The Effect of Normal Development and of Undernutrition on the Exchange of [³²P]Orthophosphate with Bone Powder *in vitro*

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1. The effect of age and undernutrition on the exchange of phosphate in the cortical bone powder of pigs with [³²P]orthophosphate was investigated *in vitro*.
2. The extent of exchange fell steadily from birth to old age.
3. The bones of animals undernourished for 1 year behaved like those of animals 3½ years old.

The capacity of the phosphate of developing bone to exchange with [³²P]orthophosphate has been investigated from several angles in the past. Bonner (1950), for example, studied it *in vivo*, Amprino (1952) and Belanger (1952) *in vitro* by using sections of bone and radioautography, and Armstrong (1940) and Barnum & Armstrong (1941–42) *in vitro* with bone powders. Most of the work has been done with rats. Smeenk (1961) standardized the bone-powder technique and with it studied the exchange in both rats and man. The general conclusions reached by these and other investigators (Neuman & Neuman, 1953, 1958) have been that the amount of phosphate that is exchanged decreases (a) with age, whether with reference to the bones of the organism as a whole or to the more recently formed portions of a single bone, and (b) with increasing size of the bone crystals. Dickerson & McCance (1961) found that during undernutrition the calcium/collagen ratio of the cortex of pig bone increased more than the age of the animal would have led one to expect and this was interpreted to mean that chemically the bone of the undernourished animal was behaving like that of an older one. It was therefore decided to explore the effects of aging and undernutrition on the phosphate exchange of bone in the pig and the present paper describes some of the results.

MATERIALS AND METHODS

The humeri of ten undernourished pigs aged 11–14 months and weighing 4.5–6 kg. were compared with the humeri from eight pigs of about the same weight aged 3–4 weeks and with the humeri from six normal pigs aged 12–14 months and weighing 160–180 kg. The undernourished animals were reared as described by McCance (1960) and by Cabak, Gresham & McCance (1962). In addition, the humeri from the following normal pigs were investigated to bring out the natural effects of aging more completely: nine at birth before being fed, three aged 4–6 months, three

aged 8–10 months, four aged 3–3½ years and three aged 5–8 years.

The bones were removed, cleaned, dried and defatted as described by McCance, Southgate, Spencer & Weston (1966). They were then once more scraped clean of marrow, powdered and passed through a sieve (150 B.S. mesh; diameter of pore 104μ) to obtain a uniform powder.

Measurement of phosphate exchange. The procedure used was based on that of Smeenk (1961). Portions (10 mg.) of dry bone powder were placed in centrifuge tubes and incubated in duplicate in a Mickle shaker at 37° for 24 hr. at 120 excursions/min. with 4 ml. of 5 mM-Na₂HPO₄-KH₂PO₄ buffer, pH 7.3, containing [³²P]orthophosphate (0.02 μCi/ml.). The tubes were then centrifuged at 3500 rev./min. in an MSE Medium centrifuge for 15 min. and the supernatant was carefully removed. The residual powder was washed three times with small amounts of water and the supernatant and washings were made up to 14 ml. The powder was heated at 100° with 2 ml. of 3 N-HCl for 10 min. and the solution that resulted was also made up to 14 ml. Then 4 ml. of the buffer solution and washings and 4 ml. of the solution of bone powder were placed in a Geiger-Müller liquid-scintillation counter (type M6H; 20th Century Electronics Ltd., New Addington, Surrey) and the activity was recorded. Between 10000 and 15000 counts were made in 100 sec. and the counting error was less than 1%. Phosphorus in the two solutions was determined by the method of Fiske & Subbarow (1925).

Expression of results. The proportion of the bone phosphate involved in the exchange reaction can be calculated from the radioactivity and phosphorus determinations (see Neuman & Neuman, 1958). It is expressed in the present study as a percentage of the proportion that was involved when the exchange reaction was carried out with a 'standard' powder made from the humerus of a healthy pig of unknown age (Smeenk, 1961). Thus the relative exchange percentage is given by:

$$\frac{P_i C_B}{C_i P_B} \times 100$$

where P_i is the phosphorus content of the incubation medium (mg.), P_B is the phosphorus content of the sample of bone powder (mg.), C_i are the counts recorded for the incubation medium, C_B are the counts recorded for the

Fig. 1. Effect of normal development and undernutrition on the exchange of [^{32}P]orthophosphate with bone powder *in vitro*. The method used to calculate the ^{32}P relative exchange percentage is described in the Materials and Methods section. Mean values are plotted for each age group and the vertical lines show the s.d. of the mean where the group contained enough animals for this to be calculated. ●, Normal animals; ○, undernourished animals.

bone solution and f is the exchange proportion found with the standard bone powder, usually about 0.2.

RESULTS

The results given in Fig. 1 show that under the conditions of these experiments the exchange of bone phosphate with [^{32}P]orthophosphate fell quite regularly from birth to old age. Theoretically they could have been accounted for by an increase in the amount of phosphorus in the bone with age. This, however, was not the case for there was no significant change in the phosphorus content of the bone with age or undernutrition. A curve has been drawn through the points to show the general trend. The differences between birth and 4 weeks, 4 weeks and 1 year, and 1 year and the older animals are all significant, the P values as determined by the t test being <0.001 , <0.001 and <0.005 respectively. The average relative exchange percentage obtained for the undernourished animals, namely 75.9 ± 8.7 , is significantly lower than the one obtained for animals of the same age ($P < 0.005$) and much lower ($P < 0.001$) than the one for animals of the same size. It is almost the same as the average obtained for animals 3–3½ years of age.

DISCUSSION

These results bring out two points that require some comment. (1) The bones of the under-

nourished animals behaved like those of old animals. This is in keeping with all the results, histological and chemical, previously obtained in this Laboratory (Dickerson & McCance, 1961; Pratt & McCance, 1964*a,b*). It is not in line with the opinion that undernutrition may maintain all the tissues of the body in a state of perpetual youth, although it in no way contradicts the idea that continuous undernutrition may prolong the life of the body as a whole (McCay, Maynard, Sperling & Barnes, 1939; Ross, 1959). (2) The effect of age was continuous from birth. There was no indication of any disturbing influence at birth (or at 4 weeks), as the evidence compiled by McCance *et al.* (1966) indicated that there was in the calcium, magnesium, sodium and citric acid metabolism of bone. For the moment these differences must remain unexplained but they must clearly be reckoned with by those interested in the metabolism of calcified tissues.

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