Phosphate minerals in a calculus from the reticulo-rumen of the sheep

With Plate IV

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Summary. Brushite occurs as radial-concentric layers of prismatic crystals deposited on baked pellets of bentonite impregnated with cobalt oxide, placed in the reticulo-rumen of lambs as a preventive of cobalt-deficiency diseases. Collophane-like whitlockite, apparently pseudomorphous after brushite, occurs underneath the brushite layers, and struvite is found as rare minute crystals between the whitlockite and brushite layers. Analytical and physical data are given for the first two minerals and the origin of the deposit is discussed.

DEWEY, Lee, and Marston (1958) reported the use of pellets ('cobalt bullets'), made of cobalt oxide bound with bentonite and baked at 1000° C, for maintaining a slow continuous supply of cobalt to lambs in areas where the 'pining' disease, or the nervous disorder known as Phalaris staggers, both due to Co-deficiency, are enzoötic. The bullets are administered before weaning into the reticulo-rumen, and have made it possible to achieve effective control of these diseases in Australia. In five out of some hundreds of cases, however, they had no effect and this appeared to be due to an incrustation of calcium phosphates on the 'bullets'. The application of the same technique in Scotland yielded a larger crop of phosphate-incrusted 'bullets'. Nine of these, taken at slaughter at the end of 1958 from lambs to which they had been administered at the preceding midsummer, were sent by Mr. A. L. Wilson, Chief Veterinary Investigation Officer of the West of Scotland Agricultural College, Auchincruive, Ayr, to Dr. E. C. Owen of the Hannah Dairy Research Institute, Kirkhill, Ayr, who made chemical analyses for Ca, PO_4''', and water, which with his density measurements suggested brushite as the chief constituent with tricalcic phosphate as a minor component. Mr. Voss of the Spectrographic Unit at the West of Scotland Agricultural College found up to 1 % of Mg in some of these incrustations. Dr. Owen therefore sent the samples to the author for

mineralogical examination. Of these 9 'bullets', one (C.B. 1) was from the Glen Farm, Gatehouse of Fleet, Kirkcudbrightshire, and the rest (C.B. 2 to C.B. 9) from Ardneil, Portincross, Ayrshire.

The deposit consists in most cases of two distinct creamy-white layers. X-ray powder photographs confirmed the identification of the mineral of the outer layers as brushite, and identified the collophane-like dominant mineral of the inner layers as whitlockite by comparison with the original material from Groton, New Hampshire (Frondel, 1941). In C.B. 2 and C.B. 6 both layers are duplicated, and in C.B. 2 there is a further innermost thin brown layer of whitlockite. In C. B. 5 the only complete layer is similar to this latter, the outer layer being represented by isolated tufted clumps of brushite crystals.

Cobalt bullet no. 2 was studied in some detail, and the mineralogical data given below refer to material from this source unless otherwise stated (see figs. 1–3). Its five layers are as follows: (T), about 0·1 mm thick, brown, impersistent, consisting of whitlockite in tufts of prismatic pseudomorphs lying tangentially; (A) (absent from, or very thin above, the edges of the cobalt bullet cylinder), thickness 0·05–0·7 mm, porous, composed of whitlockite in tufts of prismatic pseudomorphs and interstitial masses, with some brushite, mostly optically anomalous; (B) continuous, thickness up to 0·8 mm, porous, of whitlockite only, resembling that of layer A; (C) continuous, thickness c. 1·2 mm, composed of brushite in prismatic crystals in sub-parallel tufted groups, and occasional interstitial whitlockite; minute struvite crystals are very occasionally present at the base; (D) continuous, compact, thickness c. 0·6 mm, of brushite in radially sub-parallel prismatic crystals. In addition to the minerals mentioned, one pollen grain and several skins of pollen grains or eggcases of worms (unidentified) were observed in crushed material.

Description of the minerals

Brushite. Most of the material used for measurement came from layer C of C.B. 2, but this was compared with material from all the other bullets.

Brushite occurs as colourless monoclinic prismatic crystals with perfect cleavage parallel to {010}, the basal cleavage being only very poorly developed. In view of the fact that few crystal faces are seen and that brushite has been given three different crystallographic orientations in recent literature (Dana's System, 6th edn, p. 828, also used by Larsen, 1921 and by several later authors; Terpstra, 1937; and Dana's System,

7th edn, vol. 2, p. 703) it was essential to determine the crystallographic and optical orientation by X-ray single crystal photographs. This was done by Dr. R. J. Davis (B.M.(N.H.) X-ray no. 8716 a–e) who found that the prismatic crystals are elongated parallel to [001], the main crystal face visible in cleavage fragments being (100) and the crystal elements (a:b:c = 0·384:1:0·411, β = 116$\frac{1}{2}$°) close to those given in Dana, 7th edn (1951).

The crystals contain abundant brown blebby rod-like inclusions of lower refractive index, elongated parallel to the c-axis and sometimes laterally connected. Groups of these begin at definite planes, which make angles of 6°–13° with α (see below) and form concentric surfaces, obviously marking successive growth levels, in the deposit as a whole (figs. 3 and 4). They are assumed to be organic in nature, possibly bacterial.

The bulk of the material varies only within narrow limits in optical properties: $\gamma \parallel$ [010]; β:[001] 21°±1° (19$\frac{1}{2}$° red, 22$\frac{1}{2}$° violet; cf. Köhler, 1926) in the obtuse angle β. α 1·539 (observed range, 1·5379–1·5401), β 1·545 (1·542–1·5461), γ 1·551 (1·5500–1·5518), all ±0·0004, for Na-light. The mean values are based on 76 separate determinations on all the cobalt bullets, the majority of which gave figures close to the mean. The typical material from layer C, C.B. 2, does not vary by more than 0·0005 from the mean. $2V_\gamma$ (ordinary light, direct readings with the universal stage) 87°±1°.

Dispersion crossed, $r > v$; extinction angle for r less than for v.

Slight variations in refractive index occur along the length of the crystals, marking successive stages in growth; the total range shown by particular indices is less than 0·001 (i.e. < ±0·0005). In C.B. 5 the clear material immediately below each new crop of inclusions has an extinction angle, β:[001], of 18° instead of 21°. In some cases (e.g. layer A of C.B. 2, the corresponding layer in C.B. 6, and the outer layer of C.B. 5) much wider anomalies occur; the crystals show undulose extinction and some contain minute spherulitic growths, and variations in 2V (down to c. 70°) and in sign occur (fig. 4). The divergent tufted brushite of C.B. 5 shows the highest range in refractive index (1·540–1·552; cf. Murray and Dietrich's material from Pighole Cave, Virginia, 1956) and when powdered gives rise to a large proportion of fragments flattened approximately normal to an optic axis, suggesting a parting parallel to a clinodome. These irregularities may be related to a break-down of the lattice due to dehydration, and perhaps transition to whitlockite.

The density was determined by flotation, giving the following values: By E. C. Owen using unpicked material: C.B. 1: 2·33; 2: 2·19; 3: 2·25; 4: 2·32; 5: 2·37; 7: 2·26; 8: 2·20; 9: 2·20. By D. I. Bothwell, using picked material from layers C and D, C.B. 2: 2·29 ± 0·01, in both cases, with identical results when finely powdered (cf. Dana, 7th edn, 2·328 natural crystals, 2·326 artificial, 2·257 calculated; Murray and Dietrich, 1956, 2·30).

The brushite dissolves very rapidly in dilute (2 N) HCl without evolution of gas. A microanalysis on 3·3 mg of material separated by flotation from layer C, C.B. 2, gave CaO 32·6 %, Fe_2O_3+FeO not sought, MgO nil, P_2O_5 40·8; total loss on ignition (on an unseparated sample of 27 mg) 25·9; separate determination of H_2O by Penfield method (on an unseparated sample of 59 mg), 24·4 %. It is to be presumed that the low summation is due to the omission of organic matter, as the material smells of burnt protein (and in some cases of H_2S) when heated in an ignition tube. Apart from this, the analysis indicates a pure brushite, the water content being very slightly low.

Whitlockite ('Martinite'). This is collophane-like, almost always isotropic; it forms interlacing collapsed-looking fibres, apparently originating as tufts, in the inner layers of the deposit; and it also occurs as formless masses between these fibres and, occasionally, between the brushite fibres of the outer layers. It is translucent, colourless to deep green or brownish. Its density appears to be variable: some material floats with brushite in a mixture of density 2·4, while the rest ranges fairly evenly up to more than 2·9. The refractive index of material from C.B. 2, layer B, ranges from 1·578 to 1·601; a single crystalline fragment found in C.B. 5 gave a pseudo-uniaxial negative figure and a refractive index just above 1·62, and a similar one, uniaxial negative, was found in C.B. 6. An attempt to correlate density and refractive index in material from C.B. 2, layer B by determining the ranges in R.I. of successive fractions differing in density by 0·1 indicated that within each fraction, the typical or modal value lay near the top of a wide range; if successive modal rather than mean or medium values are taken, they are seen to range from 1·593 at $D = 2·3$ to 1·596 at $D = 2·9$, but the total range within each density fraction is much wider than this. The refractive index range in whitlockite from other cobalt bullets may differ somewhat from this: e.g. a range of 1·56–1·58 was noted for C.B. 5.

In warm dilute (4 N) HCl, the whitlockite dissolves very slowly with the evolution of a few bubbles, indicating probable CO_2 content. A microanalysis of 1·2 mg of gravity-separated ($D > 2·4$) material from layer

B, C.B. 2, gave CaO 42·5 %, MgO 3·3 %, Fe_2O_3+FeO not detected, P_2O_5 42·8 %. The figures are comparable to those of an analysis by Hendricks *et al.* (1931), quoted by Frondel (1943), of martinite (carbonate-whitlockite) from Curaçao, which contains solid impurities in addition to considerable CO_2, H_2O, and organic matter; the low summation is probably due to the omission of the last three items on account of the minute quantity of material available. The $MgO/(CaO+MgO)$ ratio is high, exceeding 10 % (atomic), near the maximum possible (said by Jensen and Rowles, 1957, to be 9·5 %) and above that (8 %) of the so-called magnesium-whitlockite found by these authors in dental calculi; as, however, the type whitlockite of Frondel from Groton, New Hampshire, contains 7 % (atomic) of MgO in $(CaO+MgO)$, this name would not appear to be justified. From analogy with collophane (Frondel, 1943) and the low density and refractive index, it can be assumed that the water content is high.

Struvite. Minute pale brown crystals, *c.* $10 \times 25\,\mu$, are found very occasionally near the base of the brushite prisms of the outer layers of the deposits. Ten have been recorded, from cobalt bullets nos. 1, 2 (layer *C*), 4, 5, and 6. They are rectangular or hemimorphic (pinacoids, with domes at one end only) and show extinction parallel to length, elongation $+$ or $-$; they disappear in liquid of R.I. 1·4975. Their identification as struvite has not been confirmed by chemical tests or X-rays because of their minuteness and scarcity, but there can be little doubt of its correctness.

Origin of the deposits. Brushite, whitlockite, and struvite all occur most commonly in guano deposits (Dana, 1951; Frondel, 1943; Murray and Dietrich, 1956); brushite has also been found on skeletons of historic age (Köhler, 1926; Chudoba, 1928). The main source of the material in these cases is obviously bone apatite broken down by acid compounds resulting from putrefaction. The occurrence here described has nothing in common with this, nor has it many parallels among ordinary calculi; brushite was found in only 12 out of 784 urinary calculi by Prien and Frondel (1947), while struvite occurred in 113, and they describe whitlockite as 'very rare'. There is, however, a close similarity to dental calculus, which is to be expected since the main source of the rumen fluid is saliva, which contains phosphate in alkaline solution; phosphate is also secreted by the rumen wall itself (Barnett and Reid, 1961). The calcium, and the magnesium of the whitlockite and struvite, must be presumed to come from the ingested herbage, since it has been shown that the calcium content of saliva is increased by 15 times and the

magnesium content 6 times, in a 5-hour infusion with hay (Garton, 1951, quoted by Barnett and Reid, 1961).

The brushite is clearly a primary precipitate, probably deposited during the fall in pH following feeding (Barnett and Reid 1961); according to Britton (1955) brushite begins to precipitate when the pH falls below about 6·7. The banding due to layers of inclusions is probably to be interpreted as a diurnal rhythm. The number of bands (about 50 in layer C and 30 in layer D of C.B. 2) is consistent with this explanation.

The whitlockite is obviously pseudomorphous after a prismatic mineral, its porosity indicating considerable shrinkage on conversion. The only likely precursor is brushite; if the conversion were due to enrichment in lime, addition of magnesia and dehydration, it would give rise to a shrinkage of about one-third to yield crystals or one-quarter to yield metacolloidal whitlockite of the density found. To the extent that it may be due to loss of phosphoric acid, the volume contraction would be greater. Another point in favour of origin from brushite is that relics, apparently of brushite, are present in some of the whitlockite fibres of the deposit on C.B. 5; these fibres are associated with brushite showing signs of collapse of the crystal structure, and their habit is as would be expected in a pseudomorph after brushite with considerable reduction in volume. Such pseudomorphs of whitlockite after brushite are, moreover, known in nature: e.g. the zeugite of Julien (1865) from the guano of Sombrero (Frondel, 1943). The only other precursors to whitlockite the replacement of which would involve considerable volume reduction are monetite, which occurs overlying whitlockite in the phosphate rock of Los Monges, Caribbean Sea; and the tetracalcium hydrogen tri-phosphate trihydrate, $Ca_4H(PO_4)_3 . 3H_2O$, found in association with brushite and whitlockite in dental calculus by Jensen and co-workers (1952, 1954, and 1957). In both these cases the volume reduction would be much smaller than in the case of brushite; in the case of any crystalline member of the apatite group there would be a slight expansion. There is, moreover, no evidence that any calcium phosphate other than brushite has been present.

The major succession in the more complex deposits would therefore seem to be: first brushite layer; conversion to whitlockite; second brushite layer; conversion to whitlockite; and third and fourth brushite layers. The third stage may precede the second; the fifth certainly follows the fourth. Precipitation of brushite is clearly interrupted between the stages, probably because the pH did not fall sufficiently low; conversion of existing brushite to whitlockite would seem to require an increase

in pH. This could result from periods of feeding on rich new herbage, which would yield both an increased supply of calcium and magnesium and an excess of protein, which tends to break down with the formation of ammonia (Barnett and Reid, 1961). The presence of struvite in association with the whitlockite bears out this interpretation, according to which the whitlockite layers may represent either movements from dry feeding or old pasture to new pasture, or flushes of new grass due to spells of rainy weather.

This study has not solved the mystery of the non-occurrence of the deposits in a majority of cases. Dewey et al. (1958) suggest that as all the five deposits reported by them are on cobalt bullets made with china clay from Modbury, South Australia, their formation must be due to some factor derived from the clay. This, however, does not explain their formation only in a few cases, and the present work has shown that brushite has begun to precipitate on whitlockite as well as on the bentonitic cobalt bullets and on brushite. Attempts (Dewey et al., 1958) to produce deposits on cobalt bullets artificially by 'generous additions' of calcium hydrogen phosphate or calcium carbonate to the rations of sheep have consistently failed. This suggests that saturation is not enough and that some idiosyncratic factor is involved. Perhaps, as Dr. E. C. Owen suggests, some of the lambs have a more efficient Krebs cycle than others and so produce citric acid at a greater rate. The formation of the deposit seems now to have been brought under control by the addition of citric acid to the cobalt bullets; nearly all the bullets so treated are free of deposit. (E. C. Owen, private communication, 1961.)

References

BARNETT (A. J. G.) and REID (R. L.), 1961. Reactions in the Rumen, London.
BRITTON (H. T. S.), 1955. Hydrogen Ions, vol. 1, p. 213.
CHUDOBA (K.), 1928. Centr. Min., Abt. A, p. 291.
DANA (J. D.), 1892. Syst. Min., 6th edn, p. 828.
—— (1951). Syst. Min., 7th edn, vol. 2, p. 703 (brushite), p. 684 (whitlockite), p. 716 (struvite).
DEWEY (D. W.), LEE (H. J.), and MARSTON (H. R.), 1958. Nature, vol. 181, p. 1367.
FRONDEL (C.), 1941. Amer. Min., vol. 26, p. 145.
—— 1943. Ibid., vol. 28, p. 215.
GARTON (G. A.), 1951. Journ. Exp. Biol., vol. 28, p. 358.
HENDRICKS (S. B.), HILL (W. L.), JACOB (K. D.), and JEFFERSON (M. E.), 1931. Ind. Eng. Chem., vol. 23, p. 1413.
JENSEN (A. T.), 1954. Journ. Dental Research, vol. 33, p. 741.

JENSEN (A. T.) and DANØ (M.), 1952. Journ. Dental Research, vol. 31, p. 620.
—— and ROWLES (S. L.), 1957. Acta Odont. Scand., vol. 15, p. 121.
—— 1957. Nature, vol. 179, p. 912.
—— and HANSEN (K. G.), 1957. Experientia, vol. 13, p. 311.
JULIEN (A. A.), 1865. Amer. Journ. Sci., ser. 2, vol. 40, p. 373.
KLOOS (J. H.), 1888. Neues Jahrb. Min., vol. 1, p. 41.
KÖHLER (A.), 1926. Tschermaks Min. Petr. Mitt., ser. 2, vol. 37, pt. 2, p. 93.
LARSEN (E. S.), 1921. Microscopic determination of the non-opaque minerals, U.S. Geol. Surv. Bull. no. 679, pp. 211 and 248.
MELLOR (J. W.), 1929. Inorganic and theoretical chemistry, vol. 3, p. 866 (calcium phosphates); vol. 4, p. 385 (struvite).
MURRAY (J. W.) and DIETRICH (R. V.), 1956. Amer. Min., vol. 41, p. 616.
OWEN (E. C.), ELLIS (S. E.), VOSS (R. C.), WILSON (A. L.), and ROBERTSON (J.), 1960. Proc. Nutr. Soc., vol. 19, p. xxi (of abstracts).
PRIEN (E. L.) and FRONDEL (C.), 1947. Journ. Urology, vol. 57, p. 949.
TERPSTRA (P.), 1937. Zeits. Krist., vol. 97, p. 229.

PLATE IV

FIG. 1. 'Cobalt bullet' no. 2, section with part of the deposit broken away to show the four main layers. (B.M. (N.H.) no. 24401.)

FIG. 2. Thin section of the deposit on 'cobalt bullet' no. 2; plane polarized light. (B.M. (N.H.) no. 24726B.)

FIG. 3. Single flake of brushite; a cleavage fragment parallel to (010) from layer C, 'cobalt bullet' no. 2; plane polarized light.

FIG. 4. Single flake of altered brushite; cleavage fragment parallel to (010) from layer B, 'cobalt bullet' no. 2, to show spherulites; crossed nicols.
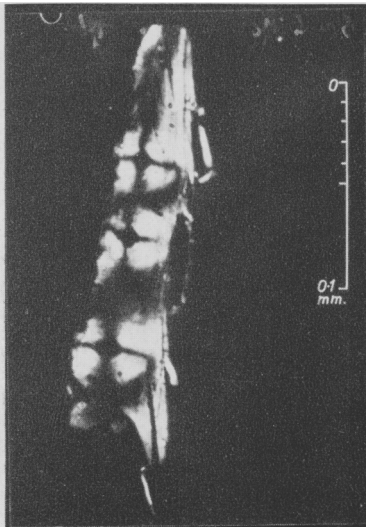
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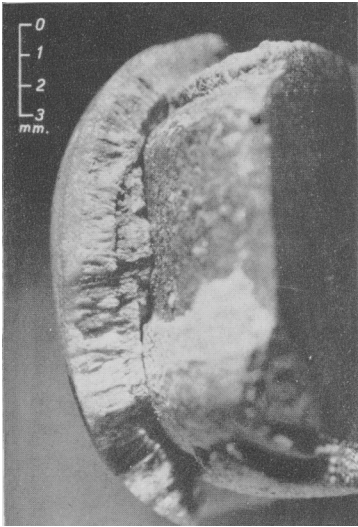
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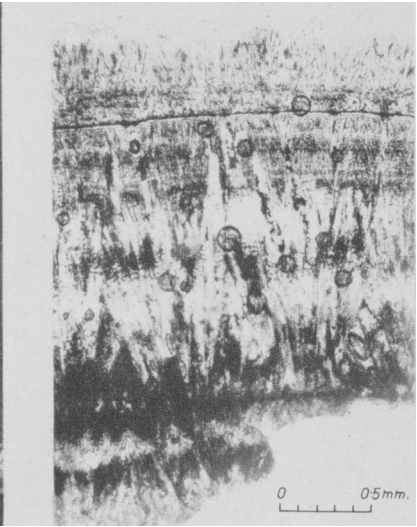
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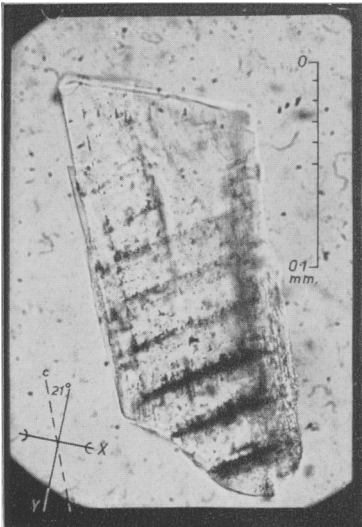
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