

Changes in the Composition of the Human Femur During Growth

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Rickets used to be a common disease of young children, and a number of the early investigators of bone chemistry (Friedleben, 1860; Schabad, 1910) were primarily interested in the changes induced by this disease. Studies of the effect of development have more recently been reported, but, for the most part, these have been limited to a particular part of the life-span or to a particular part of a bone. The study of Swanson & Iob (1937) was confined to the mineral composition of the bone and cartilage of the human foetus. Baker, Butterworth & Langley (1946) reported on the calcium and nitrogen in human bone tissue; they found that the calcium/nitrogen ratio of the cancellous bone of the rib was higher in adults than it was in infants. This aspect of the problem was again taken up by Rogers, Weidmann & Parkinson (1952), who analysed 40 samples of femoral cortical bone from persons varying in age from 8 months to 90 years. They found that there was a rise in the calcium/nitrogen ratio between the ages of 8 months and 20–30 years and, examining samples from a few of the subjects, found that collagen accounted for 89–96% of the total nitrogen at all ages. Similar information about the changes in composition of human bone during foetal and early postnatal life does not appear to be available (Wallgren, 1957).

The purpose of the present paper is to report the results of a study of femora from 62 human subjects ranging in age from 12 weeks' gestation to 35 years. At all ages cortical bone from the middle third of the shaft and, up to 12 years of age, the epiphyses, have been separated and analysed. The remainder of each bone has also been analysed and the composition of the whole bone obtained. The study is similar to that reported on the long bones of the pig, rat and fowl (Dickerson, 1962).

METHODS

Femora were obtained from 21 foetuses of 12–28 weeks' gestation, which were delivered for a variety of reasons, but all were anatomically normal. The approximate age of these foetuses was calculated from the crown-heel length according to the formula derived by Scammon & Calkins (1929). Femora were also obtained from six foetuses of 30–34 weeks' gestation, eight full-term stillborn babies, 22 babies aged 2 days to 24 months, two children of 11 and 12 years respectively, two youths of 16 years, four women aged 18–34 years and four men aged 20–35 years. At the

postnatal ages all the bones were obtained from persons who died as the result of accidents or acute illnesses which were unlikely to have affected the composition of the bones. There was no difference between the composition of the bones of the two sexes in any of the age groups and the results for the two sexes have therefore been averaged. The femora were dissected and analysed as described by Dickerson (1962).

RESULTS

Cortical bone

Table 1 sets out the composition of the bone tissue of the cortex, and ratios of the amounts of the constituents at each age. The percentage of total N in the dry fat-free bone fell and that of collagen rose during prenatal development so that there was a rise in the proportion of the total N accounted for by the collagen. The individual values in each of the age groups were somewhat variable, but the changes were nearly complete by 25–28 weeks' gestation.

The percentage of Ca in the tissue increased during growth and so did the Ca/N ratio. The Ca/N ratio, however, did not change significantly during the first 9 months of postnatal life, for the combined mean value for the six foetuses of 30–34 weeks' gestation (which were, in fact, premature babies) and the eight full-term babies was 4.89 ± 0.60 , compared with a combined mean value for the 2–9 month-old babies of 4.60 ± 0.37 . There may be a tendency for the Ca/N ratio to fall a little during this period of life and this is brought out more clearly when the individual values are plotted as shown in Fig. 1. There were no regular changes with age in either the Ca/collagen or the Ca/P ratios.

Epiphyses

Table 2 shows that the contribution of the epiphyses to the wet weight of the femur fell from 73 to 31% between 12 and 14 weeks' gestation and a postnatal age of 11–12 years. The percentage of water in the fat-free epiphyses also fell. In the dry fat-free epiphyses the percentage of total N increased to a maximum value at 2–4½ months after birth. A large and more rapid rise in the percentage of collagen took place during this period, but the increase in minerals was small. After 24 months of age there was a fall in the percentage of total N, a relatively smaller one in the percentage of collagen,

Table 1. *Composition of the cortex of the human femur*

Values are the means of the number of analyses shown. Standard deviations are shown (\pm).

Age	No.	Composition (g./100 g. of dry fat-free solids)				Collagen	Ca/N	Ca/ collagen	Ca/P
		Total N	Collagen	Ca	P	N \times 100 Total N			
Foetus									
12-14 weeks	3	5.95 (± 0.29)	16.2 (± 3.64)	18.9 (± 0.93)	9.1 (± 0.20)	49.0 (± 8.0)	3.22 (± 0.53)	1.19 (± 0.21)	2.07 (± 0.08)
15-16 weeks	4	5.61 (± 0.62)	20.8 (± 1.11)	24.6 (± 0.86)	11.0 (± 0.33)	65.5 (± 6.3)	4.22 (± 0.74)	1.19 (± 0.08)	2.22 (± 0.07)
20-24 weeks	9	5.25 (± 0.35)	22.5 (± 1.56)	23.4 (± 1.37)	10.5 (± 0.59)	77.6 (± 8.4)	4.46 (± 0.30)	1.05 (± 0.10)	2.22 (± 0.09)
25-28 weeks	5	4.90 (± 0.53)	23.3 (± 0.94)	24.7 (± 0.37)	10.6 (± 0.32)	86.0 (± 5.5)	5.05 (± 0.35)	1.16 (± 0.12)	2.32 (± 0.08)
30-34 weeks	6	5.03 (± 0.37)	22.4 (± 1.56)	24.7 (± 1.03)	10.9 (± 0.30)	80.6 (± 6.9)	4.90 (± 0.64)	1.11 (± 0.08)	2.26 (± 0.09)
Newborn, full-term	8	5.06 (± 0.41)	23.3 (± 1.51)	24.6 (± 1.94)	10.8 (± 0.59)	83.0 (± 5.3)	4.88 (± 0.48)	1.06 (± 0.13)	2.27 (± 0.12)
Infant									
2-17 days	5	5.03 (± 0.24)	22.9 (± 1.46)	24.2 (± 0.81)	10.8 (± 0.33)	82.5 (± 7.0)	4.82 (± 0.36)	1.05 (± 0.13)	2.25 (± 0.15)
2-4½ months	7	5.28 (± 0.25)	24.4 (± 1.55)	23.7 (± 0.65)	10.8 (± 0.45)	83.4 (± 6.5)	4.52 (± 0.36)	0.98 (± 0.05)	2.21 (± 0.12)
5-9 months	5	5.31 (± 0.31)	23.7 (± 1.59)	24.9 (± 0.60)	11.0 (± 0.37)	80.6 (± 5.4)	4.69 (± 0.29)	1.06 (± 0.15)	2.26 (± 0.30)
12-24 months	5	5.24 (± 0.08)	24.3 (± 1.64)	24.6 (± 1.22)	11.1 (± 0.97)	83.2 (± 5.9)	4.70 (± 0.25)	1.02 (± 0.10)	2.21 (± 0.04)
11 and 12 years	2	4.92 (4.46, 5.38)	25.4 (24.4, 26.4)	25.3 (25.2, 25.3)	11.5 (11.4, 11.5)	93.4 (88.4, 98.4)	5.18 (4.70, 5.65)	1.00 (0.96, 1.03)	2.21 (2.19, 2.22)
16 years	2	4.48 (4.27, 4.68)	24.0 (22.1, 25.8)	25.1 (24.4, 25.8)	11.3 (11.2, 11.4)	96.4 (93.3, 99.5)	5.62 (5.20, 6.04)	1.06 (0.95, 1.17)	2.22 (2.14, 2.30)
Adult 18-35 years	8	4.74 (± 0.14)	23.0 (± 0.57)	26.4 (± 0.62)	11.3 (± 0.57)	87.5 (± 0.11)	5.58 (± 0.22)	1.14 (± 0.04)	2.29 (± 0.11)

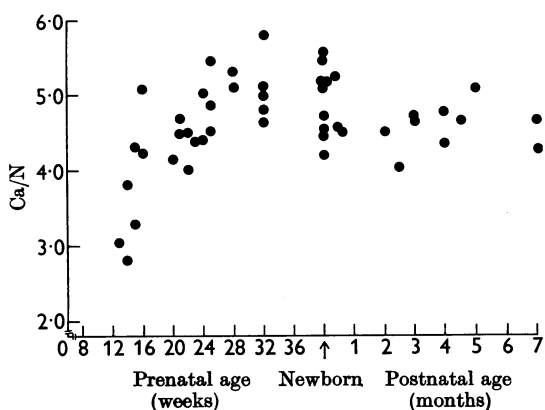


Fig. 1. Calcium/nitrogen ratio of the cortex of the human femur during pre- and early post-natal life.

phates hydrolysed by the treatment with 6N-HCl at 100°. The Ca/P ratio reached its mature value at 5-9 months of age.

Whole bone

Table 3 shows the composition of the femur as a whole at the various ages. The changes shown in this Table are those of a composite structure, the different parts of which are themselves changing both in composition and relative size. Between 12-24 months and 11-12 years the changes in the relative weight and composition of the epiphyses accounted for a large part of the fall in water in the whole bone. They contributed N and collagen, but up to 4½ months of age very little Ca and P. The rise in the percentage of these minerals in the femur which takes place during development was not apparent during the first 2-4½ months of post-natal life. There may even have been a fall in the percentage of Ca during this period, but the individual values varied and the differences were not statistically significant. The magnitude of the changes in the degree of calcification of the non-epiphysial parts of the femur during early

and a rise in the percentages of Ca and P. The Ca/P ratio rose during foetal development and early postnatal life. This rise was probably due to a fall in the proportion of phosphate from ester phos-

Table 2. *Composition of the epiphyses of the human femur*

Values are the means of the number of analyses shown. Standard deviations are shown (\pm).

Age	No.	Epiphyses (% of femur wt.)	Water (% of fat-free fresh wt.)	Composition (g./100 g. of dry fat-free solids)				Ca/N	Ca/P	Collagen N \times 100 Total N
				N	Collagen	Ca	P			
Foetus										
12-14 weeks	3	73.0	85.6	8.8 (± 0.28)	15.5 (± 0.99)	0.50 (± 0.24)	0.88 (± 0.20)	0.058 (± 0.03)	0.58 (± 0.30)	31.9 (± 2.3)
15-16 weeks	4	67.7	89.2	9.4 (± 0.14)	21.9 (± 1.55)	1.29 (± 0.27)	1.24 (± 0.37)	0.14 (± 0.09)	1.09 (± 0.34)	42.2 (± 3.4)
20-24 weeks	9	61.4	84.7	10.4 (± 0.44)	29.7 (± 2.56)	1.08 (± 0.23)	0.87 (± 0.15)	0.12 (± 0.02)	1.36 (± 0.21)	51.6 (± 4.5)
25-28 weeks	5	57.4	85.1	10.8 (± 0.51)	34.2 (± 2.96)	0.92 (± 0.19)	0.68 (± 0.23)	0.085 (± 0.04)	1.35 (± 0.33)	56.9 (± 3.4)
30-34 weeks	5	53.1	83.7	11.6 (± 1.10)	40.3 (± 2.17)	0.69 (± 0.55)	0.51 (± 0.08)	0.060 (± 0.05)	1.37 (± 0.16)	64.1 (± 4.3)
Newborn, full-term	8	50.4	79.2	11.5 (± 0.89)	42.2 (± 4.2)	1.28 (± 0.27)	0.66 (± 0.15)	0.113 (± 0.05)	1.84 (± 0.23)	67.1 (± 4.3)
Infant										
2-17 days	5	51.0	81.2	11.1 (± 0.87)	41.5 (± 3.0)	1.44 (± 0.84)	0.68 (± 0.27)	0.127 (± 0.08)	1.98 (± 0.43)	67.5 (± 3.6)
2-4½ months	6	48.5	79.2	12.4 (± 0.96)	48.8 (± 5.2)	1.56 (± 0.53)	0.80 (± 0.19)	0.127 (± 0.05)	1.91 (± 0.39)	70.8 (± 8.8)
5-9 months	5	47.1	77.3	11.6 (± 0.47)	48.0 (± 5.6)	2.97 (± 1.00)	1.42 (± 0.44)	0.273 (± 0.09)	2.22 (± 0.18)	74.6 (± 6.1)
12-24 months	5	42.9	72.5	11.7 (± 0.86)	52.9 (± 3.7)	4.42 (± 1.72)	1.94 (± 0.88)	0.386 (± 0.54)	2.29 (± 0.14)	81.7 (± 2.9)
11 and 12 years	2	31.3	50.1	8.24	41.4	15.45	7.11	1.88	2.17	90.7

development is shown in Fig. 2. The values plotted in this graph have been calculated from the mean values for the different age groups. Each of the parameters shown in Fig. 2 increased during foetal development to a peak value which was reached between 32 weeks' gestation and full term. The value of each parameter then fell during postnatal growth to a minimum value in the bones of the babies of 5-9 months. Subsequent increase was slow, and in the children of 11 and 12 years the mean value for the Ca/N ratio was a little higher, and that of the Ca/collagen ratio a little lower, than in the femora of the newborn babies. The percentage of Ca in the dry fat-free bone at 11-12 years of age was almost the same as it was in the premature babies.

No fat was found in the femora of foetuses up to 28 weeks' gestation, and at term it only amounted to 0.14%. Its deposition was slow during the early months of postnatal life and not until after 4½ months did the fat in the bones exceed 0.7%.

DISCUSSION

The mean value of 25.3 obtained by Rogers *et al.* (1952) for the percentage of calcium in dry defatted human cortical bone at 20-40 years of age

was significantly lower ($P < 0.01$) than that of 26.4 reported in this paper. The value obtained by Rogers *et al.* for the percentage of total nitrogen (4.41) was also lower. These differences between the results obtained by Rogers *et al.* and those obtained in the present study are possibly to be accounted for by the different temperature at which the bone was dried, and by the use of different fat solvents. The difference in the calcium/nitrogen ratio 5.73 (± 0.16) compared with 5.58 (± 0.22) is not statistically significant. In bone from three persons aged 27-65 years Rogers *et al.* found that collagen accounted for 88.5-95.6% of the total nitrogen. The mean value of 87.5% obtained in the present study is in reasonably good agreement with these results and there does not seem to be any real difference between the results obtained for collagen in bone by the gelatin-tannic acid precipitation method used by Rogers *et al.* and by the Neuman & Logan procedure, depending on the percentage of hydroxyproline. The youngest bone analysed by Rogers *et al.* was from a baby of 8 months and from the results obtained in the present study this would appear to be the age when the calcium/nitrogen ratio is at its lowest during postnatal life. The rather smaller differences between the degree of calcification of adult and late foetal and neonatal bone are

in agreement with the results of Wallgren's (1957) study with biophysical methods.

The growth of a bone differs from that of almost every other tissue in the body in that deposition and resorption are actively taking place at the same time. During the first 5-9 months of post-natal life, the suckling period, however, the cortex of the femur is practically in a 'steady state'. It therefore contributes little to the fall in the calcium/nitrogen ratio during this period and in the percentage of calcium in the dry fat-free non-epiphyseal parts of the femur. It may be concluded that the major part of the fall in the degree of calcification of the human femur during the suckling period is due to a fall in the degree of calcification of the cancellous bone of the metaphyses. This

conclusion is similar to that reached about the changes in the composition of the pig humerus during the latter part of gestation (Dickerson, 1962), and during the postnatal growth of other species, particularly the kitten (Bauer, Aub & Albright, 1929; Burns & Henderson, 1936; Weidmann & Rogers, 1958; Slater & Widdowson, 1961). Uncalcified bone matrix has been observed histologically in the shaft of bones from rapidly growing premature infants (Follis, 1953). Stettner (1931) described a 'physiological osteoporosis', as judged by radiographic density, in the hands of healthy children; the incidence of this 'osteoporosis' increased between the ages of 3 months and 2 years and then gradually decreased up to 8 years of age.

Table 3. *Composition of the human femur (whole bone)*

Values are the means of the number of analyses shown. Standard deviations are shown (\pm).

Age	No.	Mean femur wt. (g.) (range)	Composition of fresh bone (g./100 g.)		Composition of fat-free bone (g./100 g.)					
			Water	Fat	Water	Total N	Collagen	Ca	P	Ca/N
Foetus										
12-14 weeks	3	0.11 (0.05-0.16)	77.8	Nil	77.8 (± 3.3)	1.61 (± 0.24)	3.37 (± 0.25)	2.42 (± 0.41)	1.50 (± 0.12)	1.50 (± 0.27)
15-16 weeks	4	0.22 (0.19-0.24)	78.4	Nil	78.4 (± 3.3)	1.66 (± 0.22)	4.50 (± 0.84)	3.47 (± 1.02)	1.61 (± 0.37)	2.09 (± 0.49)
20-24 weeks	9	1.96 (1.14-2.75)	72.9	Nil	72.9 (± 2.0)	2.01 (± 0.32)	6.17 (± 0.52)	4.33 (± 0.52)	1.97 (± 0.22)	2.18 (± 0.29)
25-28 weeks	4	4.7 (3.1-6.8)	68.4	Nil	68.4 (± 1.2)	2.19 (± 0.17)	7.54 (± 0.56)	5.25 (± 0.22)	2.36 (± 0.13)	2.40 (± 0.14)
30-34 weeks	5	9.2 (8.5-10.0)	63.4	0.15	63.8 (± 6.7)	2.35 (± 0.96)	8.46 (± 0.35)	5.63 (± 0.31)	2.59 (± 0.74)	2.42 (± 0.48)
Newborn, full-term	6	16.6 (15.1-18.0)	63.8	0.14	63.9 (± 2.3)	2.71 (± 0.55)	9.28 (± 0.64)	6.06 (± 0.46)	2.84 (± 0.72)	2.24 (± 0.13)
Infant										
2-17 days	5	17.7 (14.2-19.9)	64.3	0.28	64.5 (± 6.3)	2.57 (± 0.56)	9.29 (± 0.83)	5.78 (± 0.68)	2.66 (± 0.81)	2.25 (± 0.19)
2-4½ months	6	26.4 (22.2-37.7)	63.7	0.65	64.1 (± 3.6)	3.04 (± 0.29)	10.7 (± 1.71)	5.39 (± 0.73)	2.66 (± 0.62)	1.78 (± 0.22)
5-9 months	5	38.2 (30.3-48.6)	57.9	2.09	59.8 (± 3.4)	3.32 (± 0.27)	12.5 (± 1.57)	6.01 (± 0.60)	2.75 (± 0.30)	1.81 (± 0.14)
12-24 months	5	63.7 (36.0-85.5)	52.1	7.54	55.4 (± 4.7)	3.43 (± 0.11)	14.1 (± 2.12)	6.97 (± 1.12)	3.25 (± 0.60)	2.04 (± 0.12)
11 and 12 years	2	326 (286, 366)	27.0	26.0	36.4 (33.0, 39.7)	3.82 (3.70, 3.95)	16.3 (15.4, 17.3)	13.8 (13.1, 14.6)	6.25 (5.96, 6.55)	3.62 (3.54, 3.70)
Adults 18-35 years	8	646 (513-786)	15.6	31.8	22.7 (± 4.01)	4.02 (± 0.17)	17.3 (± 1.53)	19.4 (± 1.8)	8.30 (± 0.49)	4.82 (± 0.33)

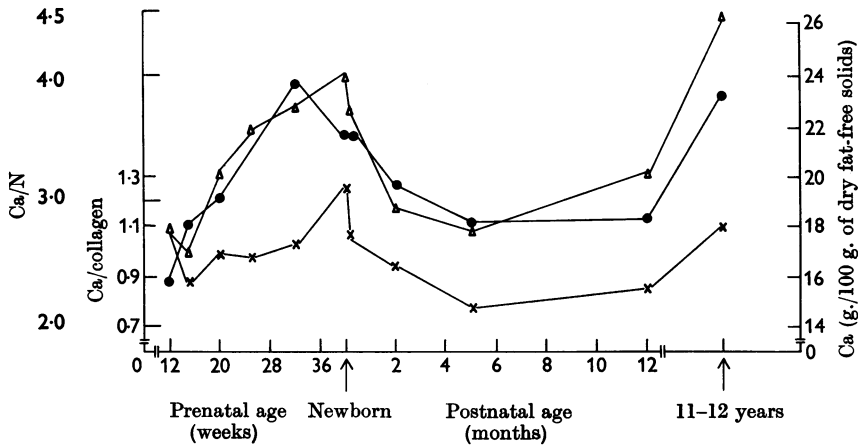


Fig. 2. Calcification of the non-epiphyseal parts of the human femur. Values are calculated from the mean values for the different age groups. Δ , Ca/N ratio; \times , Ca/collagen ratio; \bullet , Ca.

The observations on the human femur appear to support the conclusion arrived at by Brock (1932) and Stearns (1939) on the basis of balance experiments that the percentage of calcium in the whole bodies of babies falls during the suckling period. Care must, however, be exercised in extrapolating results obtained on a single long bone to the entire skeleton. In the kitten Slater & Widdowson (1961) have shown that the calcium/nitrogen ratio of the parietal bones of the skull falls during the first 7 days after birth and that this fall can to some extent be prevented by giving calcium phosphate. Even between different long bones, the humerus and the femur, for example, there are known to be differences in the rate of calcification in some species (Hammett, 1925). It might therefore be expected that when supplies of calcium or phosphorus, or both, are insufficient to meet all the demands, those bones which mature more rapidly would also calcify more rapidly. This kind of competition for the constituents of bone mineral may also, to some extent, take place between different parts of the same bone for, whilst the percentage of Ca in the dry fat-free solids of the non-epiphyseal parts of the femur (shown in Fig. 2) fell from 19.6 to 18.1 between 2-4½ months and 5-9 months of age, the percentage of calcium in the epiphyses, expressed on the same basis (shown in Table 2), increased from 1.56 to 2.97.

The amount of fat in the femora did not exceed 0.7% until the babies were 5-9 months, and even then it only amounted to 2.1%. This finding is a little surprising in view of the fact that the full-term newborn human baby has 16% of fat in its body (Widdowson & Spray, 1951). A little of this fat is probably lost during the first few days after birth, but it is a matter of empirical observation

that babies deposit fat subcutaneously during suckling. Very little, however, goes into the femur. The whole question of the changes in the amount and distribution of fat in the human body during postnatal growth is one about which there is very little information.

SUMMARY

1. The human femur has been analysed at various stages of growth between 12 weeks' gestation and 35 years.

2. In dry fat-free cortical bone the percentage of nitrogen fell and that of collagen rose. The contribution of the collagen nitrogen to the total nitrogen had nearly reached its mature value by 25-28 weeks' gestation. The calcium/nitrogen ratio rose during prenatal growth but, during the first 9 months of post-natal life, it did not rise further and may have fallen.

3. The contribution of the epiphyses to the weight of the wet bone fell during growth. The percentage of collagen nitrogen in the epiphyses increased relative to that of total nitrogen, but there was little increase in the percentage of calcium until 5-9 months after birth. The calcium/phosphorus ratio in the epiphyses rose to its adult value by 5-9 months of age.

4. The effect of the epiphyses on the composition of the whole bone is discussed. The rises in the percentage of calcium and in the calcium/nitrogen ratio in the whole bone were delayed during the first 2-4½ months after birth. The calcium/nitrogen and calcium/collagen ratios of the non-epiphyseal parts of the femur fell during the first 5-9 months after birth and rose again during later growth. This fall was attributed to a decrease in the degree of calcification of the metaphyses.

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A Zinc-Dependent Lactate Dehydrogenase in *Euglena gracilis*

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Although deprivation of metals is known to result in striking and selective changes in the activities of certain metalloenzymes (cf. McElroy & Nason, 1954; Baumeister, 1955; Hewitt, 1959; Vallee, 1959), a number of basic questions remain unanswered: What is the role of the metal in the synthesis of the apo- or holo-enzyme? What constitutes a critical concentration of metal? What causes the activity of one metalloenzyme to decrease in metal deficiency and not another?

In order to obtain answers to some of these questions we have been studying the biochemistry and physiology of zinc metalloenzymes in the alga, *Euglena gracilis*, grown under conditions where the metal content of the cells is rigidly controlled.

In the work reported here, we have sought an enzyme in *Euglena* that might be under the control of zinc. The oxidation of lactic acid by intact cells is virtually abolished in zinc deficiency and the activity of an enzyme, tentatively identified as a D-lactate dehydrogenase, is decreased.

METHODS

Abbreviation. - q_{O_2} , μ l. of oxygen consumed/hr./mg. of protein nitrogen.

Organism. *Euglena gracilis*, 'z' strain, was kindly provided by Dr S. Hutner of the Haskins Laboratory, New York, N.Y.

Special chemicals. The iron, manganese, copper and zinc sulphates employed in the growth media were Johnson Matthey and Co. Ltd. SpecPure salts. Ammonium glutamate was prepared from L(+)-glutamic acid (Eastman Kodak). Metal-free water ammonia solution and hydrochloric acid were obtained as described by Thiers (1957). D(+)- and L(-)-Lactic acid were prepared by passage of the corresponding calcium salt (California Corp. for Biochemical Research) through a column of Dowex 50 (Brin, 1953).

Analyses. Total nitrogen was determined by acid digestion followed by nesslerization. Non-protein nitrogen was determined by treating cell samples with 4% trichloroacetic acid at 0° for 1 hr., followed by centrifuging and analysis of nitrogen in the supernatant. Protein nitrogen was estimated by difference. Trichloroacetic acid