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Metabolic acidosis, nitrogen balance and weight gain in preterm infants

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Nitrogen balance, urinary NAE and the acid base parameters in the blood of 15 male preterm infants with birth weights of 1000—2370 g (mean 1715 g) and gestational age of 29—37 weeks (mean 33.3 weeks) were determined weekly, in the first six weeks of life.

The sum of NAE plus acid retention, as "total acid", was used to investigate the relationship between nitrogen balance, weight gain and acid-base homeostasis.

During the first three postnatal weeks, nitrogen intake, urinary and faecal loss of nitrogen as well as the nitrogen retention were rapidly increasing. Later, urinary excretion continued to rise, the intake remained unchanged and as a result, the amount of retained nitrogen decreased slightly.

Urinary NAE was steadily increasing to reach the maximum of 2.8 mEq/kg by the fourth week. Acid retention was the most pronounced in the second and third week, thereafter it fell until the end of the study. The importance of NAE relative to acid retention was continuously increasing throughout the observation period.

The "total acid" increased gradually and reached its peak value in the third week of life. Subsequently a continuous fall was seen.

During the first three ;weeks of life there was a significant positive correlation between "total acid" and nitrogen intake and urinary nitrogen extraction. Since the increase in "total acid" went parallel with the increasing nitrogen retention, the latter may be assumed to be an additional factor in producing acidosis.

Calculated per 100 mg nitrogen ingested, retained or excreted with urine, „total acid" was decreasing with the increasing rate of weight gain. This indicates that the growth process — irrespective of the postnatal development of renal H^+ handling — is also involved in the elimination of acids.

It is generally accepted that during the first postnatal weeks the increasing acid input and the limited renal capacity to excrete hydrogen ions lead to a positive acid balance and to maintenance of the acidotic state in low-birth-weight neonates [10, 11]. Under usual conditions the net non-volatile acid (NVA) input mainly originates from protein catabolism [14]. Net urinary acid excretion (NAE) of premature infants varies to a great extent from week to week depending on the maturity of the kidney and on the acid load to be excreted [5, 7, 8, 9, 10, 11, 18, 22, 23, 25, 26].

The present longitudinal study was undertaken to investigate the relationship between some well-known factors such as protein catabolism, weight gain and urinary NAE, all

affecting the acid-base homeostasis of premature infants during the first six weeks of life.

In an effort to overcome the disturbing effect of the rapidly changing renal contribution to the control of acidosis, the sum of NAE and acid retention (called "total acid") was used to assess the influence of different growth-rates and the components of nitrogen balance.

Material and Methods

A group of male preterm infants was selected for the study. Pregnancy, delivery and perinatal course were uneventful in all cases. The birth weights and gestational ages ranged from 1000 to 2370 g (mean, 1715 g) and from 29 to 37 weeks (mean, 33.3 weeks), respectively.

The infants were fed appropriate cow's milk formulas. Food intake was gradually increased nearly at the same rate in every infant to attain a calorie and fluid intake of 120—140 cal/kg and 180—200 ml/kg, respectively, by the end of the second week of life. Nitrogen balance, urinary net acid excretion and the acid-base parameters of blood were determined on the 7th day and thereafter weekly for 6 weeks.

Urine was collected fractionally under toluene for a period of 24 hours. The specimens were refrigerated, pooled and analysed for pH, titratable acidity, ammonia and total nitrogen.

Analytical procedures

Arterial blood acid-base status was determined by the method of Astrup [2]. Urinary pH was measured at 38 °C, titratable acidity according to Folin (end point of titration, pH 7.4), urinary ammonia according to McCullough [16]. Urinary bicarbonate was calculated from Gamble's nomogramme using the corresponding pH value [6]. Kjeldahl's method was used to determine the total nitrogen content of urine and that of the cow's milk formulas.

Calculations

Net acid excretion consisted of the sum of urinary titratable acid plus ammonium ion minus bicarbonate.

The amount of retained acids was calculated from the base excess by an arbitrary assessment. According to this a change of one mEq/l of base excess in the premature infant corresponds to about 0.5 mEq of acid/kg body weight [9, 12].

Nitrogen retention was calculated as the difference between the nitrogen intake and the sum of the urinary and faecal nitrogen losses. The nitrogen lost in the stools was approximated to be 15% of the nitrogen ingested irrespective of birth weight, postnatal age or protein intake [27, 28].

Statistical analysis was performed by calculating the coefficient of correlation, the equation of regression, the mean and the standard errors. The p values presented were determined by Student's t test.

Results

Postnatal changes in nitrogen balance

Intake, urinary excretion and faecal loss of nitrogen as well as nitrogen retention are shown in Figure 1 and Table I. It can be seen that during the first three weeks, nitrogen intake was rapidly increasing, but thereafter it remained practically unchanged.

Urinary nitrogen excretion showed a moderate stepwise increase throughout the whole observation period.

Faecal nitrogen was approximated to account for 15% of the ingested nitrogen irrespective of postnatal age, therefore its postnatal changes were similar as those of the nitrogen intake.

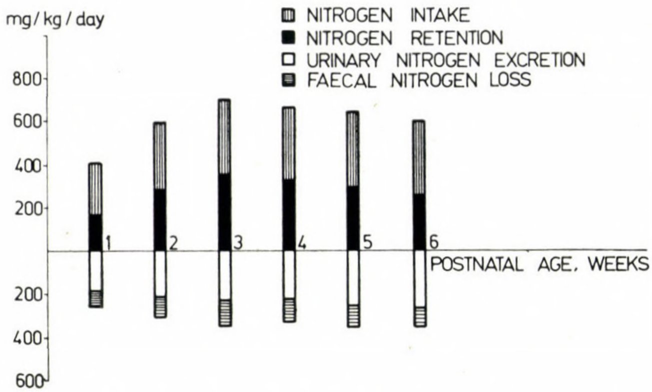

FIG. 1. Nitrogen balance of preterm infants during the first six weeks of life

TABLE I

Postnatal changes in nitrogen balance of preterm infants during the first six weeks of life

Age, weeks		Nitrogen intake mg/kg/day	Nitrogen loss (mg/kg/day) urinary	fecal	Nitrogen retention mg/kg/day
1	mean	410.4	189.4	61.6	160.3
	n	15	15	15	15
	S. E.	24.2	18.2	3.6	26.8
2	mean	594.2	221.8	89.1	282.3
	n	15	15	15	15
	S. E.	27.9	17.8	4.1	28.5
3	mean	698.1	239.9	104.7	348.8
	n	14	14	14	14
	S. E.	25.6	27.6	3.8	26.6
4	mean	656.4	234.1	98.5	318.5
	n	13	13	13	13
	S. E.	26.0	22.9	3.9	10.0
5	mean	646.2	256.5	96.9	292.5
	n	11	11	11	11
	S. E.	29.4	18.8	5.0	28.9
6	mean	598.4	263.1	89.8	247.8
	n	10	10	10	10
	S. E.	32.8	28.0	4.9	41.4

Significance brackets printed in the table: intake weeks 1–2: 0.001; intake weeks 3–6: 0.01; urinary weeks 1–5: 0.01; urinary weeks 1–6: 0.025; fecal weeks 1–2: 0.001; fecal weeks 3–6: 0.01; retention weeks 1–2: 0.025; retention weeks 1–3: 0.005; retention weeks 3–6: 0.05.

As a result of these changes a continuously positive nitrogen balance developed. The amount of retained nitrogen increased at a slow rate until the third week followed by a slight gradual fall during the further periods of observation.

Postnatal changes in retained acids and NAE

As shown in Figure 2 and Table II the urinary NAE is steadily increasing and reaches its maximum of 2.8 mEq/kg/day at the end of the fourth

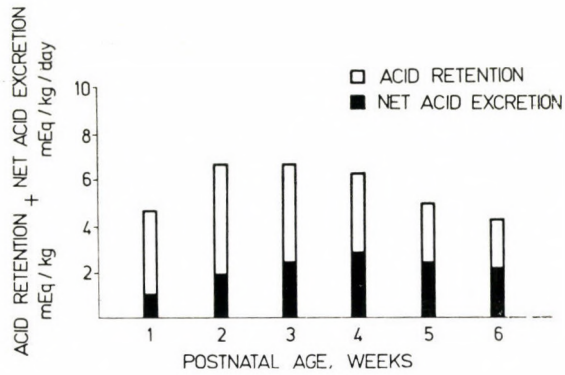

FIG. 2. Retained acids and urinary NAE of preterm infants during the first six weeks of life

TABLE II

Postnatal changes in urinary NAE, acid retention and "total acid" of preterm infants during the first six weeks of life

Age, weeks		1	2	3	4	5	6
urinary NAE	mean	$-0.94^{4'4'4}$	1.83^{4}	2.35^{4}	2.78^{4}	2.35	2.20
mEq/kg/day	n	15	15	14	13	11	10
	S. E.	0.15	0.25	0.33	0.35	0.25	0.32
acid retention	mean	$3.71^{1'2'3}$	4.71^{1}	4.50	3.37	2.61^{2}	2.04^{3}
mEq/kg/day	n	15	15	14	13	11	10
	S. E.	0.30	0.50	0.44	0.50	0.43	0.48
"total acid"	mean	$4.61^{2'2}$	6.57^{2}	$6.81^{2'3}$	6.20	4.96	4.25^{3}
	n	15	15	14	13	11	10
	S. E.	0.49	0.64	0.78	0.60	0.56	0.70

$^{1}p < 0.05$
$^{2}p < 0.025$
$^{3}p < 0.01$
$^{4}p < 0.005$

week. Later it persists at this level or undergoes a slight, statistically insignificant decrease.

Acid retention is the most pronounced in the second and third week and it falls to about 2 mE/kg by the end of the study. The amount of acids represented by the sum of the NAE and the retained acids gradually increases and reaches its peak value in the third week of life. Subsequently, a marked continuous fall occurs.

Figure 3 illustrates the relationship between NAE and retained acids. The importance of NAE against acid

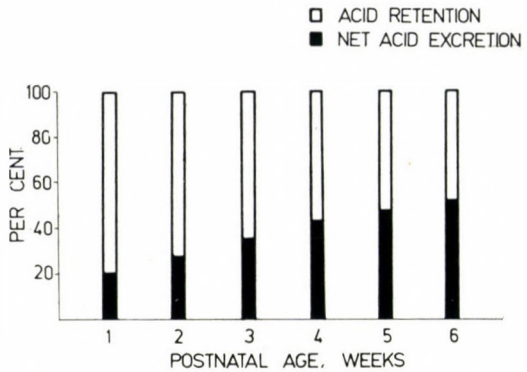

FIG. 3. Postnatal changes in the importance of urinary NAE in relation to acid retention in preterm infants

retention is continuously increasing even in the 5th and 6th weeks of life when the absolute value of NAE is practically unchanged, due to the decreased acid load to be excreted. This suggests that in the disappearance of late metabolic acidosis, factors other than renal acid excretion must also be involved.

Relationship between nitrogen balance and NAE + acid retention

During the first three weeks of life, when late metabolic acidosis develops, there is a significant positive correlation between nitrogen intake and the sum of NAE plus acid retention (Fig. 4).

Figure 5 shows the amount of "total acids" *vs.* urinary nitrogen excretion. A significant positive relationship may also be seen indicating that the development of acidosis is due to the increased protein catabolism. When total acids are plotted as a function of nitrogen retention, such a relationship cannot be found (Fig. 6). However, mean nitrogen

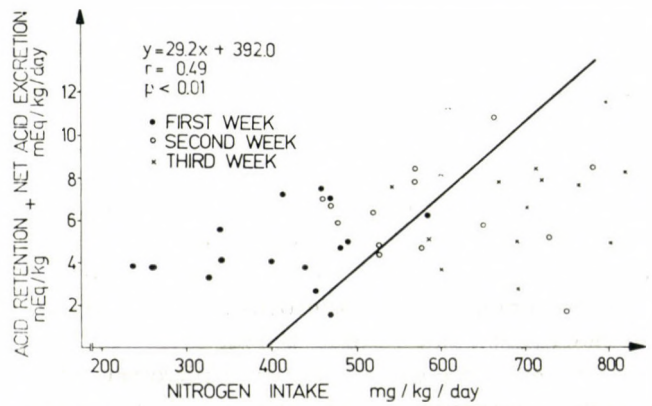

FIG. 4. Relationship between "total acids" and nitrogen intake of preterm infants during the first three weeks of life

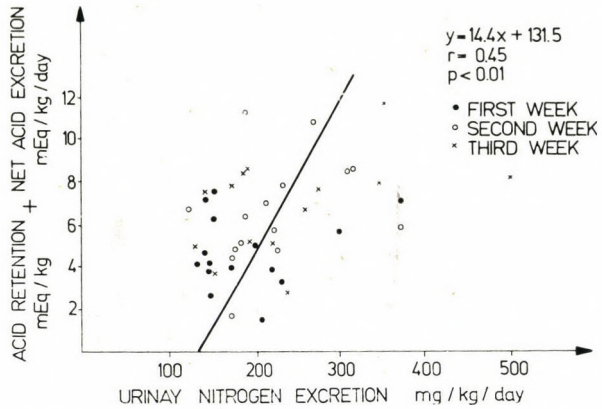

FIG. 5. Relationship between ' total acids" and urinary nitrogen excretion of preterm infants during the first three weeks of life

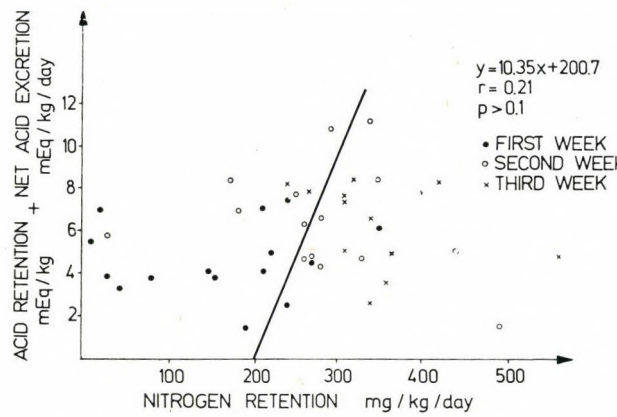

FIG. 6. Relationship between "total acids" and nitrogen retention of preterm infants during the first three weeks of life

retention and "total acids" show a similar tendency to rise during the first three postnatal weeks.

Calculated per 100 mg nitrogen ingested, retained or excreted in urine, the amount of "total acids" is gradually decreasing from the second week of life and the lowest values are attained by the 5th and 6th weeks (Fig. 7). There is an inverse relationship between the fall of these ratios and weight gain: the more pronounced the fall, the higher the rate of weight gain. These findings mean that the acid load derived mainly from protein catabolism is partly eliminated by processes involved in growth.

The pattern of "total acids" per 100 mg nitrogen retained is slightly different from that related to the ingested or excreted nitrogen. It dis-

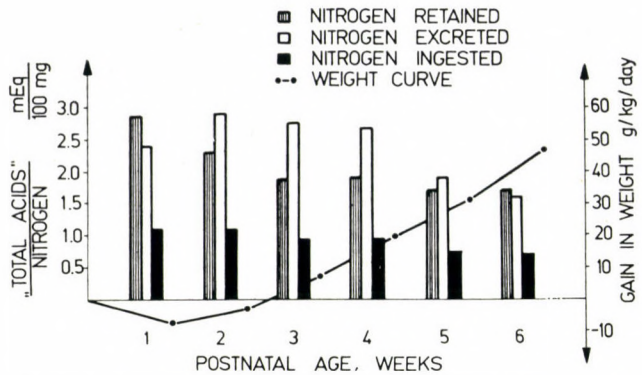

FIG. 7. Postnatal changes in "total acids" per 100 mg nitrogen ingested, excreted and retained, and their relation to the weight gain of preterm infants during the first six weeks of life

plays a marked fall in the second week and after the third week remains nearly unchanged indicating that during the period of initial weght loss the retained nitrogen may cause acidosis.

DISCUSSION

The development of metabolic acidosis in preterm infants is ascribed to an imbalance between metabolic acid production and the efficiency of the processes involved in the elimination of the endogenous and exogenous acid load.

On the basis of their 2 to 3 times higher protein intake, metabolic hydrogen ion production per kg of body weight for infants was assumed to exceed that for adults [1]. Chan [3] studied the endogenous acid production of 1 to 2 weeks old preterm infants by measuring the urinary concentration of sulphate and organic anions. The rate of endogenous acid production was 2–3 mEq/kg/day, and the kidney excreted only about 65% of the acid produced, even if the acid-base status was in equilibrium.

In the present study, endogenous acid production was not measured directly, but the data concerning "total acids" allowed to draw some conclusions as to metabolic hydrogen ion production.

(i) During the first three weeks of life, urinary net acid excretion is steadily increasing and at the same time acid retention is slightly increased or unchanged. This provided indirect evidence of the rising rate of endogenous acid production.

(ii) Under our nursing conditions the rate of metabolic acid production reached the level of 2–3 mEq/kg/day measured by Chan only after the second postnatal week, even when it was taken into account that in a steady acid-base status about 35% of the acid production is not excreted by the kidneys.

(iii) In good agreement with previous reports, the increasing protein intake resulted in an increase of

acidosis and/or urinary acid excretion. The rise is thought to be due to an increased protein catabolism [4, 5, 8, 9, 10, 11, 24, 26]. The increased protein intake resulted not only in enhanced urinary nitrogen excretion, but also in an increase of nitrogen retention. Since during the first three weeks of life, mean nitrogen retention and "total acids" showed a similar rising tendency, the nitrogen retained may also be considered an additional factor in producing acidosis. In support of this assumption it must be mentioned that in this early period of life only part of the nitrogen is incorporated to form new protein, whereas a considerable amount is accumulated in the body fluids in the form of free amino acids. The higher the protein intake, the higher the level of total free amino acids [15, 21]. It was shown that in low-birth-weight neonates fed a standard formula, the plasma amino acids were constantly rising and reached the maximum by the second and third week of life [29]. These values proved to be considerably higher than those found for older infants [21], and the question remains whether the elevated free amino acid level may interfere with cellular metabolism possibly leading to acidosis.

(iv) In spite of the marked decline in "total acids" and the steady level of urinary net acid excretion after the third week of life, endogenous acid production may be assumed to continue to rise. This is supported by the fact that protein catabolism, measured as urinary nitrogen excretion, and the basal metabolic rate [17, 20] are further increasing during the same period of life.

As a result of the constantly increasing acid production the disappearance of late metabolic acidosis may only be due to the more rapid increase in the efficiency of compensatory mechanisms.

The role of limited renal capacity for hydrogen ion excretion was repeatedly studied [5, 7, 8, 9, 10, 11, 18, 22, 23, 26] and its postnatal development was considered to be the main factor leading to a reestablishement of the normal acid-base status [9, 10, 11, 22, 25, 26].

In the present longitudinal study the importance of renal contribution was indicated by the progressive increment in net acid excretion relative to acid retention over the whole period of observation. It was, however, also suggested that a considerable amount of acid must be eliminated by other mechanisms.

On the basis of theoretical and experimental evidence, growth processess may also affect acid-base homeostasis [11]. The skeletal growth due to deposition of solid base [12, 13] and the formation of new body water due to its bicarbonate content, liberates hydrogen ions [19]. On the contrary, the synthesis of new cell solids is considered to be an acid consuming process [19]. Unfortunately, no direct quantitative information is available as to the overall effect of the growth rate on acid-base status.

The present findings are consistent

with earlier observations that a failure to grow results in development of metabolic acidosis [10, 11] by demonstrating a higher ratio between the "total acids" and the nitrogen ingested, excreted or retained during the period of slow weight gain. The decrease of these ratios may be the result of the growth process itself i.e. of the postnatal change in body composition, but it may also be due to an increased contribution of non-renal mechanisms accompanying growth. The importance of transintestinal ion transport in the regulation of acid-base homeostasis has been shown [12] but further studies are needed to quantitate its participation in the control of acidosis in infants of various ages with various degrees of metabolic acidosis.

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References

1. Albert, M. S., Winters, R. W.: Acid-base equilibrium of blood in normal infants. Pediatrics **37,** 728 (1966).
2. Astrup, P., Jorgensen, K., Siggaard-Andersen, O., Engel, K.: The acid-base metabolism. A new approach. Lancet **1,** 1035 (1960).
3. Chan, J. C. M.: The influence of synthetic amino acid and casein hydrolysate on the endogenous production and urinary excretion of acid in total intravenous alimentation. Pediat. Res. **6,** 789 (1972).
4. Edelmann, C. M., Wolfish, N. M.: Dietary influence on renal maturation in premature infants. Pediat. Res. **2,** 421 (1967).
5. Fomon, S. J., Harris, D. M., Jensen, R. L.: Acidification of the urine by infants fed human milk and whole cow's milk. Pediatrics **23,** 113 (1959).
6. Gamble, J. L.: Chemical anatomy, physiology and pathology of extracellular fluid. Harvard University Press, Cambridge 1958, P. 75.
7. Gordon, H. H., McNamara, H., Benjamin, H. R.: The response of young infants to ingestion of NH_4Cl. Pediatrics **2,** 290 (1948).
8. Hatemi, N., McCance, R. A.: Renal aspects of acid-base control in newly born. III. Response to acidifying drugs. Acta paediat. (Uppsala) **50,** 603 (1961).
9. Kerpel-Fronius, E., Heim, T., Sulyok, E.: The development of the renal acidifying process and their relation to acidosis in low brith weight infants. Biol. Neonat. **15,** 156 (1970).
10. Kildeberg, P.: Disturbances of hydrogen ion balance occuring in premature infants. II. Late metabolic acidosis. Acta paediat. scand. **53,** 517 (1964).
11. Kildeberg, P.: Clinical acid-base physiology. Munksgaard, Copenhagen 1968.
12. Kildeberg, P., Engel, K., Winters, R. W.: Balance of net acid in growing infants. Endogenous and transintestinal aspects. Acta paediat. scand. **58,** 321 (1969).
13. Lemann, J., Lennon, E. J.: Role of diet, gastrointestinal tract and bone in acid-base homeostasis. Kidney Int. **1,** 275 (1972).
14. Lennon, E. J., Lemann, J., Relman, A. S., Connors, H. P.: The effects of phosphoproteins on acid balance in normal subjects. J. clin. Invest. **41,** 637 (1962).
15. Levy, H. L., Shih, V. E., Madigan, P. M., Karolkewitz, V., Carr, J. R., Lumm, A., Richards, A. A., Crawford, J. D., MacCready, R. A.: Hypermethioninemia with other hyperaminoacidemias. Studies in infants on high protein diet. Amer. J. Dis. Child. **117,** 96 (1969).
16. McCullough, H.: The determination of ammonia in whole blood by a direct colorimetric method. Clin. chim. Acta **17,** 297 (1967).
17. Mestyán, J., Fekete, M., Bata, G., Járai, I.: The basal metabolic rate of premature infants. Biol. Neonat. **7,** 11 (1964).
18. Rubin, M. I., Calcagno, P. L., Ruben, B. L.: Renal excretion of H^+ ions. A defense against acidosis in premature infants. J. Pediat. **59,** 848 (1961).
19. Sinclair, J. C., Driscoll, J. M., Heird, W. C., Winters, R. W.: Supportive

management of the sick neonate. Parenteral calories, water and electrolytes. Pediat. Clin. N. Amer **17**, 863 (1970).
20. Sinclair, J. C.: Metabolic rate and temperature control in low-birth-weight infants: longitudinal study. Annual meeting of European Society for Pediatric Research, Budapest 1975.
21. Synderman, S. E., Holt, L. E., Norton, P. M., Roitman, E., Phansalkar, S. V.: The plasma aminogram. I. Influence of the level of protein intake and a comparison of whole protein and amino acid diet. Pediat. Res. **2**, 131 (1968).
22. Sulyok, E., Heim, T.: Assessment of maximal urinary acidification in premature infants. Biol. Neonat. **19**, 200 (1971).
23. Sulyok, E., Heim, T., Soltész, G., Jászai, V.: The influence of maturity on renal control of acidosis in newborn infants. Biol. Neonat. **21**, 418 (1972).
24. Svenningsen, N. W., Lindquist, B.: Incidence of metabolic acidosis in term, preterm and small-for-gestational age infants in relation to dietary protein intake. Acta paediat. scand. **62**, 1 (1973).
25. Svenningsen, N. W.: Renal acid-base titration studies in infants with and without metabolic acidosis in the postnatal period. Pediat. Res. **8**, 659 (1974).
26. Svenningsen, N. W., Lindquist, B.: Postnatal development of renal hydrogen ion excretion capacity in relation to age and protein intake. Acta paediat. scand. **63**, 721 (1974).
27. Valman, H. B., Aikens, R., David-Reed, Z., Garrow, J. S.: Retention of nitrogen, fat and calories in infants of low birth weight on conventional and high-volume feeds. Brit. med. J. **3**, 319 (1974).
28. Widdowson, E. M.: Absorption and excretion of fat, nitrogen and minerals from "filled" milks by babies one week old. Lancet **2**, 1099 (1965).
29. Wong, P. W. K., Pildes, R. S.: Plasma amino acids in low-birth-weight infants treated with intravenous amino acid infusion. Biol. Neonate. **25**, 300 (1974).

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