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Optimum Protein Supply of Prematures

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Four groups of premature infants, each comprising 20 members, have been studied for a month. Each group contained babies of the same birth weight and intrauterine development. The total amount of calories supplied with the food was the same for all groups, and the amount of protein contained therein varied from group to group (2.5, 4.1, 6.1 and 6.4 g/kg, respectively). It was found that any food richer in protein than breast milk (with 4.1 g/kg) ensured the most satisfactory rate of growth and reduced the degree of physiological hypoproteinaemia. Supply of protein considerably in excess of daily 4 g/kg not only failed to have a better effect but the ingestion of excessive amounts of protein (above 6 g/kg) produced phenomena to show that a supply of this magnitude is unnecessary and may even be harmful. (Impairment of nitrogen retention and the efficiency of protein utilization; elevation of the blood NPN level; decrease of urinary urea-N and increase of ammonia-N; increase of urinary acidity and increased urinary output of alpha-amino-N.) Ingestion of protein at the rate of 4 g/kg/day is recommended for premature infants.

Physiological, pathophysiological and biochemical results produce changes in the principles governing the diet of infants. Absorption and the optimum requirement of the paramount foodstuffs are frequently arising problems in this respect. There is nowadays an upsurge of interest concerning an increase in the protein supply of both mature and immature babies and the present investigations were designed to determine the amount of protein required by prematures and to ascertain the effect of proteins exceeding the physiologically necessary amount.

Material and Method

Groups consisting of 20 one-month-old premature babies were studied. Each group contained infants of equal birth weight and intrauterine development. The diet of all groups (breast milk, cow's milk, cow's milk formula) was so composed as to ensure a uniform intake of 130 calories per day. The protein content of the food, systematically determined by Kjeldahl's method, amounted to 2.5, 4.1, 6.1 and 6.4 g/kg per day, respectively, in the four groups. The period of observation lasted 4 weeks, and we determined every week the nitrogen equilibrium, the amount of alpha-amino-N, ammonia-N and NPN in the serum as also the titratable acidity of urine, the amount and composition of serum proteins, further the degree of protein utilization [8, 11, 12, 14, 29].

Results

Fig. 1 illustrates the amount of N introduced with the food and excreted with the urine and the faeces. The values represent, for each group separately, the average of results obtained from four consecutive weekly measurements. It can be seen that, while increasing intake of N had no effect on its excretion through the bowels, its urinary output was increased, a phenomenon which shows that the amount of protein supplied with the food was completely absorbed. If, however, the amount of absorbed protein exceeded a certain limit, it was not completely assimilated so that much nitrogen was eliminated with the urine.

Fig. 2 shows the retention of N as percentage of the proteins supplied.

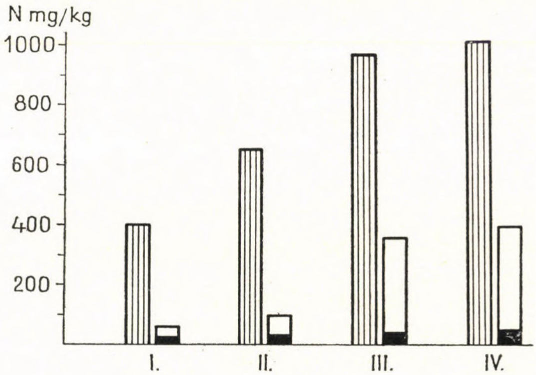

Fig. 1. Daily amount of nitrogen (shaded columns) ingested with food containing 2.5, 4.1, 6.1 and 6.4 g protein per kg body weight (I—IV); amount of N excreted with faeces (black columns) and urine (white columns)

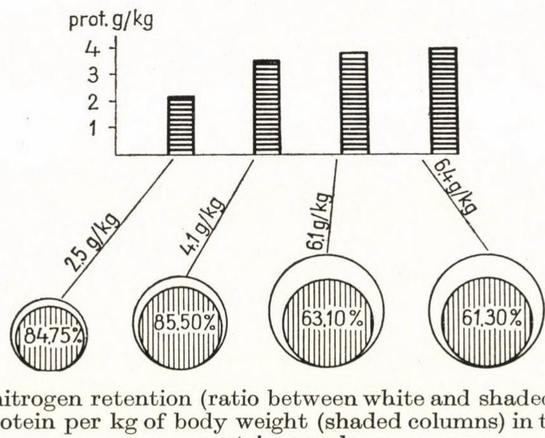

Fig. 2. Degree of nitrogen retention (ratio between white and shaded circles) and actual incorporation of protein per kg of body weight (shaded columns) in the function of daily protein supply

As can be seen, more than 80% was retained if the ingested protein amounted to 2.5 or 4.1 g/kg, whereas retention was hardly more than 60% after the ingestion of more than 6 g/kg. Retention after the ingestion of, for example, 6.1 g/kg was practically the same (3.62 g/kg) as after that of 4.1 g/kg (3.34 g/kg). The fact that the excess is excreted with the urine raises the question as to how far the kidney is capable of satisfying the increased requirement. Fluctuations of the NPN level were studied to this end.

It is evident from Table I that, if the amount of ingested protein exceeds 6 g/kg, the level of NPN is significantly higher than after a lesser protein supply: the concentration of NPN reaches and even exceeds 40 mg/100 ml. Considering that urea-N is mainly responsible for the increase in the concentration of NPN in cases of this nature, it is obvious that the quota of urea-N in the total protein content of the urine becomes less if the ingested food is rich in protein and that, at the same time, the urinary output of ammonia-N is increased (Table II), a phenomenon usual in cases with acidosis. An increased titratable acidity of the urine points likewise to the presence of compensated metabolic acidosis, the possibility of which, in cases of excessive protein supply, has been stated also by other authors [21, 22].

The values for alpha-amino-N are represented in Table III. The given

TABLE I

Correlation between NPN and protein content of the food

Protein uptake, g/kg	2.5	4.1	6.1	6.4
NPN mg%	21.8 ± 4.7	25.2 ± 5.3	39.4 ± 5.1	42.6 ± 6.4
	$p < 0.1$	< 0.001	< 0.5	

TABLE II

Effect of protein loading on the urinary output of urea-N and ammonia-N, further on the titratable acidity of urine

Amount of supplied protein, g/kg	Urea nitrogen excreted with urine		Ammonia nitrogen excreted with urine		Titratable acidity, mEq/day
	Total N%	mg/kg/day	Total N%	mg/kg/day	
2.5	81	31	10	4	12.3
4.1	86	58	8	6	19.7
6.1	78	255	14	46	32.5
6.4	74	258	15	52	29.8

TABLE III

Effect of increasing protein ingestion on the serum and blood alpha-amino-N level

Protein ingested, g/kg	Alpha-amino-nitrogen		
	In the serum, mg/100 ml	In the urine	
		Total N%	mg/kg/day
2.5	6.1	5.3	2
4.1	6.3	4.5	3
6.1	7.9	6.0	18
6.4	7.8	7.4	25

amounts of protein left the serum alpha-amino-nitrogen practically unchanged, whereas in cases of loading the excreted total nitrogen was increased. The increased excretion of a few essential amino acids is visible in a chromatogram.

It seems to follow from the foregoing that a supply of protein exceeding 6 g/kg puts the protein metabolism of premature babies out of gear. One is therefore tempted to ask if it is necessary to supply this much or even more protein. Information in this respect is provided by the weight gain per 1 g of protein introduced with the food.

Foodstuffs richer in proteins than mother's milk enhance the gain in body weight (Table IV). This effect is, however, significant only if the supply of protein amounts to 4.1 g/kg, whereas more than this amount does not essentially increase the rate of weight gain. This is evidently connected with the fact that, as has been noted, the value of N-retention is optimal if the amount of protein taken up with the food is 4.1 g/kg. Introduction of more protein produces no further effect because the rate of assimilation decreases with each additional g of protein (Table V).

TABLE IV

Daily gain in weight in the function of ingested protein

Protein ingested, g/kg	2.5	4.1	6.1	6.4
Weight gain, g/day	15.7 ± 3.8	17.3 ± 4.2	17.5 ± 3.9	17.8 ± 4.6
	$p > 0.01$	$p > 0.8$	$p > 0.7$	

TABLE V

Daily gain in weight per 1 g of protein (effect of utilization of protein)

Protein ingested, g/kg	2.5	4.1	6.1	6.4
Utilization of protein body weight/g protein/day	3.2	2.1	1.4	1.4

TABLE VI

Practically equal retention of nitrogen, NPN level and assimilation of protein after the consumption of equal volumes of breast milk and cow's milk

Food	Ingestion of N mg/kg	N-retention, per cent	NPN, mg/100 ml	Effect of protein utilization, body weight/g protein/day
Breast milk	400	84.75	21.8±4.7	3.2
Diluted cow's milk	480	81.30	23.2±5.1	2.6

A decrease of protein utilization might be due to differences in the quality of the various foodstuffs. It is, however, clear from Table VI that the values for N-retention, NPN and assimilated protein are much the same, whether the babies ingest approximately equal amounts of breast milk or cow's milk. The quantity and not the quality of ingested proteins is therefore responsible for the observed difference.

Neither do conditions regarding the level of serum proteins justify the introduction of protein exceeding about 4 g/kg. This amount reduces the physiological hypoproteinaemia of prematures to an extent which cannot be improved by any amount of additional protein (Fig. 3).

DISCUSSION

Our results are in agreement with the clinical and experimental observations that the amount of protein which premature babies receive with breast milk is insufficient [7, 9, 13, 17, 30]. More protein has to be provided in order to achieve optimum growth. It would, however, be erro-

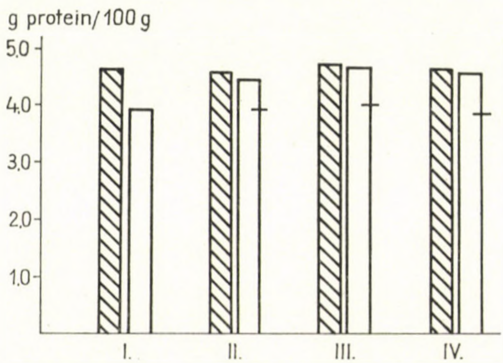

FIG. 3. With a daily protein supply of 2.5 g/kg the total serum proteins show a pronounced decrease during 4 weeks (I). Food containing 4.1, 6.1 and 6.4 g/kg protein (II–IV) considerably reduces the degree of physiological hypoproteinaemia (horizontal marks of the white columns). Total amounts of protein at the outset are indicated in respect of each group by shaded columns, those at the end of the observation period by white columns

neous to underrate the value of breast milk. Mother's milk has a composition similar to that of plasma protein owing to which it shows the best rate of absorption and utilization, and promotes moreover immunological ripening. Breast-fed infants are better provided with certain specific antibodies than bottle-fed babies [5, 10], and also their physiological hypogammaglobulinaemia is less pronounced [6]. Clinical observations, too, point to a better immunity of infants kept on mother's milk. It has been found by numerous authors [15, 16, 20, 24, 25, 31] that the gastrointestinal tract of infants does not absorb the immune proteins of the mother's milk in a native form. The supposition is therefore justified that the amino acids whose proportion is optimal in cases of breast feeding promote the synthesis of immune globulins. One should moreover bear in mind that the IgA content of the breast milk ensures enhanced immunity for the babies' intestinal tract [1, 3, 4]. Mother's milk is still the best food in many respects, although — to achieve better growth — prematures require a surplus amount of protein.

We have found that foodstuffs — if equal as regards caloric value but different as regards protein contents — have different biological effects. Food containing about 4 g/kg body weight of protein seems to be ideal: it ensures adequate nitrogen retention, a better growth than breast milk alone and mitigates the physiological hypoproteinaemia of the newborn. The level of NPN is normal and neither the urinary output of urea-N, alpha-amino-N and ammonia-N nor the titratable acidity of the urine point to increased renal activity.

The amount of protein supplied is sometimes much in excess of that regarded by us as optimal [19, 28]. Considerably more protein than about 4 g/kg does not essentially increase the value of nitrogen retention or the progress of growth; the efficiency of protein utilization is diminished, and the degree of physiological hypoproteinaemia remains unchanged, while the elimination of the waste products of unnecessarily introduced protein means a stress for the kidneys. Not only the high NPN value but also the changed composition of protein eliminated with the urine points to the impairment of excretion. Normally, urea-N accounts for some 85 to 90% of the excreted protein; this percentage gradually diminishes and the urinary output of ammonia-N as well as the acidity of the urine increase in cases of excessive protein supply. Acidification of the urine indicates a disturbance of protein metabolism and creates moreover conditions favourable for various infections.

Increased urinary excretion of alpha-amino-N in cases of protein loading is especially worthy of note because, according to several authors [2, 23, 26, 27], the amount of protein in the food does not essentially influence the degree of aminoaciduria, provided the renal apparatus is fully developed. The renal tubules of prematures are, however, immature, the reason why some investigators [18, 23] observed the highest degree of amino-N excretion in prematures, even without a preceding excessive supply of protein. If the "physiological" hyperaminoaciduria of biologically immature individuals is due to inadequate tubular activity, it is natural that loading will further aggravate tubular insufficiency and increase the urinary output of alpha-amino-N.

It follows from the foregoing that daily 4 g/kg of protein ensure the best conditions for growth, development and immunological ripening of premature babies, and that the introduction of protein amounting to or exceeding 6 g/kg is unnecessary and may even be harmful.

References

1. Bellanti, J. A., Washington, D. C.: Role of local gamma-A-immune globulins in immunity. Amer. J. Dis. Child. **115,** 239 (1968).
2. Berger, H.: Die Amino-Stickstoff-Ausscheidung im Harn in Abhängigkeit vom Lebensalter. Ann. paediat. (Basel) **186,** 338 (1956).
3. Berger, R., Ainbender, E., Hodes, H. E., Zepp, H. D., Hevizy, M. M.: Demonstration of IgA polyantibody in saliva, duodenal fluid and urine. Nature (Lond.) **214,** 420 (1967).
4. Crable, P. A., Carbonara A. O., Heremans, J. F.: The normal human intestinal mucosa as a major source of plasma cells containing gamma-A-immunoglobulin. Lab. Invest. **14,** 235 (1965).
5. Csorba, S., Jezernicky, J., Harsányi, M., Dvorácsek, É.: Különböző infekciók immun-háttere újszülött- és csecsemőkorban. Gyermekgyógyászat **22,** 489 (1971).
6. Csorba, S.: A táplálékfehérje szerepe a fiziológiás hypoproteinaemia mérvének alakulásában. Gyermekgyógyászat. In press.
7. Davidson, M., Levine, S. Z., Bauer, C. H., Dann, M.: Feeding studies in low-birth weight infants. J. Pediat. **70,** 695 (1967).
8. Dickmann (Dán), S., Braun, M.: Jodometrische Reststickstoffbestimmung ohne Veraschung. Wien. Z. inn. Med. **29,** 460 (1948).
9. Dobszay, L., Sárkány, J.: A csecsemőtáplálás. Medicina, Budapest 1961.
10. Dóbiás, Gy., Balló, T., Keményvári, J.: A colostrum befolyása az újszülöttek staphylococcus alfa-antitoxin szintjére. Orv. Hetil. **98,** 983 (1957).
11. Fearon, W. R.: The carbamido-diacetyl reaction: a test for citrulline. Biochem. J. **33,** 902 (1939).
12. Folin, O., Bell, M.: Ammonia-meghatározás. vizeletben: Kolorimetriás módszer. Cit. Bálint, P.: Klinikai laboratóriumi diagnosztika. Medicina, Budapest 1962.
13. Frank, K.: A korszerű csecsemő- és gyermektáplálás. Magy. Pediat. **2,** 336 (1968).
14. Friedman, H. S.: Modification of the determination of urea by the diacetyl monoxide method. Anal. Chem. **25,** 662 (1963).
15. Gugler, E., Muralt, G.: Über immunelektrophoretische Untersuchungen an Frauenmilch-Proteinen. Schweiz. med. Wschr. **89,** 925 (1959).
16. Gugler, E., Bein, M., Muralt, G.: Über immunelektrophoretische Untersuchungen an Kuhmilchproteinen. Schweiz. med. Wschr. **89,** 1172 (1959).
17. Harnack, G. A.: Frühgeborenenernährung; Untersuchungen über den Einfluss unterschiedlicher Eiweisszufuhr. Mschr. Kinderheilk. **111,** 388 (1963).
18. Homolka, J.: Chemische Diagnostik im Kindesalter. Verlag Volk und Gesundheit, Berlin 1961.
19. Omans, W. B. Barness, L. A., Rose C. S., Gyorgy, P.: Prolonged feeding studies in premature infants. J. Pediat. **59,** 951 (1961).
20. Osborn, J. J.: Studies of the immunology of the newborn infants. I. Age and antibody production. Pediatrics **9,** 736 (1952).
21. Plenert, W.: Der Eiweissbedarf im Kindesalter. Kinderärztl. Prax. **32,** 363 (1964).
22. Plenert, W., Gassmann, B., Heine, W.: Über die für Säuglinge wünschenswerte Eiweisszufuhr. Dtsch. Gesundh.-Wes. **20,** 1139 (1965).
23. Schmidt, G. W.: Über die normale Aminosäuren-Ausscheidung mit dem Harn und die Aminosäuren-Verwertung beim Säugling.Med.Wschr.**12,**146(1958).
24. Schneegans, E., Muralt, G., Butler, R., Heumann, G., Geisert, J.: Im-

munologische Probleme beim Frühgeborenen. Acta paediat. Acad. Sci. hung. **7**, 213 (1966).
25. Schneegans, E.: Problèmes immunologiques chez le prématuré. Étude critique des conditions d'élevage. Strasbourg méd. N. S. **17**, 395 (1966).
26. Stein, W. H., Bearn, A. G., Moore, S.: Amino acid content of blood and urine in Wilson's disease. J. clin. Invest. **33**, 410 (1954).
27. Ströder, J., Grundhofer, H., Hein, L.: Aminosäurenausscheidung und Nierenfunktion Frühgeborener. Ann. Pediat **180**, 52 (1953).
28. Švejcar, J., Hnátek, J.: Die Bedeutung der Zufuhr von Eiweissstoffen in der Behandlung und Prophylaxe der Dystrophie bei Kindern. Z. Kinderheilk. **64**, 293 (1958).
29. Szentirmai, A., Braun, P., Horváth, I., Hank, M.: A rapid screening test for determination of total alpha-amino in urine and serum. Clin. chim. Acta **7**, 459 (1962).
30. Vaccari, A., Livini, E., Sereni, F.: Valutazione metabolica della tolleranza nell'immaturo. Minerva pediat. **18**, 1016 (1966).
31. Vahlquist, B. C.: The transfer of antibodies from mother to offspring. Advanc. Pediat. **10**, 305 (1958).

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