The short-term response to a drink of milk, lactose or casein in children with apparently normal gastrointestinal tracts

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1. Drinks of milk, lactose and casein were given to children who, though suspected of having malabsorption, were subsequently found to have normal gastrointestinal tracts. The plasma concentrations of glucose, amino acids, urea and insulin following these drinks were measured. The results can probably be taken to represent control values when investigating children with gastrointestinal or metabolic disorders.

2. The rise in plasma amino acids after giving casein and the rise in plasma glucose after giving lactose were greater than those after giving equivalent amounts of milk.

3. The absorption of an individual food constituent and its uptake by the tissues are influenced by the presence of other food constituents so that 'tolerance tests' with individual nutrients may not be truly physiological.

The immediate or short-term effects of an individual meal in childhood have been studied by such techniques as oxygen consumption (Brooke, 1972) or tracer detection (Sutton & Barltrop, 1973). Short-term changes in plasma concentrations of nutrients have usually been determined after an oral dose of a single food constituent, often given in unphysiological quantities as a tolerance test, e.g. lactose (Reddy & Pershad, 1972), casein (Douglas & Booth, 1969), amino acids (Milner, 1971) and fat (Penfold & Keynes, 1971; Robards, 1973). Less work has been done with actual food but short-term responses to the ingestion of food have been determined in animals (Chesters, 1971). Plasma amino acid concentrations have been determined in low-birth-weight infants during continuous feeding with different volumes and types of milk (Valman, Brown, Palmer, Oberholzer & Levin, 1971).

The present investigation was designed to show the short-term changes in plasma levels of glucose, amino acids, urea and insulin in apparently normal children following a physiological drink of milk or of one of its constituents, lactose or casein. The investigation was prompted by a preliminary study of the effects of milk in children with coeliac disease, in which the changes in plasma glucose following milk were found to be different from those following lactose alone, in both the subjects and a 'control' group of children (Rossiter, 1973).

EXPERIMENTAL

Subjects. The children studied were being investigated for possible malabsorption. The results presented in this paper are those observed in children subsequently

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Table 1. *Clinical indications for investigation of children with suspected malabsorption who were subsequently found to have normal gastrointestinal tracts, and anthropometric and biochemical details of the children*

(Total values in parentheses)

Clinical presentation	No. of children	Age range (years)
Failure to thrive, coeliac disease suspected	6	1·6– 4·9
'Gluten challenge', previous clinical diagnosis of coeliac disease	4	3·0–15·2
Small stature	8	1·1–13·3
Late-onset rickets	2	3·7– 8·0
	(20)	(1·1–15·2)

Investigation	Abnormal result	No. studied	No. abnormal
Weight for age	<3rd centile*	20	14
Height for age	<3rd centile*	20	11
Jejunal biopsy	Abnormal histology	20	0
Duodenal juice	Trypsin positive <1:4 dilution Amylase <4 I.U./l Lipase < 24 I.U./l	13	0
Sweat test	Sweat sodium >60 mmol/l	11	0
Fat absorption	Faecal fat excretion >4·5 g/d, or nephelometry <70 LSI, or both	13	0
Haemoglobin	<120 g/l	20	2
Serum iron	<17·9 μmol/l	16	6
	<8·9 μmol/l	16	2
Folate	Serum <5 μg/l	15	2
	Red cell <200 μg/l	9	0
Plasma calcium	<45 mmol/l	20	0
Serum cholesterol	<2·6 mmol/l	19	0
Serum albumin	<35 g/l	20	0

LSI, rise in light-scattering index 2 h after oral dose of fat (Robards, 1973).
* Tanner, Whitehouse & Takaishi (1966).

considered 'normal' because they fulfilled the following criteria: (*a*) no diarrhoea; (*b*) normal jejunal histology and disaccharidase levels; (*c*) no biochemical evidence of malabsorption; (*d*) no clinical or biochemical evidence of malnutrition. Table 1 shows the clinical indications for investigation and the anthropometric and biochemical details of the twenty children who satisfied the criteria and whose parents gave consent for the study. The majority of the children were undersized, eight had low serum iron concentrations and two had low serum folate concentrations, but more specific tests for malabsorption were normal in all the children. We considered that the low serum Fe and folate levels reflected inadequate dietary intake rather than malabsorption.

Ethical considerations. The plan of the study was approved by the hospital research committee and informed consent to the programme of investigations was obtained from all parents. The children studied had been admitted to hospital for investigations of suggested gastrointestinal disease, and it was hoped the response to the drinks

would give some indication of absorptive function and, together with the other investigations performed, e.g. jejunal biopsy, contribute to the over-all assessment of the child's gastrointestinal tract. On the basis of the other investigations we subsequently decided that these children had 'apparently normal' gastrointestinal tracts. We consider they represent a group of children as near normal as is ethically possible.

Drinks. Changes in plasma amino acid levels following protein loads are far greater than those during a similar period of fasting (Palmer, Rossiter, Levin & Oberholzer, 1973), but in view of the known circadian periodicity of plasma amino acid concentrations (Feigin, Beisel & Wannemacher, 1971), all tests were performed at the same time of day. The children drank a measured amount of milk at 09.00 hours, after a 9 h fast. On a later occasion, the test was repeated with lactose or casein. Not every child participated in two tests: in all, fifteen children took milk, nine took lactose and eight took casein. When comparing the effects of the different drinks we analysed the results by two methods; first by comparing the differences in response within the same subject (using the paired *t* test) and secondly by comparing the differences in responses of the whole groups (using the independent *t* test). Comparable results were obtained by either method. We have chosen to present only the latter since they refer to a larger number of children and will therefore be of more value for future comparison with results from ill children.

The milk used (Half Cream National Dried Milk, Welfare Foods) contained (g/l): 50 lactose, 39 casein and 20 fat. The children drank 30 ml milk/kg body-weight to a maximum of 450 ml. This gave a protein load of 1·2 g/kg but in the five children who weighed more than 15 kg, up to three eggs were added to achieve this. The dose of lactose used was the same as that in the milk, i.e. 1·6 g/kg to a maximum of 23 g. Casein was given as 'Casilan' (Glaxo Laboratories Ltd, Greenford, Middx) (calcium caseinate, contains 900 g casein/kg) and again the dose was the same as in the milk, i.e. 1·2 g/kg. A carmine marker was added to the milk and lactose drinks and the subsequent marked stool was tested for reducing substances (Kerry & Anderson, 1964).

Biochemical methods. Capillary blood samples were taken at various intervals for 3·5 h after the drink for the estimation of glucose (Marsh, Fingerhut & Kirsch, 1957), urea (Gutteridge & Wright, 1968), immunoreactive insulin (Morgan & Lazarow, 1963) and amino acids (Palmer, 1973). The insulin method is normally sensitive to approximately 1 mU/l but occasionally due to poor reproducibility of low level duplicates on the standard curve, certain values are recorded as < 5 mU/l. Levels of taurine and glutamic acid can be affected by haemolysis, and levels of threonine, serine and ornithine can be affected by contamination with sweat to a degree which masks changes following meals (Dickinson, Rosenblum & Hamilton, 1965; Perry & Hansen, 1969; Palmer, 1973). These amino acids have therefore not been considered in this study.

RESULTS

Fig. 1 shows the rise in plasma glucose levels following drinks of milk, casein and lactose. The mean maximum rise in plasma glucose after milk (mean and SD,

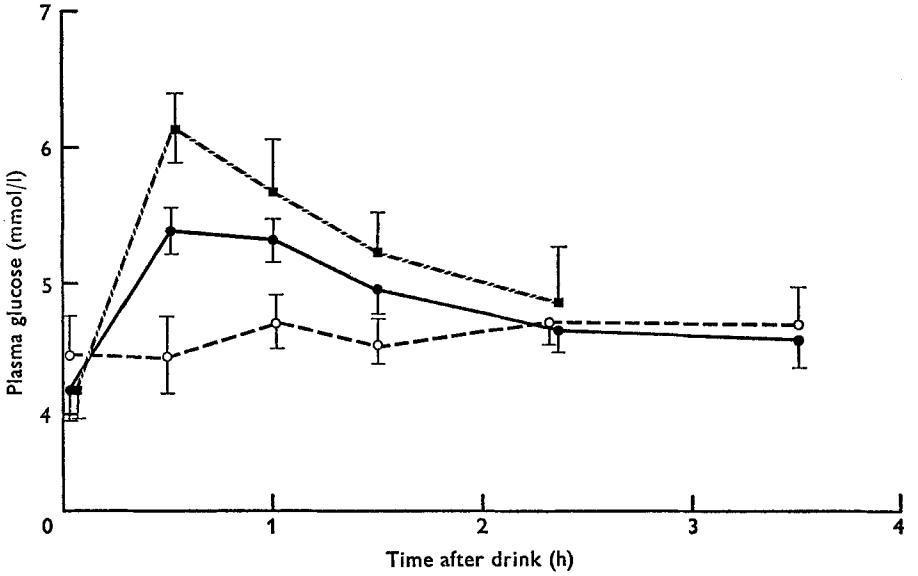

Fig. 1. Plasma glucose levels in samples taken from children at various intervals after drinks of milk, casein or lactose. After milk (● — ●), fifteen subjects; casein (○ – – ○), eight subjects; and lactose (■ – - – ■), nine subjects. Each point is the mean value with its standard error, represented by the vertical bar, for the number of subjects mentioned above.

1·38 ± 0·67 mmol/l) was significantly less than that following lactose alone (2·04 ± 0·59 mmol/l; $P < 0·02$) but significantly greater than the small rise after a casein drink (0·19 ± 0·89 mmol/l; $P < 0·001$). Table 2 shows the changes in plasma amino acid concentrations following the milk and casein drinks, and the results for a representative amino acid, valine, are illustrated in Fig. 2. Most amino acids increased after the milk drink and the greatest changes were seen in the levels of essential amino acids, such as valine. In contrast, the levels of glycine, and in some instances alanine, fell. All amino acids reached a maximum or plateau by 3·5 h after the milk was taken. By chance, fasting levels of many amino acids were greater before the casein was given than before milk even in tests performed on the same child, but this was significant only in the instance of alanine. Whether the results are expressed as absolute concentrations, maximum change compared with the fasting concentration or as 'area under the curve', there was a greater rise in the levels of proline, leucine, isoleucine, valine, lysine, arginine, phenylalanine, tyrosine and histidine following casein than after milk. Although the initial rise in amino acid levels (e.g. valine, Fig. 2) was similar for either load, the levels continued to rise for a longer time after casein so that the difference in the amino acid response was only apparent in the final samples. Changes in alanine and glycine concentrations were similar after the two loads. Fig. 2 also shows the changes in plasma urea following the two drinks. The urea level rose steadily after casein (mean maximum rise, 2·42 ± 0·85 mmol/l) whereas there was only an early and significantly smaller rise after milk (mean maximum rise, 1·45 ± 0·80 mmol/l; $P < 0·02$). The changes in urea appeared to mirror those in the amino acids. Plasma urea fell slightly after lactose, a change which was significantly different

Table 2. *Plasma levels (mmol/l) of neutral, branched-chain, dibasic and aromatic amino acids in samples taken from children at intervals after drinks of milk or casein*

(Mean values with ranges in parentheses)

	Time of sampling (h)								Change in plasma amino acid concentrations†			
	0		1·5		2·5		3·5		Maximum change		Area under curve	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Neutral:												
Glycine												
Milk	0·21	(0·16–0·36)	0·20	(0·16–0·36)	0·24	(0·13–0·33)	0·20	(0·09–0·23)	−0·03	(−0·16–0·32)	−0·01	(−0·21–0·25)
Casein	0·28	(0·17–0·37)	0·24	(0·13–0·35)	0·27	(0·16–0·41)	0·25	(0·11–0·33)	−0·03	(−0·50–0·07)	−0·09	(−0·05–0·21)
*P**		NS		NS		NS		NS		NS		NS
Alanine												
Milk	0·28	(0·18–0·42)	0·38	(0·24–0·61)	0·35	(0·22–0·55)	0·31	(0·20–0·51)	0·11	(−0·07–0·29)	0·38	(−0·20–1·0)
Casein	0·38	(0·20–0·49)	0·38	(0·25–0·61)	0·44	(0·22–0·67)	0·40	(0·25–0·52)	0·06	(−0·16–0·25)	0·19	(−0·27–0·72)
P		<0·05		NS		NS		NS		NS		NS
Proline												
Milk	0·18	(0·07–0·30)	0·28	(0·18–0·39)	0·31	(0·18–0·55)	0·31	(0·18–0·49)	0·15	(0·05–0·33)	0·58	(0·21–1·30)
Casein	0·19	(0·14–0·37)	0·40	(0·23–0·55)	0·41	(0·22–0·57)	0·43	(0·26–0·55)	0·23	(0·11–0·42)	1·06	(0·42–1·70)
P		NS		<0·02		0·08		0·08		0·06		<0·01
Branched-chain:												
Valine												
Milk	0·23	(0·14–0·37)	0·40	(0·26–0·63)	0·42	(0·22–0·61)	0·42	(0·26–0·57)	0·21	(0·10–0·34)	0·85	(0·34–1·54)
Casein	0·26	(0·15–0·40)	0·44	(0·29–0·61)	0·56	(0·37–0·67)	0·62	(0·44–0·90)	0·36	(0·24–0·48)	1·52	(0·77–1·91)
P		NS		NS		<0·02		<0·005		<0·001		<0·005
Leucine												
Milk	0·11	(0·05–0·18)	0·22	(0·10–0·37)	0·21	(0·10–0·31)	0·22	(0·12–0·30)	0·14	(0·07–0·18)	0·52	(0·24–0·92)
Casein	0·13	(0·07–0·24)	0·24	(0·16–0·34)	0·29	(0·22–0·34)	0·33	(0·20–0·50)	0·21	(0·15–0·31)	0·76	(0·42–1·12)
P		NS		NS		<0·05		<0·005		<0·005		<0·05
Isoleucine												
Milk	0·08	(0·05–0·13)	0·16	(0·09–0·24)	0·16	(0·07–0·23)	0·17	(0·10–0·22)	0·09	(0·05–0·14)	0·37	(0·19–0·60)
Casein	0·09	(0·05–0·18)	0·19	(0·13–0·27)	0·22	(0·15–0·24)	0·25	(0·13–0·38)	0·17	(0·09–0·28)	0·57	(0·25–0·82)
P		NS		NS		<0·01		<0·01		<0·05		<0·02
Methionine												
Milk	0·01	(0·004–0·017)	0·018	(0·009–0·030)	0·019	(0·011–0·037)	0·019	(0·011–0·044)	0·011	(0–0·028)	0·045	(0–0·089)
Casein	0·01	(0·006–0·016)	0·018	(0·012–0·030)	0·024	(0·012–0·034)	0·022	(0·012–0·044)	0·014	(0·003–0·034)	0·056	(0·011–0·114)
P		NS		NS		NS		NS		NS		NS

Table 2 (*cont.*)

	Time of sampling (h)								Change in plasma amino acid concentrations†			
	0		1·5		2·5		3·5		Maximum change		Area under curve	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Dibasic:												
Lysine												
Milk	0·13	(0·05–0·25)	0·18	(0·06–0·36)	0·17	(0·06–0·38)	0·16	(0·08–0·34)	0·06	(0·01–0·12)	0·25	(0·08–0·55)
Casein	0·15	(0·11–0·21)	0·23	(0·16–0·29)	0·25	(0·17–0·31)	0·26	(0·17–0·40)	0·12	(0·01–0·16)	0·46	(0·05–0·69)
P		NS		0·09		<0·05		<0·02		<0·01		<0·02
Arginine												
Milk	0·04	(0·03–0·09)	0·06	(0·03–0·11)	0·06	(0·03–0·11)	0·06	(0·02–0·14)	0·03	(−0·01–0·05)	0·09	(−0·01–0·19)
Casein	0·04	(0·02–0·07)	0·06	(0·04–0·10)	0·09	(0·06–0·11)	0·07	(0·05–0·10)	0·05	(0·02–0·09)	0·16	(0·01–0·33)
P		NS		NS		0·07		NS		0·08		0·06
Glutamine												
Milk	0·66	(0·36–0·91)	0·71	(0·44–0·91)	0·73	(0·46–0·96)	0·66	(0·43–0·82)	0·04	(−0·24–0·34)	0·14	(−0·88–1·15)
Casein	0·60	(0·43–0·77)	0·69	(0·41–0·87)	0·68	(0·39–0·88)	0·70	(0·42–1·0)	0·08	(−0·16–0·34)	0·12	(−0·40–1·34)
P		NS		NS		NS		NS		NS		NS
Aromatic:												
Phenylalanine												
Milk	0·06	(0·04–0·08)	0·09	(0·05–0·16)	0·08	(0·04–0·14)	0·08	(0·05–0·15)	0·04	(0·01–0·08)	0·14	(0·03–0·35)
Casein	0·07	(0·04–0·14)	0·10	(0·04–0·19)	0·13	(0·05–0·26)	0·12	(0·05–0·27)	0·05	(0·01–0·18)	0·21	(0·02–0·46)
P		NS		NS		0·06		NS		NS		NS
Tyrosine												
Milk	0·07	(0·03–0·08)	0·12	(0·07–0·08)	0·12	(0·07–0·16)	0·11	(0·07–0·15)	0·07	(0·01–0·09)	0·26	(−0·02–0·51)
Casein	0·06	(0·04–0·08)	0·11	(0·07–0·13)	0·16	(0·12–0·22)	0·15	(0·12–0·21)	0·10	(0·07–0·17)	0·43	(0·23–0·71)
P		NS		NS		<0·02		<0·05		<0·05		<0·02
Histidine												
Milk	0·08	(0·03–0·11)	0·08	(0·03–0·12)	0·08	(0·05–0·13)	0·08	(0·06–0·12)	0·01	(−0·02–0·30)	0·05	(−0·08–0·09)
Casein	0·09	(0·06–0·12)	0·11	(0·05–0·17)	0·10	(0·08–0·14)	0·10	(0·08–0·13)	0·03	(0·01–0·06)	0·07	(0–0·17)
P		NS		NS		NS		NS		<0·05		NS

NS, not significant ($P > 0·1$).
* Statistical significance of difference between means obtained using Student's *t* test.
† Values for each individual.

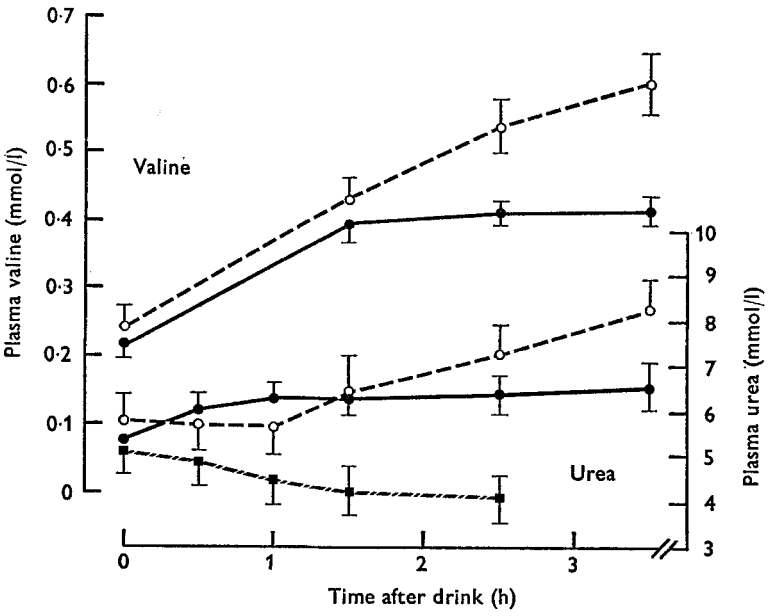

Fig. 2. Plasma valine and urea levels in samples taken from children at various intervals after drinks of milk, casein, or lactose. After milk (● — ●), fifteen subjects; casein (○ – – ○), eight subjects; and lactose (■ - - - ■), nine subjects. Each point is the mean value with its standard error, represented by the vertical bar, for the number of subjects mentioned above.

Table 3. *Plasma levels of immunoreactive insulin (mU/l) in samples taken from children at various intervals after drinks of milk, lactose or casein*

	Time of sampling (h)					Maximum change in plasma insulin concentrations†
	0	0·5	1·0	1·5	2·5	
Milk						
Mean	5	26	18	12	9	25
Range	<5–58	<5–58	6–37	<5–78	<5–34	10–59
Lactose						
Mean	5	16	14	9	3	14
Range	<5–13	<5–43	<5–61	<5–32	<5–12	−6–51
*P**	NS	NS	NS	NS	NS	NS
Casein						
Mean	7	8	8	10	10	5
Range	<5–21	<5–26	<5–26	<5–27	<5–24	−1–11
P	NS	<0·05	0·09	NS	NS	<0·05

NS, not significant ($P > 0·1$).
* Statistical significance of difference between means, compared with milk, using Student's *t* test.
† Values for each individual.

from that after milk (mean maximum change after lactose, $-0·35 \pm 0·87$ mmol/l; $P < 0·001$). Table 3 shows the plasma concentrations of insulin after the three drinks. Although the mean maximum change after milk was numerically greater than that after lactose, there was a very wide range of response and the difference was not

significant. Low insulin levels were found particularly in the younger children, as has been observed previously (Grant, 1968).

No child had reducing substances in the marked stool following milk or lactose.

DISCUSSION

The results show the short-term response of probably normal children to a physiological drink of milk. Comparison of the effects of the whole milk with those of lactose or casein, however, showed that glucose and many amino acids rose less after milk than after a milk constituent given alone. These differences could be due to variations in gastric emptying, in absorption or in intermediary metabolism.

When absorption is normal, the speed of gastric emptying determines the rate at which materials enter the blood stream (Hunt & Knox, 1968; Nimmo, Heading, Tothill & Prescott, 1973). It seems likely that the osmotic stimulus from sugar, fat and protein in whole milk, being greater than that from lactose or casein alone, would slow gastric emptying, resulting in lower concentrations of glucose and amino acids in the blood after milk, as we observed. This does not, however, explain why only some amino acids rose less after milk than after casein, nor why amino acid concentrations continued to rise for a longer time after casein, which should theoretically leave the stomach more rapidly than milk.

Alternatively, the results could be discussed in terms of rate of absorption from the small intestine. The mutual inhibition of active transport of amino acids and monosaccharides (Schultz & Curran 1970; Cook 1974), could explain the finding that the rise in plasma glucose after milk was less than that after lactose. Moreover it would explain why the reduced amino acid response to milk was most marked in the instance of those amino acids which are normally most actively transported and whose absorption might therefore be most inhibited by the presence of sugars (Adibi, Gray & Menden, 1967; Milne, 1971).

However, our findings could be explained without reference to the gastrointestinal tract. The absence of carbohydrate in a feed would result in increased utilization of amino acids, especially alanine, by the liver for glycogenesis, and in decreased uptake of amino acids such as the branched-chain ones which are normally metabolized by the extrahepatic tissues (Exton, 1972; Felig, 1973). This would explain why the branched-chain amino acids showed a greater rise after the pure protein than after milk, and why this did not occur with alanine. The higher urea concentrations found after casein might reflect increased gluconeogenesis in the absence of carbohydrate; the lower insulin levels after casein are also compatible with this explanation (Levin, Grodsky, Hagura, Smith & Forsham, 1972; Malaisse, 1972). Metabolic and hormonal mechanisms could similarly account for the smaller rise in plasma glucose after milk than after lactose (Catt, 1970).

Although the results in this study are compatible with concepts of gastric emptying, transport of amino acids and sugars by the intestine, and the handling of these nutrients in intermediary metabolism, they provide no information concerning the relative importance of the mechanisms in determining the short-term response to a

meal. The results may, however, provide a useful standard of reference when investigating a child with a suspected metabolic or gastrointestinal disorder. The study has shown that the response to a nutrient when given singly may be different from that when it is given in normal food so that many tolerance tests used in clinical practice can only be regarded as guides to diagnosis, rather than as a reflexion of the true situation.

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REFERENCES

Adibi, S. A., Gray, S. J. & Menden, E. (1967). *Am. J. clin. Nutr.* **20**, 24.
Brooke, O. G. (1972). *Br. med. J.* i, 331.
Catt, K. J. (1970). *Lancet* ii, 353.
Chesters, J. K. (1971). *Proc. Nutr. Soc.* **30**, 1.
Cook, G. C. (1974). *Gut* **15**, 239.
Dickinson, J. C., Rosenblum, H. & Hamilton, P. B. (1965). *Pediatrics, Springfield* **36**, 2.
Douglas, A. P. & Booth, C. C. (1969). *Clin. Sci.* **37**, 643.
Exton, J. H. (1972). *Metabolism* **21**, 945.
Feigin, R. D., Beisel, W. R. & Wannemacher, R. W. Jr (1971). *Am. J. clin. Nutr.* **24**, 329.
Felig, P. (1973). *Metabolism* **22**, 179.
Grant, D. B. (1968). *Acta paediat., Stockh.* **57**, 297.
Gutteridge, J. M. C. & Wright, E. B. (1968). *J. med. Lab. Technol.* **25**, 385.
Hunt, J. N. & Knox, M. T. (1968). *Am. J. dig. Dis.* **13**, 372.
Kerry, K. R. & Anderson, C. M. (1964). *Lancet* i, 981.
Levin, S. R., Grodsky, G. M., Hagura, R., Smith, D. F. & Forsham, P. H. (1972). *Endocrinology* **90**, 624.
Malaisse, W. J. (1972). *Proc. Nutr. Soc.* **31**, 213.
Marsh, W. H., Fingerhut, B. & Kirsch, E. (1957). *Am. J. clin. Path.* **28**, 681.
Milne, M. D. (1971). *Scient. Basis Med. a. Rev.* **4**, 162.
Milner, R. D. G. (1971). *Archs Dis. Childh.* **46**, 301.
Morgan, C. R. & Lazarow, A. (1963). *Diabetes* **12**, 115.
Nimmo, J., Heading, R. C., Tothill, P. & Prescott, L. F. (1973). *Br. med. J.* i, 587.
Palmer, T. (1973). Amino acid levels in blood, urine and CSF of normal and sick children. PhD Thesis, University of London.
Palmer, T., Rossiter, M. A., Levin, B. & Oberholzer, V. G. (1973). *Clin. Sci. mol. Med.* **45**, 827.
Penfold, W. A. F. & Keynes, W. M. (1971). *Ann. Surg.* **173**, 157.
Perry, T. L. & Hansen, S. (1969). *Clinica Chim. Acta* **25**, 53.
Reddy, V. & Pershad, J. (1972). *Am. J. clin. Nutr.* **25**, 114.
Robards, M. F. (1973). *Archs Dis. Childh.* **48**, 656.
Rossiter, M. A. (1973). *Archs Dis. Childh.* **48**, 79.
Schultz, S. G. & Curran, P. F. (1970). *Physiol. Rev.* **50**, 637.
Sutton, A. & Barltrop, D. (1973). *Nature, Lond.* **242**, 265.
Tanner, J. M., Whitehouse, R. H. & Takaishi, M. (1966). *Archs Dis. Childh.* **41**, 454.
Valman, H. B., Brown, R. J. K., Palmer, T., Oberholzer, V. G. & Levin, B. (1971). *Br. med. J.* iv, 789.

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