WATER EXCHANGE OF PREMATURE INFANTS—COMPARISON OF METABOLIC (ORGANIC) AND ELECTROLYTE (INORGANIC) METHODS OF MEASUREMENT

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Research Article

WATER EXCHANGE OF PREMATURE INFANTS—COMPARISON OF METABOLIC (ORGANIC) AND ELECTROLYTE (INORGANIC) METHODS OF MEASUREMENT [1,2]

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In 1923 Gamble, Ross, and Tisdall (1) presented data correlating the fixed base and water balance of two fasting epileptic children. In these observations, the water balance was represented as the difference between total body weight loss and the sum of the protein and fat loss, the latter calculated from the urinary nitrogen and ketones. In 1929 Newburgh (2) and his coworkers described a method for the calculation of the water exchange of normal adults which depended on a knowledge of the total caloric expenditure and the composition of the metabolic mixture. The limitations of this so-called metabolic method have been discussed in detail for adults by Lavietes (3), Peters (4), and Newburgh *et al.* (5) and for infants by Levine *et al.* (6). In 1935, Lavietes, D'Esopo, and Harrison (7) proposed a method for estimating water balance from base balance and changes in concentration of base in serum. This method was suggested by the earlier observations of Gamble and his coworkers (1) that water and base are lost from the body in approximately the proportions in which they appear in body fluids. In recent years the "electrolyte" method for measuring water exchange has been applied, particularly by Darrow, Yannet, and Harrison (8) and Hastings and his coworkers (9), to quantitative studies of the relative distribution of intracellular and extracellular water.

Both the "metabolic" and the "electrolyte" methods of measuring total water exchange are open to criticism on theoretical grounds since the former assumes that the respiratory exchange is a reliable index of the composition of the metabolic mixture and the latter, that certain electrolytes are retained solely as extracellular or intra-

cellular components in uniform concentrations throughout the body. Moreover, both methods are subject to errors in application in the presence of perceptible perspiration. In the organic method, perspiration may interfere with prediction of the total calories from the insensible water loss; in the electrolyte method, unmeasured skin excretion will give falsely high values for retention of minerals.

This report presents concurrent measurements of the water, organic, and electrolyte balances of four premature infants which permit a critical comparison of the above methods for determining total water exchange. Premature infants are particularly suited for such studies since interference by visible perspiration is reduced to a minimum. This tendency to minimal perspiration is dependent on poorly developed sweat glands, relatively large surface area for loss of heat by radiation, scanty adipose tissue, and feeble muscular movements.

METHODS

Four healthy premature male infants were studied in eight observations of from 3 to 11 days for a total of 48 days. The infants ranged from 17 to 31 days and from 1900 to 2600 grams at the onset of observations. They resided in a constant temperature and humidity room and were exposed in customary clothing to environmental temperatures of from 72 to 87° F. and to relative humidities of from 40 to 80 per cent in the different observations. No difficulty was encountered in maintaining body temperature.

Diet

All food mixtures were prepared by the nurse in charge and were fed to the infants by trained assistants. The infants took their feedings well; their lips and the inside of nipples were wiped with sterile gauze which was immediately placed in weighed sealed jars to provide a quantitative estimate of the portion of the formula not swallowed. In these observations an average of 5 to 10 per cent of the initial formula adhered to the sides

of utensils, bottles, bottle caps, and nipples, or was regurgitated. The amount actually ingested and retained was determined in each observation.

The diets consisted of boiled human milk, evaporated or powdered cow's milk to which water, dextrimaltose, olive oil, casein or calcium paracaseinate were added in varying amounts in the different observations. The caloric fluid and protein intake were adequate to permit satisfactory weight gains of 25 to 49 grams daily. Adequate concentrates of vitamins C and D were added to each infant's diet.

Thoroughly mixed samples of the powdered preparations and pooled daily aliquot samples of the liquid cow's and human milk were analysed for water, nitrogen, chloride, potassium, sodium, calcium, and phosphorus, and where indicated for fat and carbohydrate. All analyses were made in triplicate; the methods used are given at the end of the report.

Urine and feces

Urine and feces were collected separately and analysed for water, nitrogen, chloride, potassium, sodium, and phosphorus. Fecal calcium and fat were determined, and for Infants L. O. and E. M., urinary calcium as well.

Changes in body weight and total insensible perspiration

The infants were weighed daily with a balance having a capacity of 10 kgm. and an accuracy of 0.05 gram (10). The weight of the total insensible perspiration was determined for each 24 hours by subtracting from the total weight of the intake the sum of the weights of urine and feces and the change in body weight. The water lost through the skin and lungs was obtained by subtracting from the total insensible loss the portion due to $CO_2 - O_2$, calculated from the metabolic mixture (2). In two observations (7 and 8 on Infants L. O. and H. L., Table II) a filter paper (11) was applied for 60 seconds to the forehead and abdomen one to three times daily,

and then exposed to silver nitrate and sunlight as an index of chloride excretion from the skin.[8]

Total caloric expenditure and composition of metabolic mixture

The total caloric expenditure was determined for Infants L. O. and H. L. by combining 24-hour minute to minute records of activity with calorimeter observations of 2 to 5 hours made in a small respiratory chamber attached to a Benedict universal respiration table (12). The total caloric expenditure for Infants N. O. and E. M. was predicted from the insensible perspiration on the assumption that approximately 25 per cent of the total heat production of premature infants (13) is lost by vaporization of water. While the latter is admittedly not an ideal method of estimating total calories, the diminished tendency of premature infants to perspire and their low activity permits a satisfactory approximation, especially under constant environmental conditions. Furthermore, because of their low total caloric expenditure (150 to 200 calories per 24 hours) an error of as much as 20 per cent in prediction would introduce a maximum deviation of only 4 to 5 grams in the daily water balance.

The urinary nitrogen was used as a measure of protein catabolism and the dietary carbohydrate as a measure of carbohydrate combustion (2, 14). The calories derived from fat were obtained by subtracting the protein and carbohydrate calories from the total.

Methods of calculating water balance

The methods used for calculating the water balance are presented in Table I. According to the direct or-

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[8] This procedure has now been made routine in all mineral balance observations, and whenever the test proves positive the room temperature is lowered or clothing is removed to prevent appreciable excretion of chloride through the skin.

TABLE I

Methods of estimating water balance

"ORGANIC" METHODS

	Direct (2)			Indirect
Water intake	minus	Water output	= Water balance	= Total body weight change minus change in weight due to solids:
1. Ingested as such		1. Urine		Protein = Intake minus urine, feces
2. In solid foods		2. Feces		Fat = Intake minus feces, metabolized
3. Oxidation		3. Skin and lungs		(CHO) = Intake minus metabolized
Protein $\times 0.41$		$H_2O = I.L. - (CO_2 - O_2)$		(Minerals) = Intake minus urine, feces
Fat $\times 1.07$		$CO_2 - O_2 = (P \times 0.08)$		(skin)
CHO $\times 0.60$		$- (F \times 0.08) + (C \times 0.41)$		

"ELECTROLYTE" METHODS

Anion			Cation (7)
$\dfrac{\text{Chloride retained, mM.}}{120} \times 1000 = \text{Extracellular } H_2O \text{ grams } (8d)$			
plus			
$\dfrac{\text{Nitrogen retained, grams}}{54} \times 1000 = \text{Intracellular } H_2O \text{ grams}$		= Water balance grams =	$\dfrac{\text{Sodium} + \text{Potassium retained, mM.}}{160} \times 1000$

TABLE II

Detailed results in terms of 24 hours

Subject	Observation number	Days	Room Temperature	Room Relative humidity	Intake Fat	CHO	N	Cl	Na	K	Ca	P	H₂O*	Total calories†	Balance Fat	CHO	N	Cl	Na	K	Ca	P	H₂O‡	Total weight
			°F.	per cent	grams	grams	grams	mm.	mm.	mm.	mm.	mm.	grams		grams	grams	grams	mm.	mm.	mm.	mm.	mm.	grams	grams
N. O.	1	6	77	50	12.1	23.6	0.84	4.5	3.5	6.0	3.9	3.0	332	114	6.8	0.0	0.41	1.1	1.5	1.2	0.7¶	1.4	17	25
N. O.	2	6	77	50	12.2	24.3	1.44	6.8	4.6	9.6	6.6	6.1	292	152	6.5	0.0	0.64	2.0	2.2	1.7	2.4¶	2.1	28	38
E. M.	3	11	77	50	12.0	24.8	1.66	8.0	6.9	11.3	9.1	8.3	297	163	1.6	0.0	0.66	1.3	3.0	1.3	1.2¶	2.1	24	32
L. O.	4	6	77	40	9.8	44.2	1.78	6.8	4.8	9.5	8.9	7.5	431	183	7.5	4.5	0.88	1.9	1.8	3.3	6.6	5.3	31	49
L. O.	5	3	87	40, 80	10.3	46.3	1.86	7.1	5.0	9.9	9.4	7.9	468	186	8.0	5.1	0.94	4.5§	4.4	4.9	7.5	5.7	23	45
L. O.	6	4	68, 72	40	12.4	55.3	2.25	8.5	6.0	12.0	11.3	9.5	531	270	9.4	0.0	0.82	0.7‖	1.3	1.3	9.7	6.4	14	35
L. O.	7	5	77, 72	40	15.4	68.5	2.78	10.6	7.4	14.8	14.0	11.8	542	337	11.5	0.3	1.01	1.4‖	1.2	3.3	11.9	8.2	12	40
H. L.	8	7	72	40	13.8	38.5	2.49	9.5	6.7	13.3	12.5	10.5	319	199	8.1	0.0	0.83	1.8‖	3.2	3.1	7.9	5.3	13	25

* Includes water of oxidation (2).
† Total calories were predicted from insensible perspiration in Observations 1, 2, 3, and estimated from observations in calorimeter of 2 to 5 hours combined with 24-hour minute to minute records of activity in remaining observations.
‡ Direct organic method (2).
§ Perspiring profusely.
‖ Filter paper test (11) positive.
¶ Urinary calcium not determined.

ganic method (2), the water intake consists of water ingested plus water of the food plus water of oxidation, the latter derived from knowledge of the metabolic mixture. The water output consists of water excreted through the urine, feces, and skin and lungs, the latter derived from the total insensible weight loss and the metabolic mixture. According to the indirect organic method, the water balance is calculated by subtracting from the total weight change the weight of retained solids, of which the organic solids, protein and fat, constitute the chief items. Although the indirect method uses the same fundamental assumptions as the direct in deriving the composition of the metabolic mixture, it involves the additional determination of dietary and fecal fat, and fecal nitrogen. The calculation is simpler than in the direct method.

Two methods of constructing the water balance from electrolyte balance were used, one from the chloride and nitrogen balance (1, 8d) and the other from the sodium and potassium balance (7). In the anion method, the total water was partitioned into extracellular and intracellular phases on the basis of chloride and nitrogen retention. It was here assumed that chloride was retained extracellularly and nitrogen intracellularly in uniform concentrations of 120 mm. (8d) and 54 grams [4] per liter of water respectively. The use of nitrogen (1) as a measure of intracellular water accretion seemed particularly desirable because the uniformity in nitrogen retention from period to period fitted with the concept that these rapidly growing premature infants were adding protoplasm at a regular rate. In the cation method it was assumed that no changes in body concentration

[4] This represents a recently determined concentration of nitrogen in the intracellular water of human muscle (15).

of base took place from the beginning to the end of an observation and that the total water balance therefore equalled the sum of sodium and potassium retained, divided by the concentration of total base in intra- and extracellular water, namely, 160 mm. per liter (16). No analyses of serum were made.

RESULTS

The detailed results of the observations are presented in Table II, and a summary of the water balances in Table III. Comparison of the results of direct and indirect organic methods (Columns 1 and 2, Table III) shows that in six

TABLE III

Summary of results of water balance calculated according to both organic and electrolyte methods

Subject	Observation number	Days	Organic method Direct 1	Indirect 2	Electrolyte method Anion Extracellular (from Cl) 3	Intracellular (from N) 4	Total (3+4) 5	Cation From Na and K Total 6	Average organic 7	Average electrolyte 8
			grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours	grams per 24 hours
N. O.	1	6	17	16	9	8	17	17	17	17
N. O.	2	6	28	27	16	12	28	24	28	26
E. M.	3	11	24	26	11	13	24	27	25	26
L. O.	4	6	31	31	15	17	32	32	31	32
L. O.	5	3	23	26	37	18	55	58	25	57
L. O.	6	4	14	21	6	16	22	16	18	19
L. O.	7	5	12	22	11	20	31	28	17	30
H. L.	8	7	13	11	15	16	31	39	12	35

of eight observations (Observations 1, 2, 3, 4, 5, and 8) the water balances differ by 3 grams or less per 24 hours. Comparison of the results of the two electrolyte methods (Columns 5 and 6) shows that in all but two of the observations (Observations 6 and 8) the difference between the water balances is 4 grams or less per 24 hours. This agreement of results within each pair of methods serves as a check on the accuracy of the different analytical procedures used, although it is no measure of the validity of the assumptions underlying each pair of methods. The same assumptions (17) concerning the reliability of the respiratory quotient as an index of intermediary metabolism are used for both organic methods; and assumptions concerning the uniformity of concentration of electrolytes throughout various body fluids are used in both electrolyte methods.

To test the validity of both sets of assumptions we have in Table III, Columns 7 and 8, compared the average results of the organic methods with the average results of the electrolyte methods. It is seen that in 5 of the 8 observations (1, 2, 3, 4, and 6) the difference between results was 2 grams or less per 24 hours. In the remaining three observations (5, 7, and 8) the water balance as calculated from the electrolyte retention was from 13 to 32 grams too high. Appreciable excretion of chlorides through the skin was present in all three observations in which disagreement of results was found, suggesting that falsely high retentions of electrolytes were credited to the infants because no measure of skin excretion had been made (18).

To determine whether the agreement between results of the organic and electrolyte methods represented a valid support of the two methods or merely a fortuitous coincidence, we have in Table IV related the water balance to the body weight change. These infants were all receiving diets adequate to produce a satisfactory gain in weight. Under these conditions the percentage of weight gain consisting of water might be reasonably expected to approximate 60 to 80 per cent, since water comprises 70 per cent of infantile tissue by actual analysis. It is seen that, according to the direct organic method in six of eight observations, water comprised 51 to 75 per cent of the weight gain and averaged 61 per cent for the whole group. According to the indirect organic

TABLE IV

Relation of water balance to weight gain

Subject	Observation number	Weight gain	Amount of body weight as water				Remarks
			Organic method		Electrolyte method		
			Direct	Indirect	Anion	Cation	
		grams per 24 hours	*per cent*	*per cent*	*per cent*	*per cent*	
N. O.	1	25	68	64	68	68	
N. O.	2	38	74	71	74	63	
E. M.	3	32	75	81	75	84	
L. O.	4	49	63	63	67	67	
L. O.	5	45	51	58	122	129	Perspiring profusely. Room temperature 87° F.
L. O.	6	35	40	60	63	46	
L. O.	7	40	30	55	78	70	Filter paper test positive
H. L.	8	25	52	44	124	156	Filter paper test positive
Average (weighted)		61	63		72*	70*	

* Observations 5 and 8 omitted from these averages.

method, water comprised 55 to 81 per cent of the weight gain in seven of eight observations and averaged 63 per cent for the whole group. According to the anion method, water comprised 63 to 78 per cent of the weight gain in six observations, and according to the cation method 63 to 84 per cent in five of eight observations. In Observations 5 and 8, the water as predicted from both anions and cations represented 122 to 156 per cent of the weight gain, impossible results which were undoubtedly due to the unmeasured excretion of electrolytes through the skin.

The evidence suggests that under normal conditions of weight gain, either method of assessing water balance gives results consistent with the concept that infantile tissue is 70 per cent water, but that in the presence of perspiration the excretion of electrolytes through the skin gives falsely high values for retention and therefore interferes with an accurate prediction of water balance.

In Table V is presented a comparison of the found and "theoretical"[5] retention of sodium,

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[5] The "theoretical" sodium retention was calculated from the chloride on the assumption that for every 120 mM. of chloride retained 148 mM. of sodium (8d) were retained, these being the approximate concentrations per liter of extracellular water. The "theoretical" potassium was calculated from the nitrogen retention by as-

TABLE V

Comparison of found and "theoretical" retentions of sodium, potassium, and phosphorus[*]

Subject	Obser-vation number	Sodium		Potassium		Phosphorus	
		Found	"Theo-ret-ical"[†]	Found	"Theo-ret-ical"[‡]	Found	"Theo-ret-ical"[§]
		mm. per 24 hours	*mm. per 24 hours*	*mm. per 24 hours*	*mm. per 24 hours*	*mm. per 24 hours*	*mm. per 24 hours*
N. O....	1	1.5	1.4	1.2	1.3	1.4	1.2
N. O....	2	2.2	2.5	1.7	2.0	2.1	2.7
E. M....	3	3.0	1.6	1.3	2.0	2.1	2.0
L. O....	4	1.8	2.4	3.3	2.7	5.3	5.7
L. O....	6	1.3	.9	1.3	2.5	6.4	7.5
Average (weighted)		2.1	1.7	1.7	2.1	3.2	3.5

[*] Observations 5, 7, and 8 omitted because of perceptible perspiration.

[†] Na mM. $= \frac{148}{120}$ Cl mM.

[‡] K mM. $= 3.1$ N grams.

[§] P mM. $= 0.6$ Ca mM. $+2$ N grams.

potassium, and phosphorus. Although they do not coincide in individual observations, the weighted averages show a fair agreement for the 33 days of observation in which three infants gained a total of 1164 grams. The found and " theoretical " sodium retentions were 2.1 and 1.7 mM.; the found and " theoretical " potassium retentions were 1.7 and 2.1 mM., and the found and " theoretical " phosphorus retentions were 3.2 and 3.5 mM. per 24 hours, respectively. If allowance be made for excess sodium (8d) present in bone in a concentration of 1 mM. of sodium for every 30 mM. of calcium (20), the agreement between found (2.1 mM.) and " theoretical " (1.9 mM.) is closer.

The evidence suggests that over long periods of observation electrolytes are probably stored in the growing infant in concentrations approximating those demonstrated by tissue analyses. More exact definition of the range within which they vary may depend on further analyses of human tissues and carefully controlled balance studies.

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suming that for each gram of nitrogen retained, 3.1 mM. of potassium were retained, this being the approximate K : N ratio of human and dog muscle (8d, 16). The " theoretical " phosphorus retention was calculated from the calcium and nitrogen retention using a formula derived from the P : Ca ratio of bone (19) and the P : N ratio of muscle (8d, 16).

DISCUSSION

The two chief methods used for determining the total water exchange of these thriving premature infants are based on entirely different principles. In the organic method, the water balance is determined in part on the basis of the relation of water exchange to the metabolism of organic substances, protein, fat, and carbohydrate, accepting the respiratory quotient and the urinary nitrogen as indices of the composition of the metabolic mixture; in the second method, water exchange is related to the metabolism of inorganic substances. Because of the fundamental needs for maintaining proper relations in the body between electrolytes and water (21), one might a priori consider a method of measuring water balance based on electrolytes as more desirable than one based on assumptions concerning organic metabolism, in the use of which increasing caution is being advised (17). In practice, however, there is great difficulty in measuring electrolyte balance accurately, both because of the small absolute amounts normally retained and because unmeasured skin excretion results in crediting the subjects with false retentions of electrolytes and therefore of water. Under such conditions, the use of the method based on calculation of the composition of the metabolic mixture gives a more accurate measure of the water balance, provided the total 24-hour caloric expenditure has been reasonably approximated. The chief reason for this, as the method applies to infants, has already been pointed out (6b), namely, that water comprises by far the largest part of the intake, urine, feces, insensible weight loss, and of shifts in body weight so that by accurate weighing of the subjects, his actual intake, and carefully collected samples of urine and feces, one directly arrives at an approximation of the water intake, partition of outgo between urine, feces, and skin and lungs, and the water balance.

It should be noted, however, that if special precautions are taken to prevent appreciable excretion of electrolytes through the skin by lowering room temperature, shedding clothes or limiting activity, methods based on electrolyte exchange will give data not only concerning total water exchange but also on its partition into intracellular and extracellular compartments.

The agreement between the average found and "theoretic" balances of sodium, potassium, and phosphorus and the fact that the water balance as predicted from electrolyte balances represented approximately 70 per cent of the body weight gain demonstrate that in the tissue accretion of premature infants, just as in the loss of tissue by older children (1), electrolytes are added to the body in approximately the proportions normally present in body fluids.

SUMMARY AND CONCLUSIONS

Eight observations of water and electrolyte balance, totalling 48 days, were concurrently made on four premature male infants on diets adequate to produce weight gains of 25 to 49 grams daily. The water balance was calculated from both the metabolic mixture, *i.e.*, from an estimate of the protein, fat, and carbohydrate oxidized, and from the electrolyte exchange. The close agreement between the results of both methods in five of eight observations suggests that under carefully controlled environmental and dietary conditions, either method of predicting the water balance of thriving premature infants is satisfactory. In two of three observations in which perceptible perspiration was present, the unmeasured excretion of electrolytes through the skin presumably resulted in crediting the subjects with a falsely high retention of electrolytes and water. Under such conditions the organic method yielded better estimates of water balance.

The similarity between the actual retentions of sodium, potassium, and phosphorus and "theoretic" retentions calculated from the chloride, nitrogen, and calcium and nitrogen retentions, respectively, indicate that in normal growth electrolytes are retained in approximately the relations to each other that exist in body tissues.

APPENDIX

Protocols

1. Infant N. O., white, aged 17 days, weight 1912 grams at the onset of observation, was studied in two observations (Numbers 1 and 2) of six days each. He gained an average of 25 and 38 grams daily in these two observations. The room temperature was 77° F. and the relative humidity 50 per cent in the two observations.

In Observation 1, he received human milk for three days and a diet of evaporated milk, water, dextrimaltose, and olive oil of similar organic and fluid content for the succeeding three days. In Observation 2, he received a formula of human milk, casein, and dextrimaltose for three days, followed by a formula of evaporated milk, water, and dextrimaltose of similar organic and fluid content.

2. Infant E. M., negro, aged 25 days, weight 2083 grams at the onset of Observation 3, was studied for eleven consecutive days. He gained an average of 32 grams per day during this observation. The room temperature was 77° F. and the relative humidity 50 per cent during the observation.

During the first three days he received a formula of human milk, dextrimaltose, and calcium paracaseinate (casec); during the following eight days, a diet of evaporated milk, dextrimaltose, and water of similar organic and fluid content.

3. Infant L. O., negro, aged 31 days, weight 2365 grams at the onset of observation, was studied in four observations under varying environmental conditions. In all observations he received a diet of a powdered skim-milk-olive oil preparation (olac), reinforced with dextrimaltose and water. His daily weight gain in the four observations averaged 49, 45, 35, and 40 grams respectively.

The first observation (Observation 4) consisted of three two-day periods at a temperature of 77° F. and 40 per cent relative humidity. Between the second and third period of this observation an interval of three days elapsed during which he was exposed to a temperature of 87° F., with a relative humidity of 40 per cent for two days and 80 per cent for the final day. These three days in which visible perspiration was present constitute Observation 5.

In Observation 6, the subject, now 51 days of age and weighing 3197 grams, was studied for two two-day periods at temperatures of 72 and 68° F., respectively, with the relative humidity 40 per cent.

In Observation 7, L. O., now 65 days of age and weighing 3795 grams, was studid for two days at a temperature of 77° F. and for three days at a temperature of 72° F. with the relative humidity 40 per cent throughout. In both periods the elimination of water through the skin and lungs was

considerably higher than in Observations 4 and 6 at similar temperatures, and chlorides were excreted through the skin as indicated by the filter paper test.

4. Infant H. L., colored, aged 23 days and weighing 2564 grams at the onset of observation, was studied in a single observation of seven days, at an environmental temperature of 72° F. and relative humidity of 40 per cent. A high insensible perspiration, combined with a positive filter paper test, again indicated appreciable excretion of chlorides through the skin. His diet consisted of the powdered skim-milk-olive oil preparation and water in amounts adequate to permit an average daily gain of 25 grams.

Chemical methods

The following methods were employed in triplicate for analysis of the diet, urine, and feces. The latter were dried by evaporation on a steam bath for 48 to 96 hours. Aliquots of the dried stool were used for fat determination, and the fat was extracted from the remaining dried feces with a mixture of petroleum and ethyl ether. The remaining analyses were made on the resulting fat-free dried residue.

Water in urine and milk was determined by drying at 100° C. for 48 hours, nitrogen by the Kjeldahl method, fat by the Roese-Gottlieb method (22), and lactose in human and liquid cow's milk by a gravimetric method (23); for the dried cow's milk preparation, the carbohydrate content as submitted by the manufacturer was accepted.

Chlorides were determined by a modified Volhard titration method (24), phosphorus by the Tisdall method (25) and calcium by the McCrudden method (26). Samples were ashed at 500° C. in a muffle furnace prior to determination of sodium by the Butler-Tuthill (27) modification of the Barber-Kolthoff method, and potassium by the chloroplatinate method of Shohl and Bennett (28).

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