence or severity of a parasitic infestation.

The sodium chloride crystals seen in some specimens of duodenal juice demonstrated the high concentration of this salt in the pancreatic secretion. It was interesting that sodium chloride was precipitated and not sodium carbonate or sodium bicarbonate as sodium bicarbonate is present in high concentration in pancreatic juice.

C. The clinical significance of this study.

In the patient with kwashiorkor it is vital that protein should be given as soon as possible. This will provide "building bricks" for enzyme synthesis and for the restoration of tissues which have a rapid protein turnover. It is doubtful whether there is any indication for supplementing the diet with oral pancreatin or with protein hydrolysates (328a) as it has been shown that even in severely ill patients with P-C.M. there is absorption of nitrogen with a rise in the serum concentration of amino acids.(321).

Intensive after-care and follow-up of children with P-C.M. is mandatory if the late complications of chronic irreversible pancreatic disease are to be avoided. The children usually return to their homes after the successful treatment of their nutritional illness; at home food continues to be scarce and of a poor quality and the vicious circle of infection and malnutrition once again plays its destructive role. This is a sociological problem, but its significance should be appreciated by doctors and public health authorities.

All children in whom there is any suspicion of a septicemia should be treated very vigorously after a blood culture has been taken to determine the responsible organism and its antibiotic sensitivity. An inflammatory process in the pancreas, or in some other important organ, may thus be minimised or avoided and the consequences of such a process prevented.

In malnourished children who have diarrhoea which does not respond to conventional therapy, the possibility of giardiasis should be suspected and appropriate treatment with mepacrine instituted.

D. Unanswered problems.

The exact time when nutritionally-induced pancreatic atrophy becomes irreversible is not known. The tissues of the host may play an important part in determining this as well as the period of nutritional insufficiency.

For obvious ethical reasons this will probably never be determined in the human subject, but animal experiments may yield some valuable information in this respect.

The degree of enzyme insufficiency before steatorrhoea and creatorrhoea occurs is not accurately known. Balance studies from the first day of treatment with repeated PFT's might provide some information on this problem. However, this would appear to be more of a physiological exercise than of true clinical significance.

Further studies of chronically malnourished children are strongly indicated if a true incidence of chronic pancreatic disease among these patients is to be determined. Many more may be found who have pancreatic dysfunction and their response to protein repletion should be assessed. Irreversible pancreatic damage might be present in many of these children. Glucose tolerance tests should be performed at regular intervals, and their growth and development carefully followed. Chronically malnourished children dying at an older age than those with acute kwashiorkor may show a different histological picture to that found in the present study. A greater incidence of fibrosis may be discovered and involvement of the ducts and Islets may also be detectable. Special stains may be employed to study the Islets of Langerhans and the distribution of fibrous tissue.

Malnourished children without the stigmata of kwashiorkor and marasmus may provide some information as to how soon or how late the pancreas is affected by P-C.M. Such children form the bulk of the malnourished children in the population and may thus provide a more accurate index of the incidence of pancreatic dysfunction of nutritional origin.

It has been shown that bile is produced in adequate quantities in children suffering with P-C.M. A study of the bile acid and pigments in

the pancreozymin-stimulated juice may provide some qualitative information on the biliary secretion in P-C.M.

The role played by trace elements in the metabolism of the pancreas is not well defined, but this organ does have an affinity for zinc and manganese. (273, 52). Trace element studies have shown that children with kwashiorkor have a zinc deficiency. (320,344). Further studies in this field may elaborate the role of these deficiencies with regard to pancreatic function, particularly in view of the fact that certain enzymes (e.g. carboxypeptidase) are zinc dependant.(289).

Among the Cape coloured population, the intake of alcohol is high and pancreatitis is common.(263). Malnutrition is also rife among these people. This association may be more than coincidental. Experimental work in rats has demonstrated that dietary deficiency is more deleterious to the pancreas than alcohol (271); the high alcohol intake among subjects who are undernourished may, therefore, be adding insult to injury with respect to the pancreas. It is well known that alcoholism and nutritional deficiency are often associated; it would thus be interesting to trace the specific role of these factors in adults with pancreatitis.

CONCLUSIONS.

Pancreatic function may be safely and accurately estimated in children. The technique employed is that using simultaneous duodenal and gastric aspiration, stimulating the pancreas with secretin and pancreozymin, and measuring the volume, pH, and the output of a broad spectrum of enzymes in the duodenal aspirate.

In acute protein-calorie malnutrition (P-C.M.) the exocrine pancreas is affected in structure and function. The children with kwashiorkor tend to be more severely affected than those with marasmus but there is a distinct overlap between the two groups. Some degree of pancreatic reserve is present in these patients. The output of all enzymes is depressed; chymotrypsin appears to be the most susceptible and trypsin the least susceptible to protein deficiency. The pancreatic dysfunction is rapidly reversed when protein repletion is commenced. The volume output and bicarbonate secretion from the pancreas are relatively not affected.

Among chronically malnourished patients, severe pancreatic damage may occur. In some patients this is not reversed with protein repletion and it is postulated that this may herald chronic pancreatic disease. Pancreatic calcification was not found.

Despite poor pancreatic function, children with P-C.M. absorb sufficient nutrients to initiate the clinical cure of their illness when fed a high protein diet. Pancreatic insufficiency may play a part in the malabsorption syndrome of P-C.M. in the early phase of the illness, but it is not a dominant one. Pancreatin and protein hydrolysates in the diet are unnecessary in the treatment of this condition as there is a rapid recovery

with a normal protein intake. Among chronically malnourished patients with severe irreversible pancreatic damage, these dietary supplements may be indicated.

With an adequate dietary protein intake, the diet may qualitatively affect the pancreatic enzyme output; carbohydrates stimulate amylase output and certain proteins stimulate the output of proteolytic enzymes.

Bile is secreted in normal quantity in patients with P-C.M.

Giardiasis is extremely common among malnourished children. Infestation with these flagellates does not prevent the recovery of pancreatic function when patients are fed a high protein diet. The parasites did not disappear spontaneously after protein repletion in the host.

APPENDIX.

A. Laboratory Methods.

1. Amylase.

(a). Reagents:-

(i). Substrate - 2% starch solution (30 ml).
- 0.1M Phosphate buffer pH 7 (70 ml).

(ii). 0.01N Iodine solution.

(iii). 5% Sulphuric acid.

(iv). 1.5% Sodium chloride solution.

(b). Assay method.

(i). Dilute the juice : 0.1 ml. juice - glycerol mixture was made up to 50 ml. with 1.5% Na Cl in a measuring flask.

(ii). Buffered starch was placed in the water bath at 37°C. for 15 minutes; then well shaken.

(iii). One 50 ml. volumetric flask was taken for each specimen and 1 similar flask for a control for each specimen.

(iv). One ml. starch was added to all flasks.

(v). All the flasks were put in the water bath for 8 minutes.

(vi). The diluted juice was added to the test flasks - 1.0 ml. if the enzyme concentration was expected to be low, and 0.5 ml. if the enzyme concentration was expected to be high. This was timed to the second with a stopwatch.

(vii). The incubation was allowed to proceed for 30 minutes to the second.

(viii). The control flasks were completed while the test samples were in the incubator:- Diluted duodenal juice was added in the same quantity as in the test sample. Then 2 ml. 5% H_2SO_4 were added immediately (from a burette).

3 ml. water (roughly).

1 ml. Iodine solution from a burette, and water was added to the 50 ml. mark.

(ix). After the incubation of the test flasks for 30 minutes exactly:-

2 ml. 5% H_2SO_4 were added, then

3 ml. water (roughly).

1 ml. Iodine solution, and water to the 50 ml. mark.

(x). All flasks were allowed to stand for 30 minutes.

(xi). Readings were performed on a Klett-Summerson colorimeter using a red filter (No. 66), setting the machine on 3 with distilled water.

(c). Calculation.

$$\frac{C - T}{C/6} \times \frac{X}{1} = \text{units per ml. in thousands}$$

Where C = control

T = test

X = 1 if 1.0 ml. juice added to substrate

X = 2 if 0.5 ml. juice added to substrate

2. Lipase.

(a). Reagents:-

(i) Olive oil suspension: Olive oil 50%

Acacia gum 5%

Sodium benzoate 0.2%

Aqua ad 100%

(ii). Ethanol 96%.

(iii). 0.05M Phosphate buffer pH8.

(iv). Thymolphthalein indicator.

(v). 0.1.N. alcoholic potassium hydroxide.

(b). Assay method.

(i). Dilution of juice. The juice-glycerol mixture was diluted 1 in 10 with distilled water (0.4 ml. made up to 4 ml.).

(ii). Two ml. of the diluted juice was pipetted into a 50 ml. Erlenmeyer flask (test) and 2 ml. was first boiled for 5 minutes and then put into a similar flask (blank).

(iii). To each flask were added:-

2.5 ml. olive oil suspension

1.0 ml. phosphate buffer pH8.

(iv). The samples were then placed in an incubator equipped with a flask shaker and kept at 37°C. for 24 hours. (Tests and blanks).

(v). The reaction was stopped by the addition of 5 ml. 96% ethanol.

(vi). 3 drops thymolphthalein were added to each flask.

(vii). The titration was performed with 0.1.N. alcoholic KOH.

(c). Calculation.

$$(T - B) \times 290 = \text{units per ml.}$$

Where T = ml. 0.1.N. alcoholic KOH titrated in test sample.

B = ml. 0.1.N. alcoholic KOH titrated in blank.

3. Ribonuclease.

(a). Reagents:

(i). Substrate - yeast ribonucleic acid, dialysed for 48 hours against water. Final concentration 0.8%.

(ii). 0.1 M Acetate buffer pH 5.0.

(iii). 0.75% Uranium acetate in 25% perchloric acid.

(iv). Ribonuclease of known activity.

(b). Assay method.

(i). Standards were made up with 0.4375, 0.875, 1.75, 3.5 and 7.0 γ ribonuclease in 1.5 ml. acetate buffer. A blank tube contained only the buffer with no enzyme. Unknown samples were diluted 1 in 5 with acetate buffer to 1.5 ml.

(ii). 1 ml. dialysed nucleic acid was added to each tube, and timed to the second with a stopwatch.

(iii). The tubes were incubated at 30°C. for 30 minutes.

(iv). The reaction was stopped by adding 0.5 ml. of 0.75% uranium acetate in perchloric acid.

(v). Precipitated protein and substrate was removed by centrifugation.

(vi). 0.10 ml. of the supernatent fluid was diluted to 3.1 ml. with distilled water.

(vii). Readings were performed on a Beckman model DB spectrophotometer at 260 mμ

(c). Calculation.

(i). A standard curve was plotted from the readings of the samples of known activity. Correction was made for the reagent blank determined by incubation without enzyme.

(ii). From the graph the activity of the unknowns may be determined in γ per 1.5 ml.

(iii). A correction was applied for the dilution.

(iv). From the RNase of known activity, the activity in the unknowns was calculated in milli-Kunitz units per ml. juice.

4. Trypsin.

(a). Reagents:-

(i). Substrate: N - Benzoyl - L - arginine ethyl ester hydrochloride (BAEE). 0.00025 M solution in 0.05 M phosphate buffer pH8. (8.6 mg. BAEE per 100 ml. buffer).

(ii). 0.001. N. HCl.

(b). Apparatus.

(i). Beckman model DB spectrophotometer with recording apparatus.

(ii). Colora Ultrathermostat apparatus.

(c). Assay method.

(i). Dilution of juice. The pancreatic juice was diluted 1 in 5 to 1 in 20 with 0.001.N. HCl depending on the expected enzyme activity.

(ii). The following were placed in 10 mm. quartz cells at 25°C.

	Test sample	Control	Reference cuvette.
Substrate	3.0 ml.	3.0 ml.	3.0 ml.
0.001.N.HCl	-	0.2 ml.	-
Juice solution	0.2 ml.	-	-

(iii). The spectrophotometer was set at 253 mμ.
The reference cuvette was placed in position in the "R" cuvette chamber and left there for the duration of the whole experiment. The optical density scale was adjusted to read 0.05 with the control cuvette. The recording apparatus was set at the corresponding mark.

(iv). Immediately following the addition of the enzyme solution to the substrate, the graph was commenced. This was allowed to run for 3 minutes once the graph was recording a straight line.

(v). The spectrophotometer was re-set at 0.05 with the control cuvette between each assay, and the graph re-adjusted accordingly.

(vi). Each assay was performed in duplicate.

(vii). A standard with crystalline trypsin of known activity was assayed with each test.

(d). Calculation. One unit of activity was defined as "that activity which causes an increase in optical density at 253 mμ of 0.001 per minute" under the given standard conditions. From the graph the units per ml. juice were calculated making allowance for the dilution factor, volume of juice used and the period of assay.

5. Chymotrypsin.

(a). Reagents:-

(i). Substrate: N - Acetyl - L - tyrosine ethyl ester monohydrate (ATEE). 0.00025 M. solution in 0.05 M. phosphate buffer pH 7. (25.2 mg. ATEE per 100 ml.).

(b). Apparatus.

(i). Beckman model DB spectrophotometer with recording apparatus.

(ii). Colora Ultrathermostat apparatus.

(c). Assay method.

(i). Dilution of juice. The pancreatic juice was diluted 1 in 5 to 1 in 40 with 0.001.N.HCl depending on the expected enzyme activity.

(ii). The following were placed in 10 mm. quartz cells at 25°C.

	Test sample	Control	Reference cuvette.
Substrate	3.0 ml.	3.0 ml.	2.25 ml.
0.001.N.HCl	-	0.2 ml.	0.75 ml.
Juice solution	0.2 ml.	-	-

(iii). The spectrophotometer was set at 237 mμ . The reference cuvette was placed in position in the "R" cuvette chamber and left there for the duration of the whole experiment. The optical density scale was adjusted to read 0.200 with the control cuvette. The recording apparatus was set at the corresponding mark.

(iv). Immediately following the addition of the enzyme solution to the substrate, the graph was commenced. This was allowed to run for 3 mintues once the graph was recording a straight line.

(v). The spectrophotometer was re-set at 0.200 with the control cuvette between each assay, and the graph re-adjusted accordingly.

(vi). Each assay was performed in duplicate.

(vii). A standard with crystalline chymotrypsin of known activity was assayed with each test.

(d). Calculation. One unit of activity was defined as "that activity which causes a decrease in optical density at 237 mμ of 0.001 per minute" under the given standard conditions. From the graph the units per ml. juice were calculated making allowance for the dilution factor, volume of juice used and the period of assay.

6. Colour Index.

(a). Reagents:-

(i). 5% Sodium citrate (with a few drops chloroform added as preservative).

(ii). Potassium dichromate : Stock solution 1.57 g. $K_2Cr_2O_7$ in 100 ml. aqueous solution containing one drop concentrated sulphuric acid.

Working standard: 1 in 100 dilution of stock (pH must be 5 or < 5).

(b). Method.

(i). The juice was diluted 1 in 50 with clear 5% Na citrate solution and read against distilled water at 410 mμ in the Beckman model DB spectrophotometer.

(ii). The working standard $K_2Cr_2O_7$ solution was read at the same wavelength.

(c). Calculation.

$$\text{Units of "icterus"} = \frac{50 \times \text{Absorbance of test sample}}{\text{Absorbance of Standard.}}$$

B. Detailed results of pancreatic function tests.

(Test numbers correspond with patient numbers in Tables 2 to 6, Chapter 5).

TABLE A. KWASHIORKOR VOLUME (14 cases).

Test No.	1	4	7	9	10	14	15	16	17	18	23	27	31	35	Mean	Range	S.D.
B(ml) per 10 mins.	2.7	0.8	1.2	1.8	2.6	0.7	2.2	2.0	1.3	1.6	1.4	0.4	0.5	1.8	1.5	0.4-2.7	0.7
S1	9.0	5.1	1.7	2.7	7.0	3.2	2.0	8.2	12.7	2.5	5.3	1.2	5.1	8.5	5.3	1.2-12.7	3.4
S2	9.1	8.9	2.1	4.1	5.9	8.0	6.5	4.2	10.6	5.0	4.3	3.0	3.9	1.2	5.5	1.2-10.6	2.8
S3	5.5	5.2	7.5	2.5	4.2	6.5	1.5	5.0	13.2	5.3	11.2	1.1	3.5	2.2	5.3	1.1-13.2	3.5
S4	5.0	3.2	6.0	3.5	5.2	4.0	0.5	1.7	7.9	4.2	11.5	0.5	4.8	2.3	4.3	0.5-11.5	2.9
S5	5.2	2.1	4.3	3.0	1.6	3.8	2.8	3.6	3.5	1.8	6.0	0.1	6.5	1.3	3.3	0.1-6.5	1.8
S6	4.4	1.6	5.2	3.0	1.0	1.6	1.6	3.5	1.2	1.4	5.5	1.3	1.3	0.1	2.3	0.1-5.5	1.7
P1	11.0	6.7	10.5	1.8	3.8	2.9	2.3	3.7	8.0	7.0	6.7	3.0	7.2	4.8	5.7	1.8-11.0	2.9
P2	4.8	7.6	5.4	4.0	0.9	8.1	1.3	1.0	4.4	1.5	6.2	2.0	6.4	10.5	4.6	0.9-10.5	3.0
P3	1.4	5.5	4.5	2.2	0.7	2.7	2.3	1.0	3.0	2.0	3.1	0.8	5.9	3.2	2.7	0.7-5.9	1.6
P4	-	9.3	1.8	1.9	1.4	1.0	3.1	3.5	1.3	2.5	0.8	5.9	6.3	4.0	3.3	0.8-9.3	2.5
B/min	0.267	0.077	0.123	0.177	0.260	0.067	0.220	0.203	0.133	0.157	0.140	0.043	0.047	0.177	0.149	0.043 - 0.267	0.074
B/min/kg	0.028	0.011	0.013	0.028	0.026	0.008	0.032	0.021	0.013	0.029	0.021	0.005	0.005	0.019	0.019	0.005-0.032	0.009
S/min	0.637	0.435	0.447	0.313	0.415	0.452	0.248	0.437	0.818	0.337	0.730	0.120	0.418	0.260	0.433	0.120-0.818	0.189
S/min/kg	0.066	0.063	0.046	0.049	0.042	0.051	0.036	0.046	0.080	0.063	0.108	0.013	0.046	0.027	0.053	0.013-0.108	0.023
P/min	0.573	0.728	0.555	0.248	0.170	0.368	0.225	0.230	0.418	0.325	0.420	0.293	0.645	0.563	0.412	0.170-0.728	0.176
P/min/kg	0.059	0.105	0.057	0.039	0.017	0.041	0.032	0.024	0.041	0.061	0.062	0.032	0.071	0.059	0.050	0.017-0.105	0.023
S+P/min	0.62	0.55	0.49	0.29	0.32	0.42	0.24	0.35	0.66	0.33	0.61	0.19	0.51	0.38	0.43	0.19-0.66	0.21
S+P/min/kg	0.064	0.080	0.051	0.045	0.032	0.047	0.034	0.037	0.064	0.062	0.090	0.021	0.056	0.040	0.052	0.021-0.090	0.019

TABLE B. MARASMUS VOLUME (7 cases).

Test No.	2	3	8	26	29	46	47	Mean	Range	S.D.
B(ml) per 10 mins.	1.8	0.4	4.2	0.6	2.0	1.5	0.7	1.4	0.4 - 4.2	1.37
S1	4.7	2.9	8.9	0.8	3.1	1.2	1.5	3.3	0.8 - 8.9	2.81
S2	3.2	2.9	7.4	3.5	6.3	0.2	0.8	3.5	0.2 - 7.4	2.64
S3	3.2	3.3	5.0	12.2	9.4	0.1	2.7	5.1	0.1 - 12.2	4.21
S4	3.2	0.4	2.6	6.2	4.3	0.2	2.2	2.7	0.2 - 6.2	2.12
S5	3.2	1.5	2.8	1.7	3.8	0.4	1.5	2.1	0.4 - 3.8	1.18
S6	3.2	0.3	3.4	1.0	2.2	0.2	0.8	1.6	0.2 - 3.4	1.34
P1	1.5	4.5	6.7	3.6	6.8	1.5	2.5	3.9	1.5 - 6.8	2.24
P2	4.0	2.4	3.8	0.5	3.2	1.1	1.3	2.3	0.5 - 4.0	1.39
P3	-	0.6	4.4	0.7	1.9	0.9	0.6	1.5	0.6 - 4.4	1.50
P4	-	0.1	5.2	1.0	2.1	0.7	2.6	2.0	0.1 - 5.2	1.84
B/min	0.177	0.037	0.417	0.057	0.200	0.053	0.067	0.092	0.053 - 0.417	0.137
B/min/kg	0.028	0.008	0.082	0.007	0.027	0.012	0.011	0.016	0.008 - 0.082	0.027
S/min	0.345	0.188	0.502	0.423	0.485	0.038	0.158	0.194	0.038 - 0.502	0.180
S/min/kg	0.054	0.038	0.098	0.054	0.065	0.009	0.027	0.031	0.009 - 0.098	0.031
P/min	0.275	0.190	0.503	0.145	0.350	0.105	0.175	0.158	0.105 - 0.503	0.139
P/min/kg	0.043	0.039	0.098	0.019	0.047	0.024	0.029	0.027	0.019 - 0.098	0.026
S+P/min	0.33	0.19	0.50	0.31	0.43	0.07	0.17	0.29	0.07 - 0.50	0.15
S+P/min/kg	0.051	0.039	0.098	0.040	0.058	0.015	0.017	0.045	0.015 - 0.098	0.028

TABLE C. 5-YEAR FOLLOW-UP VOLUME (10 cases).

Test No.	39	40	41	42	43	49	50	51	52	53	Mean	Range	S.D.
B(ml)per 10 mins.	1.4	0.9	1.1	1.2	4.4	6.8	0.4	0.5	0.6	1.3	1.9	0.4 - 6.8	2.09
S1	14.0	3.3	8.0	2.9	14.0	15.0	12.5	5.7	7.2	7.9	9.1	2.9 - 15.0	4.52
S2	8.6	6.5	5.2	3.8	16.0	7.8	7.5	16.2	7.4	2.7	8.2	2.7 - 16.2	4.57
S3	12.5	7.1	4.8	2.2	6.1	6.0	7.0	5.5	4.4	16.0	7.2	2.2 - 16.0	4.08
S4	10.3	4.1	7.2	2.0	5.0	4.6	8.5	6.2	1.7	2.9	5.3	1.7 - 10.3	2.81
S5	5.0	1.6	2.3	2.0	1.7	4.8	1.1	4.2	1.6	12.0	3.6	1.1 - 12.0	3.27
S6	18.0	1.8	1.5	1.5	0.1	3.6	1.0	1.8	1.2	21.0	5.2	0.1 - 21.0	7.65
P1	21.5	4.4	10.5	3.2	16.0	8.1	19.0	5.3	6.0	21.0	11.5	3.2 - 21.5	7.21
P2	10.5	1.3	2.1	1.6	4.6	12.5	5.7	8.5	6.3	3.0	5.6	1.3 - 12.5	3.87
P3	8.2	0.5	2.0	0.6	2.4	1.8	2.0	1.3	1.5	2.8	2.3	0.5 - 8.2	2.19
P4	10.5	0.1	1.7	1.0	1.8	2.5	1.1	1.8	1.2	2.5	2.4	0.1 - 10.5	2.93
B/min	0.143	0.093	0.113	0.117	0.440	0.683	0.037	0.053	0.057	0.127	0.186	0.037 - 0.683	0.209
B/min/kg	0.007	0.006	0.006	0.006	0.020	0.041	0.003	0.003	0.004	0.008	0.010	0.003 - 0.041	0.012
S/min	1.114	0.407	0.483	0.240	0.715	0.697	0.627	0.660	0.392	1.042	0.638	0.240 - 1.114	0.278
S/min/kg	0.057	0.026	0.024	0.013	0.032	0.042	0.042	0.037	0.027	0.067	0.037	0.013 - 0.067	0.016
P/min	1.268	0.016	0.408	0.016	0.620	0.623	0.695	0.423	0.375	0.733	0.518	0.016 - 1.268	0.366
P/min/kg	0.065	0.001	0.020	0.001	0.027	0.037	0.047	0.024	0.025	0.047	0.029	0.001 - 0.065	0.020
S+P/min	1.19	0.31	0.45	0.21	0.68	0.67	0.65	0.57	0.39	0.92	0.60	0.21 - 1.19	0.29
S+P/min/kg	0.061	0.020	0.022	0.011	0.030	0.040	0.044	0.032	0.026	0.059	0.035	0.011 - 0.061	0.016

TABLE D. KWASHIORKOR AFTER TREATMENT VOLUME (11 cases).

Test No.	5	12	13	19	20	21	22	34	37	44	45	Mean	Range	S.D.
B(ml)per 10 mins.	2.5	2.4	2.1	3.1	1.9	6.7	1.2	1.2	1.8	4.0	1.3	2.6	1.2 - 6.7	1.61
S1	12.1	7.0	13.2	3.0	3.5	5.8	7.2	3.1	2.6	7.9	6.3	6.5	2.6 -13.2	3.57
S2	9.0	13.5	9.2	8.8	11.1	7.2	9.5	2.0	3.6	5.0	3.4	7.5	2.0 -13.5	3.58
S3	10.0	7.0	5.0	5.0	9.0	4.2	6.5	4.0	23.0	7.3	11.5	8.4	4.0 -23.0	5.41
S4	4.2	5.6	3.0	7.1	5.4	2.4	6.7	1.7	4.3	5.5	1.5	4.3	1.5 - 7.1	1.95
S5	6.5	2.8	3.0	11.5	2.4	1.0	1.0	2.2	1.0	4.0	5.8	3.7	1.0 -11.5	3.17
S6	3.6	4.2	1.6	6.0	8.6	2.1	2.0	2.6	0.3	2.2	1.8	3.2	0.3 - 8.6	2.35
P1	15.5	5.6	12.1	4.2	2.3	4.0	11.0	7.1	3.8	13.0	7.1	7.8	2.3 -15.5	4.41
P2	3.6	4.1	4.3	7.2	3.3	4.2	1.7	2.2	2.6	4.3	3.1	3.7	1.7 - 7.2	1.46
P3	2.4	2.5	2.0	1.2	2.2	6.9	2.5	2.1	2.1	1.5	0.7	2.4	0.7 - 6.9	1.61
P4	1.9	3.5	2.0	5.8	3.4	1.4	4.0	1.5	6.0	1.4	3.5	3.1	1.4 - 5.8	1.67
B/min	0.250	0.240	0.213	0.307	0.193	0.670	0.120	0.120	0.177	0.397	0.127	0.256	0.120 - 0.670	0.161
B/min/kg	0.027	0.024	0.019	0.034	0.020	0.103	0.011	0.011	0.019	0.034	0.011	0.028	0.011 - 0.103	0.026
S/min	0.757	0.668	0.583	0.690	0.667	0.378	0.548	0.260	0.580	0.532	0.505	0.561	0.260 - 0.757	0.144
S/min/kg	0.083	0.068	0.053	0.076	0.068	0.058	0.048	0.024	0.062	0.045	0.043	0.057	0.024 - 0.083	0.017
P/min	0.585	0.393	0.510	0.460	0.280	0.413	0.480	0.323	0.363	0.505	0.360	0.425	0.280 - 0.585	0.092
P/min/kg	0.064	0.040	0.046	0.051	0.028	0.064	0.042	0.030	0.039	0.043	0.031	0.043	0.028 - 0.064	0.012
S+P/min	0.69	0.56	0.55	0.60	0.51	0.39	0.52	0.29	0.49	0.52	0.45	0.51	0.29 - 0.69	0.11
S+P/min/kg	0.075	0.057	0.050	0.066	0.052	0.061	0.046	0.026	0.052	0.044	0.038	0.052	0.026 - 0.075	0.013

TABLE E. FEED-UP CONTROLS VOLUME (8 cases).

Test No.	25	28	30	32	33	36	38	48	Mean	Range	S.D.
B(ml)per 10 mins.	1.6	1.1	1.2	1.2	2.0	2.2	0.8	0.7	1.4	0.7 - 2.2	0.55
S1	4.5	3.7	7.3	8.0	6.5	5.5	10.0	2.5	6.0	2.5 - 10.0	2.45
S2	4.3	1.2	6.9	4.9	5.5	5.5	8.0	7.4	5.5	1.2 - 8.0	2.14
S3	10.0	5.5	6.0	5.0	3.0	11.5	7.1	3.0	6.4	3.0 - 11.5	3.06
S4	6.0	4.2	2.3	5.6	3.3	11.3	6.1	3.6	5.3	2.3 - 11.3	2.79
S5	8.4	3.3	1.3	4.8	2.5	2.1	6.8	2.5	4.0	1.3 - 8.4	2.50
S6	1.3	3.5	0.5	3.3	3.2	1.2	4.7	2.5	2.5	0.5 - 4.7	1.42
P1	6.8	2.5	3.3	10.5	6.0	9.0	3.8	4.7	5.8	2.5 - 10.5	2.82
P2	2.1	6.5	1.7	4.0	2.4	3.0	5.6	1.1	3.3	1.1 - 6.5	1.92
P3	2.5	3.5	3.6	2.5	2.0	3.8	2.5	3.0	2.9	2.0 - 3.8	0.65
P4	1.0	3.9	1.7	1.8	0.2	3.1	1.1	1.4	1.8	0.2 - 3.9	1.19
B/min	0.157	0.107	0.123	0.123	0.200	0.223	0.080	0.067	0.135	0.067 - 0.223	0.055
B/min/kg	0.016	0.008	0.011	0.012	0.019	0.020	0.006	0.005	0.012	0.005 - 0.020	0.006
S/min	0.575	0.357	0.405	0.527	0.400	0.618	0.712	0.358	0.494	0.400 - 0.712	0.133
S/min/kg	0.060	0.028	0.038	0.053	0.038	0.056	0.055	0.026	0.044	0.026 - 0.060	0.013
P/min	0.310	0.410	0.258	0.470	0.265	0.473	0.325	0.255	0.346	0.255 - 0.473	0.093
P/min/kg	0.032	0.032	0.024	0.047	0.025	0.043	0.025	0.018	0.031	0.018 - 0.047	0.010
S+P/min	0.47	0.38	0.35	0.50	0.35	0.56	0.56	0.32	0.44	0.32 - 0.56	0.10
S+P/min/kg	0.049	0.030	0.032	0.051	0.033	0.051	0.043	0.023	0.039	0.023 - 0.051	0.011

TABLE F. KWASHIORKOR pH (14 cases)

Test No.	1	4	7	9	10	14	15	16	17	18	23	27	31	35	Mean	Range	S.D.
B	8.5	7.2	7.0	7.8	7.0	7.0	7.2	7.8	4.0	8.0	7.5	8.0	8.2	8.2	7.4	4.0-8.5	1.1
S1	9.0	8.6	7.2	8.0	7.0	7.5	7.2	8.2	8.0	8.0	8.0	8.0	8.6	8.5	8.0	7.0-9.0	0.6
S2	9.5	8.7	7.5	8.5	8.5	8.3	8.0	8.6	8.6	8.5	8.5	8.5	8.8	8.6	8.5	7.5-9.5	0.4
S3	9.5	8.8	8.5	8.8	8.8	8.5	8.3	8.8	8.5	8.8	8.5	8.6	8.8	8.8	8.7	8.3-9.5	0.3
S4	9.5	8.8	8.8	9.0	8.8	8.5	8.6	9.0	8.5	8.8	8.8	8.8	8.8	8.8	8.8	8.5-9.5	0.2
S5	9.5	8.8	8.5	8.8	9.0	9.0	8.8	8.8	8.5	8.8	8.8	9.0	8.6	8.8	8.8	8.5-9.5	0.2
S6	9.5	8.8	8.2	8.7	9.0	9.3	9.0	8.5	8.8	8.8	8.6	8.8	8.8	9.0	8.8	8.2-9.5	0.3
P1	9.0	8.6	8.2	8.6	8.5	8.8	8.8	8.5	8.6	8.6	8.8	8.8	8.6	8.5	8.6	8.2-9.0	0.2
P2	9.0	8.5	8.2	8.5	8.8	8.8	8.6	8.5	8.5	8.6	8.6	8.5	8.6	8.5	8.6	8.2-9.0	0.2
P3	9.0	8.4	8.5	8.4	8.6	8.8	8.5	8.5	8.2	8.8	8.6	8.8	8.6	8.6	8.6	8.2-9.0	0.2
P4	9.0	7.2	8.5	8.4	8.8	8.8	8.8	8.2	8.2	8.6	8.6	8.0	8.6	8.6	8.5	7.2-9.0	0.5
Mean S	9.4	8.8	8.1	8.6	8.5	8.5	8.3	8.7	8.5	8.6	8.5	8.6	8.7	8.8	8.6	8.1-9.4	0.3
Mean P	9.0	8.2	8.4	8.5	8.7	8.8	8.7	8.4	8.4	8.7	8.7	8.5	8.6	8.6	8.6	8.2-9.0	0.2
Mean S + P	9.3	8.5	8.2	8.6	8.6	8.6	8.5	8.6	8.4	8.6	8.6	8.6	8.7	8.7	8.6	8.2-9.3	0.2
Peak	9.5	8.8	8.8	9.0	9.0	9.3	9.0	9.0	8.8	8.8	8.8	9.0	8.8	9.0	9.0	8.8-9.5	0.2

TABLE G.

MARASMUS pH (7 cases).

Test No.	2	3	8	26	29	46	47	Mean	Range	S.D.
B	7.2	7.2	7.2	6.8	8.5	8.0	8.0	7.6	6.8 - 8.5	0.61
S1	8.3	7.2	8.0	8.0	8.5	8.5	8.2	8.1	7.2 - 8.5	0.45
S2	8.3	8.3	8.2	8.5	8.6	8.5	8.5	8.4	8.2 - 8.6	0.15
S3	8.3	8.3	8.5	8.8	8.6	8.8	8.5	8.5	8.3 - 8.8	0.21
S4	8.6	8.6	8.6	8.8	8.8	8.8	8.8	8.7	8.6 - 8.8	0.11
S5	8.0	8.2	8.6	9.0	8.8	8.8	8.8	8.6	8.0 - 9.0	0.37
S6	8.2	8.6	8.3	9.0	8.8	8.8	9.0	8.7	8.2 - 9.0	0.32
P1	8.3	7.2	8.2	8.8	8.6	8.8	8.6	8.4	7.2 - 8.8	0.56
P2	8.0	7.5	8.5	8.8	8.6	8.8	8.6	8.4	7.5 - 8.8	0.48
P3	-	7.8	8.5	9.0	8.8	8.8	8.5	8.6	7.8 - 9.0	0.42
P4	-	8.0	8.3	8.8	8.8	8.8	8.5	8.5	8.0 - 8.8	0.33
Mean S	8.3	8.2	8.4	8.7	8.7	8.7	8.6	8.5	8.2 - 8.7	0.21
Mean P	8.2	7.6	8.4	8.9	8.7	8.8	8.6	8.5	7.6 - 8.9	0.45
Mean S + P	8.3	8.0	8.4	8.8	8.7	8.7	8.6	8.5	8.0 - 8.8	0.30
Peak	8.6	8.6	8.6	9.0	8.8	8.8	9.0	8.8	8.6 - 9.0	0.18

TABLE H.

5-YEAR FOLLOW-UP pH (10 cases)

Test No.	39	40	41	42	43	49	50	51	52	53	Mean	Range	S.D.
B	8.2	8.0	8.5	8.5	8.6	8.5	8.5	8.5	8.2	8.5	8.4	8.0 - 8.6	0.19
S1	8.5	8.5	8.5	8.5	8.6	8.5	8.6	8.8	8.5	8.2	8.5	8.2 - 8.8	0.15
S2	8.6	8.8	8.6	8.8	8.6	8.6	8.6	8.8	8.8	8.5	8.7	8.5 - 8.8	0.12
S3	8.8	8.8	8.8	9.0	8.8	8.8	8.8	9.0	8.8	8.6	8.8	8.6 - 9.0	0.12
S4	8.8	8.8	8.8	9.0	8.8	8.8	8.8	8.8	9.0	8.6	8.8	8.6 - 9.0	0.12
S5	9.0	8.8	8.8	9.2	8.8	8.8	9.0	9.0	8.8	8.8	8.9	8.8 - 9.2	0.12
S6	8.5	8.8	8.9	9.0	9.2	8.8	9.0	9.0	9.0	8.6	8.9	8.5 - 9.2	0.21
P1	8.5	8.5	8.5	8.5	8.5	8.6	8.5	8.5	8.6	8.5	8.5	8.5 - 8.6	0.05
P2	8.5	8.5	8.5	8.5	8.6	8.6	8.5	8.6	8.6	8.5	8.5	8.5 - 8.6	0.05
P3	8.5	8.5	8.5	8.5	8.5	8.8	8.6	8.5	8.5	8.5	8.5	8.5 - 8.8	0.09
P4	8.5	8.5	8.5	8.5	8.6	8.8	8.6	8.5	8.5	8.6	8.6	8.5 - 8.8	0.09
Mean S	8.7	8.8	8.7	8.9	8.8	8.7	8.8	8.9	8.8	8.6	8.8	8.6 - 8.9	0.09
Mean P	8.5	8.5	8.5	8.5	8.6	8.7	8.6	8.5	8.6	8.5	8.6	8.5 - 8.7	0.07
Mean S + P	8.6	8.7	8.6	8.8	8.7	8.7	8.7	8.8	8.7	8.5	8.7	8.5 - 8.8	0.06
Peak	9.0	8.8	8.9	9.2	9.2	8.8	9.0	9.0	9.0	8.8	9.0	8.8 - 9.2	0.15

TABLE J.

KWASHIORKOR AFTER TREATMENT pH (11 cases).

Test No.	5	12	13	19	20	21	22	34	37	44	45	Mean	Range	S.D.
B	7.5	7.2	7.2	8.0	6.5	8.0	7.5	8.5	8.2	8.2	8.5	7.8	7.2 - 8.5	0.63
S1	8.5	7.8	7.8	8.6	8.2	8.5	8.5	8.5	8.5	8.5	8.8	8.4	7.8 - 8.8	0.32
S2	8.7	8.8	8.5	8.8	8.6	8.6	8.8	8.6	8.6	8.8	8.8	8.7	8.5 - 8.8	0.11
S3	8.7	8.8	8.8	8.8	8.6	8.8	8.8	8.8	8.6	9.0	8.8	8.8	8.7 - 9.0	0.11
S4	8.8	9.0	9.0	8.8	8.6	8.8	8.8	8.8	8.8	9.0	9.0	8.9	8.6 - 9.0	0.13
S5	8.8	9.0	9.0	8.8	8.8	8.8	8.8	9.0	9.0	9.0	8.8	8.9	8.8 - 9.0	0.10
S6	8.9	8.8	9.0	9.0	8.6	8.6	9.0	8.8	8.8	8.8	9.0	8.8	8.6 - 9.0	0.15
P1	8.2	8.5	8.5	9.0	8.8	8.6	8.5	8.5	8.6	8.6	8.5	8.6	8.2 - 9.0	0.20
P2	7.7	8.5	8.5	8.8	8.5	8.8	8.8	8.5	8.5	8.5	8.6	8.5	7.7 - 8.8	0.30
P3	8.6	8.5	8.5	8.8	8.6	8.8	8.8	8.6	8.6	8.6	8.8	8.7	8.5 - 8.8	0.12
P4	8.6	8.6	8.5	8.6	8.6	8.6	8.6	8.6	8.5	8.6	8.8	8.6	8.5 - 8.8	0.08
Mean S	8.7	8.7	8.7	8.8	8.6	8.7	8.8	8.8	8.7	8.9	8.9	8.8	8.6 - 8.9	0.10
Mean P	8.3	8.5	8.5	8.8	8.6	8.7	8.7	8.6	8.6	8.6	8.7	8.6	8.3 - 8.8	0.13
Mean S + P	8.6	8.6	8.6	8.8	8.6	8.7	8.7	8.7	8.7	8.7	8.8	8.7	8.6 - 8.8	0.82
Peak	8.9	9.0	9.0	9.0	8.8	8.8	9.0	9.0	9.0	9.0	9.0	9.0	8.8 - 9.0	0.08

TABLE K.

FEED-UP CONTROLS pH (8 cases).

Test No.	25	28	30	32	33	36	38	48	Mean	Range	S.D.
B	7.8	8.5	8.5	8.2	6.0	8.2	8.2	8.5	8.0	6.0 - 8.5	0.84
S1	8.3	8.5	8.5	8.5	7.5	8.5	8.5	8.8	8.4	7.5 - 8.8	0.38
S2	8.6	8.8	8.6	8.6	8.6	8.6	8.6	8.8	8.7	8.6 - 8.8	0.29
S3	8.8	8.5	8.8	8.6	8.8	8.6	8.6	9.0	8.7	8.5 - 9.0	0.16
S4	8.8	8.8	8.8	8.6	8.8	8.8	8.5	8.8	8.7	8.5 - 8.8	0.12
S5	8.6	9.0	9.0	9.0	8.8	8.8	8.5	9.0	8.8	8.5 - 9.0	0.20
S6	8.7	8.8	9.0	9.0	8.6	8.8	8.6	9.0	8.8	8.6 - 9.0	0.17
P1	8.8	8.5	8.5	8.8	8.5	8.5	8.8	8.6	8.6	8.5 - 8.8	0.15
P2	8.6	8.5	8.5	8.8	8.5	8.5	8.6	8.6	8.6	8.5 - 8.8	0.10
P3	8.6	8.5	8.5	8.8	8.6	8.5	8.8	8.6	8.6	8.5 - 8.8	0.13
P4	8.6	8.8	8.6	8.8	8.6	8.5	8.6	8.6	8.6	8.5 - 8.8	0.11
Mean S	8.6	8.7	8.8	8.7	8.5	8.7	8.6	8.9	8.7	8.5 - 8.9	0.13
Mean P	8.7	8.6	8.5	8.8	8.6	8.5	8.7	8.6	8.6	8.5 - 8.8	0.11
Mean S + P	8.6	8.7	8.7	8.8	8.5	8.6	8.6	8.8	8.7	8.5 - 8.8	0.08
Peak	8.8	9.0	9.0	9.0	8.8	8.8	8.8	9.0	8.9	8.8 - 9.0	0.11

TABLE L. KWASHIORKOR Units per minute per Kg. (14 cases).

Test No.	1	4	7	9	10	14	15	16	17	18	23	27	31	35	Mean	Range
Amylase																
B	5	5	6	14	9	2	44	9	2	14	0	4	4	0	8	0 - 44
S	15	47	25	25	15	3	14	13	40	4	0	10	11	5	16	0 - 47
P	85	37	36	19	4	2	11	6	24	18	0	16	6	15	20	0 - 85
S+P	43	43	30	22	11	3	13	10	33	9	0	12	9	9	18	0 - 43
Peak	159	81	63	29	22	7	44	26	55	29	0	25	21	23	42	0 - 159
Lipase																
B	5	1	2	10	12	6	7	15	0	50	4	3	2	15	9	0 - 50
S	7	43	12	10	11	25	17	17	88	117	30	5	18	32	31	5 - 117
P	6	55	23	11	4	27	21	15	63	150	28	22	43	82	39	4 - 150
S+P	6	48	16	10	8	26	19	16	78	130	29	12	28	52	34	6 - 130
Peak	14	108	30	17	23	41	42	34	151	195	43	26	49	117	64	14 - 195
RNase																
B	2	1	0	1	1	1	1	0	0	1	0	0	0	1	1	0 - 2
S	4	2	1	1	1	1	0	0	2	1	1	0	0	1	1	0 - 4
P	7	3	2	1	0	2	1	0	2	1	1	1	2	3	2	0 - 7
S+P	5	2	1	1	1	1	0	0	2	1	1	1	1	2	1	0 - 5
Peak	8	4	2	1	2	2	1	1	4	2	1	1	3	4	3	1 - 8
Trypsin																
B	3	4	3	5	15	5	3	24	6	7	0	3	2	13	7	0 - 24
S	13	68	12	7	20	21	13	38	47	46	7	5	24	24	25	5 - 68
P	9	59	15	7	8	17	13	13	45	90	5	26	38	59	29	5 - 90
S+P	11	64	13	7	15	19	13	28	46	63	6	13	30	38	26	6 - 64
Peak	29	147	21	12	38	33	32	72	79	117	9	34	48	96	55	9 - 147
Chymotrypsin																
B	0	0	0	0	3	0	0	4	3	0	0	0	0	3	1	0 - 4
S	0	9	1	0	5	0	0	6	8	20	0	0	2	5	4	0 - 20
P	0	4	0	0	2	0	0	2	10	43	0	3	7	11	6	0 - 43
S+P	0	7	1	0	4	0	0	4	8	29	0	1	4	7	5	0 - 29
Peak	0	27	3	0	12	0	0	14	18	60	0	6	10	22	12	0 - 60

TABLE M. MARASMUS Units per minute per Kg. (7 cases).

Test No.	2	3	8	26	29	46	47	Mean	Range
Amylase									
B	5	21	49	2	44	5	4	19	2 - 49
S	56	43	67	41	42	2	8	37	2 - 67
P	39	40	120	20	10	12	9	36	9 - 120
S+P	52	42	88	32	29	6	8	37	6 - 88
Peak	79	103	140	87	74	18	11	73	11 - 140
Lipase									
B	46	18	75	12	17	1	5	25	1 - 75
S	72	37	84	110	31	1	14	50	1 - 110
P	53	120	105	49	35	10	23	56	10 - 120
S+P	68	70	92	86	33	5	18	53	5 - 92
Peak	142	221	107	239	52	17	24	115	17 - 239
RNase									
B	1	2	1	0	1	1	3	1	0 - 3
S	4	1	2	5	2	1	1	2	1 - 5
P	2	3	3	3	1	1	1	2	1 - 3
S+P	3	2	2	5	1	1	1	2	1 - 5
Peak	5	6	3	11	5	1	3	5	1 - 11
Trypsin									
B	30	13	77	4	21	1	2	21	1 - 77
S	47	38	93	35	19	1	11	35	1 - 93
P	43	68	121	17	37	12	15	48	12 - 121
S+P	46	50	104	28	26	5	13	39	5 - 104
Peak	68	129	141	74	56	17	17	72	17 - 141
Chymotrypsin									
B	0	7	39	1	0	0	0	7	0 - 39
S	0	16	37	1	0	0	0	8	0 - 37
P	0	27	54	4	0	0	3	13	0 - 54
S+P	0	20	44	2	0	0	1	10	0 - 44
Peak	0	50	57	5	0	0	4	17	0 - 57

TABLE N.

5 - YEAR FOLLOW-UP Units per minute per Kg.(10 cases)

Test No.	39	40	41	42	43	49	50	51	52	53	Mean	Range
Amylase												
B	74	59	51	9	176	379	3	4	24	54	83	3 - 379
S	463	227	208	23	256	271	13	14	217	287	198	13 - 463
P	419	182	132	27	279	291	12	70	218	379	201	12 - 419
S+P	445	216	178	24	265	279	12	37	218	323	200	12 - 445
Peak	538	321	278	44	552	564	26	93	522	626	356	26 - 626
Lipase												
B	31	22	19	5	76	126	5	8	11	26	33	5 - 126
S	212	97	90	12	110	119	50	64	86	245	109	12 - 245
P	270	86	86	15	119	129	115	75	99	178	117	15 - 270
S+P	235	94	88	13	113	123	76	68	91	218	112	13 - 235
Peak	373	138	138	27	248	226	201	127	163	393	203	27 - 393
RNase												
B	1	2	1	1	3	1	0	0	0	1	1	0 - 3
S	7	3	1	2	5	1	1	1	2	3	3	1 - 7
P	10	5	3	2	6	2	4	2	5	3	4	2 - 10
S+P	8	4	2	2	6	1	2	2	3	3	3	1 - 8
Peak	13	5	4	3	11	3	5	3	7	4	6	3 - 13
Trypsin												
B	17	16	4	1	43	71	2	1	2	11	17	1 - 71
S	73	30	56	5	32	30	17	18	18	131	41	5 - 131
P	190	52	69	10	143	92	34	75	94	115	87	10 - 190
S+P	120	39	61	7	76	55	24	41	48	124	60	7 - 124
Peak	308	94	123	17	277	174	55	139	155	194	154	17 - 308
Chymotrypsin												
B	42	29	5	0	64	89	2	1	3	9	24	0 - 64
S	110	46	63	1	82	27	14	8	18	94	46	1 - 110
P	165	37	60	3	131	74	33	34	68	70	68	3 - 165
S+P	132	42	62	2	101	46	22	18	38	84	55	2 - 132
Peak	212	67	133	5	245	136	56	64	117	132	117	5 - 245

TABLE O.

KWASHIORKOR AFTER TREATMENT — Units per minute per Kg. (11 cases).

Test No.	5	12	13	19	20	21	22	34	37	44	45	Mean	Range	S.D.
Amylase														
B	140	114	71	87	46	561	18	50	112	342	98	149	18 - 561	161
S	207	99	77	220	346	168	168	134	205	423	241	208	99 - 423	102
P	271	108	99	211	140	267	275	173	207	407	202	215	99 - 407	89
S+P	233	102	86	216	264	208	211	150	205	417	226	211	86 - 417	88
Peak	446	195	153	337	381	561	390	245	403	681	340	376	153 - 681	153
Lipase														
B	69	100	79	99	59	276	17	31	77	121	39	88	17 - 276	70
S	154	249	188	199	259	180	157	95	181	161	149	179	95 - 259	46
P	220	169	183	160	108	201	158	137	151	204	113	164	108 - 220	36
S+P	180	217	186	183	198	188	157	112	169	178	135	173	112 - 217	29
Peak	362	393	362	251	283	317	234	203	410	352	188	305	188 - 410	78
RNase														
B	2	3	2	3	1	4	0	1	2	5	2	2	0 - 5	1.41
S	3	5	5	3	3	3	2	1	3	2	1	3	1 - 5	1.33
P	5	4	5	5	2	3	2	3	4	6	1	4	1 - 6	1.57
S+P	3	4	5	4	2	3	2	2	3	3	1	3	1 - 5	1.14
Peak	8	8	10	7	4	4	3	4	7	11	2	6	2 - 11	3.00
Trypsin														
B	31	63	77	35	52	71	9	15	41	29	11	39	9 - 77	24
S	66	43	85	75	166	34	55	30	46	32	42	61	30 - 166	39
P	119	36	133	59	57	51	99	83	98	131	88	87	36 - 133	33
S+P	87	40	104	68	122	41	72	51	67	72	60	71	40 - 122	25
Peak	199	75	253	148	284	74	151	138	100	208	146	161	74 - 284	69
Chymotrypsin														
B	27	170	139	82	59	167	12	32	78	61	44	79	12 - 170	56
S	43	271	201	105	318	127	87	35	108	52	66	128	43 - 318	95
P	75	272	283	105	231	184	111	71	130	163	118	158	75 - 283	75
S+P	56	271	233	105	283	150	96	49	117	96	87	140	49 - 283	84
Peak	120	488	453	164	378	241	172	101	187	246	200	250	101 - 488	132

TABLE P.

FEED-UP CONTROLS Units per minute per Kg.(8 tests, 7 cases).

Test No.	25	28	30	32	33	36	38	48	Mean	Range	S.D.
Amylase											
B	114	60	85	137	63	79	46	44	79	44 - 137	33
S	413	226	253	433	365	415	458	227	349	226 - 458	98
P	287	240	156	380	262	447	233	161	271	156 - 447	101
S+P	380	231	214	411	323	427	368	200	319	200 - 427	92
Peak	620	326	519	632	541	905	625	320	561	320 - 905	188
Lipase											
B	56	27	40	40	63	64	21	12	40	12 - 64	20
S	199	88	121	212	176	185	237	70	161	70 - 237	61
P	146	119	95	195	117	176	102	77	128	77 - 176	41
S+P	178	100	111	205	152	182	183	72	148	72 - 205	48
Peak	283	131	212	308	264	365	291	96	244	96 - 365	91
RNase											
B	1	1	2	1	2	2	1	1	1	1 - 2	0.52
S	2	2	3	4	5	5	6	1	4	1 - 6	1.77
P	2	3	3	7	6	8	3	2	4	2 - 8	2.37
S+P	2	2	3	5	6	6	5	1	4	1 - 6	1.98
Peak	3	3	6	10	9	11	7	2	6	2 - 11	3.46
Trypsin											
B	44	8	12	16	47	23	13	4	21	4 - 47	16
S	77	23	35	57	58	49	240	16	69	16 - 240	72
P	151	79	129	176	98	112	98	80	115	79 - 176	34
S+P	106	45	72	104	74	74	183	42	88	42 - 183	45
Peak	191	109	134	322	181	172	277	115	188	109 - 322	76
Chymotrypsin											
B	79	12	39	112	51	45	15	8	45	8 - 112	36
S	156	40	78	227	87	85	163	27	108	27 - 227	68
P	119	77	77	218	73	229	73	54	115	54 - 229	69
S+P	141	55	77	223	81	142	127	38	111	38 - 223	60
Peak	204	92	181	491	151	335	215	76	218	76 - 491	136

TABLE Q.

KWASHIORKOR COLOUR INDEX (13 cases).

Test No.	4	7	9	10	14	15	16	17	18	23	27	31	35	Mean	Range	S.D.
410 mμ																
B	8.3	23.2	26.6	9.8	16.1	6.2	9.4	2.9	15.1	8.5	8.2	6.8	2.3	11.0	2.3 - 26.6	7.3
S1	11.6	17.4	19.3	13.0	8.6	33.0	2.7	24.7	17.1	11.2	4.5	8.2	6.7	13.7	2.7 - 33.0	8.5
S3	3.6	8.5	6.9	2.0	3.3	7.0	12.8	3.8	13.2	7.1	4.2	3.1	2.1	6.0	2.0 - 13.2	3.7
S5	6.2	10.8	6.8	0.3	3.4	5.7	2.4	1.9	5.1	8.8	4.2	2.2	1.6	4.6	0.3 - 10.8	3.1
P1	17.8	23.2	19.1	1.7	21.9	20.9	28.4	36.0	26.4	13.0	16.0	30.0	13.6	20.6	1.7 - 36.0	8.8
P3	6.6	18.1	14.6	9.5	30.0	21.9	20.9	51.2	32.5	8.6	13.7	15.4	10.2	19.5	6.6 - 51.2	12.4
Mean S	8.1	13.8	11.0	5.1	5.1	15.2	6.0	10.1	11.8	9.0	4.3	4.5	3.5	8.3	3.5 - 15.2	3.9
Mean P	12.2	20.7	16.9	5.6	26.0	21.4	24.7	43.6	29.5	10.8	14.9	22.7	11.9	20.1	5.6 - 43.6	9.9
Peak	17.8	23.2	26.6	13.0	30.0	33.0	28.4	51.2	32.5	13.0	16.0	30.0	13.6	25.3	13.0 - 51.2	10.9

TABLE R.

MARASMUS COLOUR INDEX (7 cases).

Test No.	2	3	8	26	29	46	47	Mean	Range	S.D.
410 mμ										
B	7.5	13.9	7.0	-	20.0	3.9	8.2	10.1	3.9 - 20.0	5.85
S1	7.2	13.0	7.0	19.2	20.0	4.8	3.4	10.7	3.4 - 20.0	6.81
S3	1.9	8.4	3.9	15.2	7.5	2.6	4.3	6.3	1.9 - 15.2	4.62
S5	8.7	10.4	4.4	14.6	5.2	2.6	1.4	6.8	1.4 - 14.6	4.70
P1	6.1	73.3	7.5	30.5	24.8	19.7	36.8	28.4	6.1 - 73.3	22.78
P3	-	-	5.8	-	8.9	41.1	68.5	31.1	5.8 - 68.5	29.62
Mean S	5.9	10.6	5.1	16.3	10.9	3.3	3.0	7.9	3.0 - 16.3	4.90
Mean P	6.1	73.3	6.7	30.5	16.9	30.4	52.7	30.9	6.1 - 73.3	24.73
Peak	8.7	73.3	7.5	30.5	24.8	41.1	68.5	33.3	7.5 - 73.3	27.00

TABLE S.

5 - YEAR FOLLOW-UP COLOUR INDEX (10 cases).

Test No.	39	40	41	42	43	49	50	51	52	53	Mean	Range	S.D.
410 mμ													
B	48.8	137.2	2.9	12.5	16.4	6.8	-	29.1	-	23.6	34.7	2.9 - 137.2	43.88
S1	22.6	27.9	34.6	5.7	9.1	1.0	1.7	3.1	6.0	47.1	15.9	1.0 - 47.1	16.15
S3	1.2	16.1	4.5	2.2	6.9	0.3	1.0	1.9	3.3	6.8	4.4	0.3 - 16.1	4.71
S5	2.9	24.3	1.4	2.9	7.0	0.2	-	-	-	4.8	5.0	0.2 - 24.3	7.07
P1	17.3	56.5	84.2	71.1	70.4	23.1	83.0	79.3	64.2	26.5	57.6	17.3 - 84.2	25.81
P3	3.4	-	59.6	-	13.9	3.3	55.5	39.4	-	10.3	26.5	3.3 - 59.6	24.48
Mean S	8.9	22.8	13.5	3.6	7.7	0.5	1.2	2.3	4.2	19.6	8.4	0.5 - 22.8	7.83
Mean P	10.4	56.5	71.9	71.1	42.2	13.2	69.3	59.4	64.2	18.4	47.7	10.4 - 71.9	24.85
Peak	48.8	137.2	84.2	71.1	70.4	23.1	83.0	79.3	64.2	47.1	70.8	23.1 - 137.2	30.14

TABLE T.

KWASHIORKOR AFTER TREATMENT COLOUR INDEX (11 cases).

Test No.	5	12	13	19	20	21	22	34	37	44	45	Mean	Range	S.D.
410 mμ														
B	8.8	11.2	23.1	15.6	6.2	4.3	2.9	19.9	8.6	3.1	17.6	11.0	2.9 - 23.1	7.05
S1	16.1	9.3	18.5	15.6	13.9	6.2	4.1	21.7	5.4	2.9	10.4	11.3	2.9 - 21.7	6.30
S3	4.9	9.5	2.4	13.9	5.1	6.8	7.5	4.6	1.9	0.3	2.1	5.4	0.3 - 13.9	3.93
S5	3.9	10.2	1.2	13.7	6.5	11.1	5.5	7.9	1.2	0	6.5	6.2	0 - 13.7	4.40
P1	93.5	22.4	29.2	14.7	21.9	16.1	24.3	39.7	7.0	48.1	45.9	33.0	7.0 - 93.5	23.92
P3	21.7	15.9	15.1	21.4	9.4	14.7	11.1	31.3	9.0	46.2	10.6	18.8	9.0 - 46.2	11.28
Mean S	8.3	9.7	7.4	14.4	8.5	8.0	5.7	11.4	2.8	1.1	6.3	7.6	1.1 - 14.4	3.70
Mean P	57.6	19.2	22.2	18.1	15.7	15.4	17.7	35.5	8.0	47.2	28.3	25.9	8.0 - 57.6	15.09
Peak	93.5	22.4	29.2	21.4	21.9	16.1	24.3	39.7	9.0	48.1	45.9	33.8	9.0 - 93.5	23.27

TABLE U.

FEED-UP CONTROLS - COLOUR INDEX (8 cases).

Test No.	25	28	30	32	33	36	38	48	Mean	Range	S.D.
410 mμ											
B	17.1	5.8	9.6	17.1	4.5	4.5	12.0	8.9	9.9	4.5 - 17.1	5.13
S1	7.2	4.3	11.3	12.8	6.5	8.0	21.4	3.1	9.3	3.1 - 21.4	5.86
S3	8.2	2.9	6.0	5.3	3.3	2.2	36.8	1.2	8.2	1.2 - 36.8	11.76
S5	12.3	3.3	5.0	4.5	4.8	1.0	27.7	1.5	7.5	1.0 - 27.7	8.86
P1	37.7	57.3	82.2	27.4	13.9	8.6	18.2	123.3	46.1	8.6 -123.3	39.70
P3	69.9	12.0	60.1	11.1	19.7	6.0	6.0	57.4	30.3	6.0 - 69.9	27.22
Mean S	9.2	3.5	7.4	7.5	4.9	3.7	28.6	1.9	8.3	1.9 - 28.6	8.54
Mean P	53.8	34.7	71.2	19.3	16.8	7.3	12.1	90.4	38.2	7.3 - 90.4	30.52
Peak	69.9	57.3	82.2	27.4	19.7	8.6	36.8	123.3	53.2	8.6 -123.3	37.98

c. Statistical Methods.

1. The Mann-Whitney U-test for the determination of significance. (138a)

This method was used for the non-parametric analysis of results obtained from the pancreatic function tests. The bulk of the comparisons (i.e. those shown in tables V, W and X, pages 205-207) were performed with the help of an electronic computer. Spot checks on the computer analysis and numerous additional examples were performed by the investigator.

$$U = NN' + \frac{N(N+1)}{2} - R$$

$$U' = NN' - U$$

where N = the number of observations in 1 group

$$(x_1 , x_2 \ldots\ldots\ldots\ldots x_N)$$

and N' = the number of observations in the 2nd group.

$$(x'_1 \quad x'_2 \ldots\ldots\ldots\ldots x'_{N'})$$

To determine R: (see example on following page)

a) Arrange all figures in order from smallest to largest. (A)

b) Assign a score to each digit in A, commencing with 1 at the smallest and increasing the score by 1 for each digit in A. (B)

c) List the equal A numbers in each group (C) and (D)

d) Total the B scores assigned to equal A values irrespective of the group from which they are drawn. (E)

e) Find the mean score for each C and D number. $F = \frac{E}{C + D}$ (F)

f) R (calculated for 1 group)

$$= (C_1 \times F_1) + (C_2 \times F_2) \ldots\ldots + (C_n \times F_n)$$

$$\text{or } (D_1 \times F_1) + (D_2 \times F_2) \ldots\ldots + (D_n \times F_n)$$

U and U' are then calculated from the given formula.

The smaller of the 2 U-values are used for the determination of P from tables.

Example:

Kwashiorkor males (KM) versus kwashiorkor females (KV) - basal amylase output.

KM:	0	2	2	4	5	9	44	(N = 7)
KV:	0	4	5	6	9	14	14	(N' = 7)

	0	0	2	2	4	4	5	5	6	9	9	14	14	44	(A)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	(B)
KM	1		2		1		1		-	1		-		1	(C)
KV	1		-		1		1		1	1		2		-	(D)
	3		7		11		15		9	21		25		14	(E)
	1.5		3.5		5.5		7.5		9	10.5		12.5		14	$(\frac{E}{C+D})$ =(F)

R(for KM) = 1(1.5) + 2(3.5) + 1(5.5) + 1(7.5) + 1(10.5) + 1(14) = 46

$$U = 49 + 28 - 46$$

$$= 31$$

$$U' = 49 - 31$$

$$= 18$$

From tables, P is not significant (>0.10)

2. <u>Standard deviation of a sample.</u>

n observations $(x_1 , x_2 \ldots\ldots\ldots x_n)$

$\Sigma x = x_1 + x_2 \ldots\ldots\ldots + x_n$ = sum of the x's.

$\Sigma x^2 = x_1^2 + x_2^2 \ldots\ldots + x_n^2$ = sum of x^2.

$(\Sigma x)^2 = (x_1 + x_2 \ldots\ldots\ldots + x_n)^2$

$$\bar{x} = \frac{\Sigma x}{n} = \text{mean.}$$

$$\text{Variance of } x = \frac{1}{n-1}(x_1 - \bar{x})^2 = \frac{1}{n-1}\left[\Sigma x_1^2 - \frac{(\Sigma x)^2}{n}\right]$$

$$\text{Standard deviation of } x = \sqrt{\text{Variance of } x}$$

$$= \sqrt{\frac{1}{n-1}\left(\Sigma x_1^2 - \frac{(\Sigma x)^2}{n}\right)}$$

3. <u>Students t test for the difference of two sample means.</u>

This test was used for the parametric analysis of the clinical data of the children studied.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SD(\bar{x}_1 - \bar{x}_2)}$$

n_1 observations of x_1: mean $\bar{x}_1$.

n_2 observations of x_2: mean $\bar{x}_2$.

Pooled estimate of variance $(SD)^2$

$$= \frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

where $\Sigma(x_1 - \bar{x}_1)^2 = \Sigma x_1^2 - \frac{(\Sigma x_1)^2}{n_1}$ (as above)

$$SD = \sqrt{(SD)^2}$$

Standard deviation of $(\bar{x}_1 - \bar{x}_2) = SD\left(\frac{1}{n1} + \frac{1}{n_2}\right)$

$$= SD\left(\frac{n_1 + n_2}{n_1 n_2}\right)$$

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{SD\left(\frac{n_1 + n_2}{n_1 n_2}\right)}$$ with $n_1 + n_2 - 2$ degrees of freedom.

4. <u>Regression.</u>

n pairs of observation x_1y_1 x_2y_2 ..... x_ny_n

where y is dependent on x

Regression line $y = mx + c$

$$m = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

$$c = \frac{(\Sigma x)(\Sigma xy) - (\Sigma y)(\Sigma x^2)}{(\Sigma x)^2 - n(\Sigma x^2)}$$

Correlation coefficient r

$$= \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{(\Sigma x^2 - \frac{(\Sigma x)^2}{n})(\Sigma y^2 - \frac{(\Sigma y)^2}{n})}}$$

$$= \frac{m}{\sqrt{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}}$$

5. <u>Standard deviation of the difference between duplicate enzyme estimations.</u>

Duplicate estimation a and b.

Difference (d) = a - b.

Number of duplicate estimations = n.

S.D. of single determination = $\sqrt{\frac{\Sigma d^2}{2n}}$

D. Results of detailed statistical analysis.

STATISTICAL SIGNIFICANCE - PANCREATIC STIMULATION WITH SECRETIN AND PANCREOZYMIN

	KWASHIORKOR				MARASMUS				FIVE-YEAR FOLLOW-UP				RECOVERED KWASHIORKOR				CONTROLS			
Volume	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
Basal (B)	1	1	1		-	-	-		1	-	1		1	1	1		1	1	1	
Secretin (S)		-	-			-	-			-	-			5	-			5	-	
Pancreozymin (P)			-				-				-				-				-	
Mean S + P																				
pH	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	1	1	1	1	1	2	2	1	1	-	1	1	1	1	1	1	1	1	1	1
S		-	-	1		-	-	-		1	-	1		1	-	1		-	-	1
P			-	1			-	-			1	1			-	1			-	1
S + P				1				-				1				1				1
Colour Index	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	-	2		1	-	-		5	-	-		5	-	2		1	1	5		1
S		1		1		5		5		1		1		1		1		2		1
P				-				-				-				-				-
S + P																				
Amylase	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	-	5	5	1	-	-	-	5	-	5	-	2	5	5	5	1	1	1	1	1
S		-	-	2		-	-	-		-	-	-		-	-	2		-	-	5
P			-	5			-	-			-	-			-	2			-	1
S + P				-				-				-				2				5
Lipase	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	1	1	1	1	-	-	-	5	1	1	1	1	1	1	1	1	1	1	1	1
S		-	-	5		-	-	-		-	-	5		-	-	1		-	-	5
P			-	-			-	-			-	5			-	1			-	5
S + P				-				-				5				1				5
Ribonuclease	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	-	2	-	1	-	-	-	2	5	1	1	1	-	-	-	1	2	1	2	1
S		-	-	1		-	-	-		-	-	2		-	-	1		-	-	-
P			-	-			-	-			-	-			-	-			-	-
S + P				5				-				5				1				-
Trypsin	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	1	1	1	1	-	-	-	-	1	1	1	1	-	1	2	1	2	1	1	1
S		-	-	5		-	-	-		5	-	1		5	-	1		2	-	1
P			-	-			-	-			-	-			-	1			-	5
S + P				5				-				2				1				1
Chymotrypsin	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak	S	P	S+P	Peak
B	-	-	5	5	-	-	-	-	-	5	-	1	-	2	-	1	-	5	5	1
S		-	-	-		-	-	-		-	-	5		-	-	5		-	-	-
P			-	-			-	-			-	-			-	-			-	-
S + P				-				-				5				2				-

Significance (P) : 1 = <0.01 2 = <0.02 5 = <0.05 - = Not Significant.

TABLE V.

THE EFFECT OF HORMONAL STIMULATION IN THE DIFFERENT CLINICAL GROUPS.

STATISTICAL SIGNIFICANCE OF ENZYME TESTS - VALUES FOR P

	AMYLASE				LIPASE				RIBONUCLEASE				TRYPSIN				CHYMOTRYPSIN			
Basal (B)	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	1	1	-	2	1	1	-	-	1	5	-	-	1	2	-	1	1	1
M		-	1	1		-	1	-		-	-	-		-	-	-		-	1	1
F			-	-			2	-			5	-			5	-			2	-
R				-				-				-				-				-
Secretin (S)	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	1	1	-	1	1	1	-	5	1	1	-	-	1	2	-	1	1	1
M		-	1	1		-	1	1		-	-	-		-	-	-		1	1	1
F			-	-			2	-			-	-			-	-			2	-
R				1				-				-				-				-
Pancreozymin (P)	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	1	1	-	1	1	1	-	1	1	1	-	1	1	1	-	1	1	1
M		1	1	1		-	1	5		5	5	-		-	5	1		1	1	1
F			-	-			-	-			-	-			-	-			1	-
R				-				-				-				-				-
Mean S + P	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	1	1	-	1	1	1	-	1	1	1	-	5	1	1	-	1	1	1
M		5	1	1		5	1	1		-	-	-		-	5	5		1	1	1
F			-	-			5	-			-	-			-	-			1	-
R				-				-				-				-				-
Peak	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	1	1	-	1	1	1	-	1	1	1	-	1	1	1	-	1	1	1
M		5	1	1		-	1	5		-	-	-		-	1	1		1	1	1
F			-	-			5	-			-	-			-	-			-	-
R				-				-				-				-				-
S - B	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	2	5	1	-	-	1	1	-	5	-	2	-	-	-	-	-	2	2	1
M		-	-	1		-	2	1		-	-	-		-	-	-		2	2	1
F			-	5			-	-			-	-			-	-			-	5
R				1				-				-				-				-
P - B	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	2	1	-	5	1	1	-	2	-	-	-	2	-	1	-	1	1	1
M		-	-	1		-	5	2		5	-	-		5	-	1		5	1	1
F			-	-			-	-			-	-			-	-			-	-
R				5				-				-				-				-
Peak - B	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C	M	F	R	C
K	-	1	1	1	-	1	1	1	-	2	-	5	-	1	5	1	-	1	1	1
M		2	5	1		-	2	5		-	-	-		2	-	1		1	1	1
F			-	5			-	-			-	-			-	-			-	-
R				1				-				-				-				-

K = Kwashiorkor M = Marasmus F = Five year Follow-up R = Recovered Kwashiorkor C = Controls

Significance (P) : 1 = <0.01 2 = <0.02 5 = <0.05 - = Not Significant.

TABLE W.

ENZYME OUTPUT - COMPARISONS BETWEEN EACH CLINICAL GROUP.

STATISTICAL SIGNIFICANCE - VOLUME, pH, COLOUR INDEX - VALUES FOR P

	VOLUME				pH				COLOUR INDEX			
Basal (B)	M	F	R	C	M	F	R	C	M	F	R	C
K	-	5	-	-	-	1	-	-	-	-	-	-
M		5	-	-		1	-	-		-	-	-
F			5	-			1	-			-	-
R				5				-				-
Secretin (S)	M	F	R	C	M	F	R	C	M	F	R	C
K	-	-	-	-	-	2	2	-	-	-	-	-
M		-	-	-		1	2	-		-	-	-
F			2	-			-	-			-	-
R				-				-				-
Pancreozymin (P)	M	F	R	C	M	F	R	C	M	F	R	C
K	-	-	-	-	-	-	-	-	-	5	-	-
M		-	-	-		-	-	-		-	-	-
F			-	-			-	-			-	-
R				-				-				-
Mean S + P	M	F	R	C	M	F	R	C	M	F	R	C
K	-	5	-	-	-	-	5	-	Not Calculated			
M		-	-	-		-	-	-				
F			5	-			-	-				
R				-				-				
Peak	M	F	R	C	M	F	R	C	M	F	R	C
K	Not Calculated				-	-	-	-	-	1	-	-
M						-	-	-		5	-	-
F							-	-			1	-
R								-				-
S - B	M	F	R	C	M	F	R	C	M	F	R	C
K	-	-	-	-	-	1	-	2	-	-	-	-
M		-	-	-		2	-	-		-	-	-
F			-	-			1	-			-	-
R				-				-				-
P - B	M	F	R	C	M	F	R	C	M	F	R	C
K	-	-	-	-	-	1	-	-	-	-	-	-
M		-	-	-		1	-	-		-	-	-
F			-	-			1	-			-	-
R				-				-				-
Peak - B	M	F	R	C	M	F	R	C	M	F	R	C
K	Not Calculated				-	1	-	2	-	5	-	5
M						2	-	-		-	-	-
F							1	-			-	-
R								-				-

K = Kwashiorkor M = Marasmus F = Five-year Follow-up R = Recovered Kwashiorkor C = Control

Significance (P) : 1 = $<$ 0.01 2 = $<$ 0.02 5 = $<$ 0.05 - = Not Significant

TABLE X.

VOLUME, pH, COLOUR INDEX - COMPARISONS BETWEEN EACH CLINICAL GROUP.

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