NEW ZEALAND ARCHAEOLOGICAL ASSOCIATION NEWSLETTER

If the artifacts recovered to date are a true representation of the material culture of the occupants, why were they so poor in suitable materials for tool-making? Even if the obsidian does belong to the ovens, there was very little of it. The small piece of flint was not a tool, and nor was it suitable for tool-making. To be reduced to using greywacke for a valued item indicates a distinct paucity of better materials. Even the shell fragments noted suggest imported tools rather than the remnants of a meal. The best materials found in primary association, the argillites, are available from a source which is fairly obvious and only a few days canoe journey from the site. Moreover, that source is a natural landing place for a party on a canoe journey between the two points at which a particular style of flaked knife has been noted.

The wide variations in the sizes of the ovens found indicate either corresponding variations in the numbers present to consume the meals or else variations in the amount of food to be prepared. The largest oven could provide one good meal for up to four hundred people; the smallest would be more suitable for a dozen or twenty. Did a small group occasionally entertain on a lavish scale? Did they sometimes find themselves with a large catch and cook a fortnight's meals all at once? Or is the site just a favourite camping place for travelling parties? The geography of the site as it is at present seems to make this last a poor choice. The small group with a taste for the social whirl hardly accords with the poor material culture. So what kind of small ill-equipped group would sometimes cook a little and sometimes cook a lot?

Fugitives? Hardly. The site is in plain view from dozens of natural vantage points, and probably always has been. Outcasts? A remnant of a conquered tribe, tolerated but not encouraged to encroach on the material wealth of a the area? What a waste of good slaves. Or a small isolated group in a country which they had not had time to explore thoroughly?

This last is an intriguing possibility, and it accords well with all the information gathered so far. The main difficulty is that the facts so far would also fit a small group of survivors in the late eighteen-thirties when epidemics and inter-tribal wars had almost denuded Marlborough of its Maori population.

SOME OBSERVATIONS OF RESIDUAL PROTEIN IN MOA BONE

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INTRODUCTION:

During a salvage dig on the Ngatitoa Domain, Paremata (Grid reference: N160.418447) a quantity of Moa bone was recovered. Much of this consisted of tabs and small fragments of leg bone but a few vertebrae and whole leg

bones were found also. The material was taken from two layers: a greasy, charcoal-rich stratum which, from associated artifacts, appeared to be of Moa Hunter origin and from a layer of clean wind-blown sand immediately below this occupation layer. Much of the bone from the sand layer was very well preserved. It was decided to examine some of this bone in more detail. After decalcification of pieces of the bone with mineral acid it was found that a jelly-like replica of the specimen remained. It was this observation which led to the following investigations being carried out. Sections were cut from the decalcified material and after staining by various methods these sections were examined microscopically. Some of the material was also subjected to hydrolysis and a quantitative assay of the amino acids present was made using a paper chromatographic technique.

METHODS:
Well preserved fragments of bone from the tibial shaft, identified as belonging to ANOMALOPTERYX were sawn into small pieces and decalcified with E.D.T.A. solution. The blocks of protein remaining were embedded in celloidin-paraffin wax and sections 10 microns thick were cut. These sections were stained by the following methods:-

(a) Haematoxylin and eosin
(b) Dunn-Thompson stain
(c) Verhoeff stain
(d) Feulgen stain
(e) Periodic acid - Schiff
(f) Silver impregnation

Unstained sections were also examined with polarised light. Other pieces of bone were decalcified with cold 20% hydrochloric acid and the remaining protein was hydrolysed with 2N hydrochloric acid (acid hydrolysis) and also with barium hydroxide solution (alkaline hydrolysis) at 105° C for 24 hours. Both acid and alkaline hydrolysis was carried out because there are a few amino acids which are destroyed by hot acids but not by hot alkali and vice versa. As a result of hydrolysing the protein a solution of the constituent amino acids was obtained. These amino acid solutions were analysed by two-way paper chromatography. The ascending technique was used. Sheets of paper 33 x 33 cm. were run overnight in butanol-acetic acid followed by an overnight run in phenol-ammonia. The dried sheets were developed with ninhydrin. For purposes of comparison fresh bone was obtained from the humerus of the Black Backed Gull (Larus dominicanus) found on the nearby beach. Some ordinary gelatine was hydrolysed and chromatographed in a similar manner.

RESULTS:
On examining the stained sections it was interesting to find that the protein residue did not present an amorphous appearance but still retained an organised structure after centuries of burial. Sections stained with haematoxylin and eosin gave a general idea of the structure. No trace of

nuclear material was found nor were any formed cells seen. The Dunn-Thompson stain demonstrated that most of the material present was COLLAGEN. Embedded in this collagen were structures which appeared to be blood vessels. Accordingly sections were treated with Verhoeff's stain and it was shown that ELASTIN was present in these vessel walls. In the lumen of some of these there was present a small amount of brown amorphous material. One would have liked to have shown that this was a haemoglobin derivative, a remnant of the Moa's blood, but it did not react with various reagents tried and remains unidentified.

The Feulgen reactions was tried with some sections but this was negative showing that deoxyribonucleic acid (an important constituent of nuclear material) was not present. Treatment of the material with Periodic acid-Schiff reagents showed that a few fungal hyphae were present in the outer layer of the bone. It was not possible to assess the age of this fungal material but it was probably old. Sand taken aseptically from the same positions as the bones were proved to be quite sterile - no growth of bacteria or fungi was obtained in aerobic an anaerobic cultures. Sections impregnated with silver (Gordon and Sweet's method) did not demonstrate the presence of reticulin. Finally unstained sections examined by polarised light showed that the bundles of collagen fibres were birefringent. The amino acids found in the Moa bone closely resembled those found in the modern bird bone. The accompanying table sets out the amino acids found.

	Acid hydrolysis			Alkaline hydrolysis		
	Moa Bone	Fresh Bone	Gelatine	Moa Bone	Fresh Bone	Gelatine
Alanine	+	+	+	+	+	+
Arginine	+	+	+	o	o	o
Aspartic acid	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+
Glutamine	o	o	+	o	o	+
Glycine	+	+	+	+	+	+
Hydroxyproline	+	+	+	+	+	+
Isoleucine	o	+	+	o	o	+
Leucine	+	+	+	+	+	+
Lysine	+	+	+	+	+	+
Ornithine	o	o	o	+	+	+
Proline	+	+	+	+	+	+
Serine	+	+	+	+	+	+
Threonine	o	+	+	o	o	o
Valine	+	+	+	+	o	+

+ = amino acid present, o = amino acid not detected.

REMARKS:

Of the many proteins present in the animal body collagen is the most resistant to decay. It is insoluable in the usual protein solvents and is only slightly digestable by the enzymes pepsin and trypsin. Boiling in water converts it into gelatine which is more soluable and easily digested by proteolytic enzymes. This conversion probably involves only minor molecular rearrangement and has little effect on the constituent amino acids. It is for this reason that ordinary commercial gelatine was included in the above investigations as a comparative substance. In the absence of radio-carbon dating I estimate that the age of the bone examined lies between 700 and 1,000 years. The preservation of some of its contained protein undoubtably is due to the fact that it was soon covered after death and sank in the sand to a sterile, anaerobic level free from the attacks of micro-organisms some of which secrete collagenase, an enzyme capable of breaking down collagen. Elastin, which was also present in the blood vessel walls is very similar chemically to collagen and is resistant to break-down. The other organic substances present in the original bone (fats, nucleoproteins, blood pigments, etc.) would have been broken down readily by chemical means (autolysis) and would have disappeared within a short time of burial. Undoubtably collagen disappears slowly from buried bone and the speed of disappearance will depend on the circumstances of preservation of the bone. Samples of subfossil bone have not yet been tested for protein content. Tyler (Tyler 1957) found small amounts of protein in Moa egg shell but no analysis of this protein was given in his paper. Possibly the ratio inorganic material to collagen content might be of use in relative dating of material recovered from the same site as has been attempted by estimating the conchyolin content of midden shell (Palmer 1963). Amino acid chromatograms of old and new bone were very similar and did not appear to lead to any method of dating bone recovered from an archaeological site.

REFERENCES:

Palmer, J.B. 1963 — Dating shell middens - a South African chemical aid for relative dating. N.Z.A.A. Newsletter, 6, 2: 112 - 114

Tyler, Cyril 1957 — Some chemical, physical and structural properties of Moa egg shell. J. Poly. Soc., 66, 1, : 110 - 130.