It is interesting that in the low-thiamine media pyruvate accumulates in considerable amounts (Griffiths, 1953). Whether this means that pyruvate is an intermediate in the formation of β -carotene is not known, but when tested under other conditions pyruvate did not stimulate pigment synthesis (Goodwin, Lijinsky & Willmer, 1953).

SUMMARY

1. β -Carotene synthesis by *Phycomyces blakesleeanus* was greater at 25° than at 3–5, 20 or 30°. At all temperatures, β -carotene was the major pigment produced. There was no indication of the synthesis of enhanced amounts of more saturated polyenes at temperatures where β -carotene synthesis was reduced.

2. β -Carotene synthesis is inhibited in *Phycomyces blakesleeanus* grown on media containing less than 0.2 μ g. thiamine/100 ml., whilst growth inhibition begins below a much higher level (2.0 μ g./100 ml.). No qualitative changes in polyene synthesis were associated with this inhibition of carotenogenesis.

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Chemical Analysis of Human Foetal Skull Bones

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Pressures exerted on the foetal head during labour cause it to alter its shape, and this ability of the head to 'mould' in response to external pressure becomes less marked as the foetus ages. The present work was an attempt to correlate changes in chemical composition of the bones with the increasing 'hardness' of the maturing foetal head.

There have been several investigations into the chemical composition of foetal bones, but the only reports referring to the cranial bones are those of Yoshida (1930) and Toverud & Toverud (1933). Yoshida analysed the parietal bone for calcium and phosphorus, and related his findings to the length of gestation. These showed that whereas the absolute amounts of phosphorus and calcium in this bone increased as pregnancy advanced, the percentage of phosphorus decreased while that of calcium increased. This finding was surprising as was the fact that at a foetal age of 10 months the Ca/P ratio of the parietal bones was over four.

Toverud & Toverud (1933) analysed a small portion (2 × 1.5 cm.) of the parietal bone for the percentage calcium per dry weight of the sample, and the percentage calcium and phosphorus of ash.

The findings were related to the length of gestation of the foetus, and no obvious relationship between percentage of calcium and foetal age was noted. The authors, however, found a sudden increase in percentage of calcium in the section of parietal bone of full-term babies as compared with the values found for the premature infants up to and including 38 weeks.

METHODS

Material

The bones for this study were obtained from still-births or from infants who had died very soon after birth. The age of the foetus was estimated from the menstrual history of the mother, only singletons were used and any foetus which showed obvious signs of possible skeletal deformity was discarded.

The bones were removed *post mortem* and kept in 0.9% (w/v) NaCl in a refrigerator until they were prepared for examination, which was always within 5 days of death. The parietal and frontal bones only were used. The medial border of each parietal bone was not included in the specimen, nor was the orbital part of the frontal bones included. Before the bones were examined the soft tissues on each surface of the bone were removed by stripping.

Chemical analysis

The concentration of calcium, phosphorus, carbonate and collagen present in the bones was determined, the results being expressed as % dry wt. The following procedure was adopted to prepare the bones for analysis. Each bone was cut into pieces approximately 2×1 cm. and the pieces from one bone were placed in a small beaker which was surrounded by CO_2 snow. When the pieces were hard they were pulverized to a fine powder. The powder was then placed in an H_2SO_4 desiccator, where it remained for a week or longer before being transferred to an oven whose temperature was 100° ; there was no evidence that the H_2SO_4 in the desiccator affected the carbonate content of the bone powder. After at least 6 hr. in the oven it was found that further heating did not reduce the weight of the samples.

The dry, powdered bone was then ready for the estimation of carbonate. To determine the concentrations of calcium, phosphorus and collagen, approximately 0.2 g. of the dry, powdered bone was accurately weighed into an ampoule. Exactly 10 ml. of 6N-HCl were added to the ampoule, which was then sealed. The contents of the ampoule were hydrolysed either by heating for 3 hr. in a pressure cooker at 60 lb./sq.in. or left overnight in a boiling water bath. After heating, the ampoule was opened and the contents were filtered. An ampoule to which no bone powder had been added was used as a control.

The filtrate or bone solution (1 ml.) was pipetted into a 50 ml. volumetric flask followed by 1 ml. of 6N-NaOH and 2 ml. of 8% trichloroacetic acid. The solution was made up to the 50 ml. mark with distilled water and then shaken. The addition of the trichloroacetic acid never caused any precipitation, so further filtering was, in every case, unnecessary.

Estimation of calcium. The estimation of the calcium was based on the method described by Kramer & Howland

(1926) in which the calcium was precipitated as the oxalate, the precipitate then dissolved in acid and the solution titrated while hot with potassium permanganate. The error of the method was $\pm 0.5\%$.

Estimation of phosphorus. The method of Fiske & Subbarow (1925), with modifications by King (1932), was employed to determine the phosphorus content of the bone solution. The error of the method was $\pm 1\%$.

Estimation of carbonate. Dry, powdered bone was used, and the method was that of Van Slyke (1917) with the modification suggested by Bowes & Murray (1935). The error of the method was $\pm 5\%$.

Estimation of collagen. The method of Neumann & Logan (1950a, b) for the estimation of hydroxyproline was used to determine the collagen concentration of the bone solution. The error of the method was $\pm 5\%$.

RESULTS

Table 1 shows the mean percentages of dry weight of calcium, phosphorus, carbonate and collagen for each of the twenty-one cases examined. The figures for each substance, in the majority of cases, are the mean values of twelve estimations, three on each of the two frontal and three on each of the two parietal bones. Also shown in Table 1 is the estimated time of gestation.

It will be seen that as pregnancy advances there is an overall increase in the percentage of calcium and carbonate in the foetal bones while the proportions of phosphorus and collagen are unaltered by foetal age, in the period under investigation.

Evaluation of results. There is a statistically significant increase in the percentage of calcium

Table 1. *The mean percentage (dry weight) of calcium, phosphorus, carbonate and collagen in foetal skull bones against the age of the foetus*

Each value is calculated from the mean of triplicate estimations of the parietal and frontal bones of an individual foetus. The figures within brackets are the standard error of the mean. Student's *t* test was applied in order to find the *P* values.

Foetus	Age (weeks)	Calcium (%)	Phosphorus (%)	Carbonate (%)	Collagen (%)
A	28	23.50 (± 0.17)	10.68 (± 0.10)	3.63 (± 0.16)	28.4 (± 0.33)
B	29	24.31 (± 0.12)	11.41 (± 0.06)	3.80 (± 0.15)	28.2 (± 0.13)
C	29	24.43 (± 0.32)	10.78 (± 0.14)	4.03 (± 0.11)	29.1 (± 0.90)
D	32	24.27 (± 0.34)	11.00 (± 0.08)	4.08 (± 0.10)	28.3 (± 0.40)
E	32	24.01 (± 0.21)	10.95 (± 0.05)	4.19 (± 0.05)	27.6 (± 0.13)
F	32	24.40 (± 0.08)	10.79 (± 0.10)	4.17 (± 0.05)	28.3 (± 0.45)
G	33	24.23 (± 0.27)	10.81 (± 0.07)	4.50 (± 0.04)	27.3 (± 0.30)
H	36	24.66 (± 0.05)	10.67 (± 0.13)	4.71 (± 0.02)	27.8 (± 0.75)
I	36	23.85 (± 0.15)	10.65 (± 0.06)	4.01 (± 0.09)	27.8 (± 0.30)
J	36	24.46 (± 0.12)	10.49 (± 0.02)	4.60 (± 0.02)	27.9 (± 0.24)
K	37	24.73 (± 0.07)	10.71 (± 0.10)	4.54 (± 0.10)	28.5 (± 0.47)
L	38	24.17 (± 0.20)	10.76 (± 0.04)	4.52 (± 0.04)	28.6 (± 0.15)
M	38	24.53 (± 0.14)	11.29 (± 0.03)	4.20 (± 0.04)	26.6 (± 0.33)
N	40	24.37 (± 0.10)	10.79 (± 0.03)	4.84 (± 0.04)	28.7 (± 0.24)
O	40	24.73 (± 0.22)	10.63 (± 0.15)	4.65 (± 0.06)	27.5 (± 0.22)
P	41	24.60 (± 0.20)	11.15 (± 0.07)	4.24 (± 0.08)	27.8 (± 0.95)
Q	42	24.28 (± 0.16)	10.50 (± 0.06)	4.47 (± 0.03)	29.0 (± 0.42)
R	42	24.10 (± 0.08)	10.79 (± 0.06)	4.54 (± 0.07)	29.0 (± 0.33)
S	43	24.81 (± 0.04)	10.86 (± 0.03)	4.81 (± 0.07)	27.1 (± 0.56)
T	43	24.85 (± 0.11)	10.81 (± 0.05)	4.46 (± 0.10)	29.8 (± 0.38)
U	43	24.63 (± 0.13)	10.64 (± 0.04)	4.79 (± 0.05)	27.4 (± 0.43)
P	—	0.02-0.01	0.3-0.2	0.001	0.9

($x = 23.06 + 0.037y$) and of carbonate ($x = 2.46 + 0.052y$) with foetal age, whereas the percentage of phosphorus ($x = 11.24 - 0.012y$) and collagen ($x = 28.35 - 0.005y$) show no such correlation where x = foetal age and y = percentage of the material investigated.

Differences in the rate of change of calcium, phosphorus and carbonate between the left and right parietal and left and right frontal bones were tested by comparing the slopes of regression lines. The differences found were not significant. Similarly, any differences between frontal and parietal bones were shown to be not significant.

DISCUSSION

As the foetus becomes mature there is an increase in the total amount of calcium, phosphorus, carbonate and collagen in those bones of the cranial vault examined, because there is no decrease in the proportion of these substances while the size of the head is increasing as is also the thickness of the parietal bone (Macdonald, 1953, 1954). During the period of pregnancy under review the chemical composition of the growing parietal and frontal bones must be changing, because the approach of maturity is associated with an increased percentage of calcium and carbonate while the percentage of phosphorus is unaltered. The chemical changes found in the bones of the cranial vault of the foetus are changes taking place in membrane bone, which has an entirely different method of development from the usual cartilage bone. It is of interest to see to what extent the theories of the inorganic structure of cartilage bone can be applied to the results described in this paper.

Bassett's (1917) views of bone as a mixture of apatite and calcium carbonate cannot be applied to these foetal bones, as the Ca/P ratio for non-carbonate Ca is too low for apatite. In like fashion the views of Kramer, Yuska & Steiner (1939) on the effect of age on bone do not apply. Bowes & Murray (1935) made three alternative suggestions to explain the distribution of calcium in dentine, the Ca/P ratio of which is very similar to the mean residual Ca/P ratio of foetal skull bones. Of these three possibilities the one that might be applied suggests the presence of calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) as a constituent.

It is probable that increasing amounts of calcium carbonate are laid down in the bones of the cranial vault as the foetus matures, and therefore any increased 'hardness' that is found in these bones may be, in part, due to this greater proportion of calcium carbonate present at maturity. The proportion of collagen in the bones examined did not

appear to have any relation to the age of the foetus in the period under review and as the organic matrix of bones consists of 90–96 % collagen or collagen-like proteins (Rogers, Weidmann & Parkinson, 1952) there does not appear to be any very marked change in the organic composition of the parietal or frontal bones of the foetus in the last 3 months of pregnancy.

SUMMARY

1. Chemical analysis of the frontal and parietal bones of the human foetus has shown an increase in the proportions of calcium and carbonate as the foetus approaches maturity, but no such increase in phosphorus and collagen. The mean proportional increase per dry weight of bone per week is 0.04 % for calcium and 0.05 % for carbonate.

2. The inorganic chemical composition of growing membrane bone, as exemplified by the foetal parietal and frontal bones, may consist of a mixture of apatite, brushite and calcium carbonate or of a mixture of calcium phosphate, calcium carbonate and small amounts of other calcium salts. It is impossible, from the results obtained here, to indicate which is the more likely chemical arrangement.

3. A probable increase in the proportion of calcium carbonate in the skull bones as the foetus grows may be a factor in the increased 'hardness' of the head associated with foetal maturity.

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