THE STRUCTURE OF THE WATER-HOLDING FEATHERS
OF THE NAMAQUA SANDGROUSE

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ABSTRACT

The morphology and fine structure of the feather barbules of the Namaqua Sandgrouse *Pterocles namaqua* are investigated histologically and experimentally by means of light microscopy, scanning electron micrography and X-ray diffraction. Proximally the barbule is helically coiled for three and a half turns and has a kidney-shaped, concave/convex transverse section. The inner concave surface is pitted, the outer convex surface smooth. The barbule is solid, consisting of three layers, and bears a number of appendages at its distal end, where it is more rounded in transverse section. The uncoiling of barbules from the abdominal feathers on contact with water may be initiated by water uptake and further facilitated by the number of helical coils at the base of the barbules. The keratin is fairly crystalline when dry. This crystallinity is somewhat reduced on wetting. The uncoiling mechanism is related to the expansion of the polypeptide chains of β-keratin in order to accommodate additional water bound to the side chains.

INTRODUCTION

The Namaqua sandgrouse *Pterocles namaqua* is a pigeon-sized bird of desert to semi-desert habitats in southern Africa (Maclean 1968). Cade & Maclean (1967) have given a detailed account of the method of water transport by the male Namaqua sandgrouse to its young. This transport is made possible by the water-holding properties of the abdominal feathers, the lower breast feathers and, to a reduced extent, the upper breast feathers. The structure of these feathers is the same in the female, but the area of specialized barbules is not as great, and the downy basal region is better developed.

Rijke (1972) has shown by X-ray diffraction that the mechanism of uncoiling and subsequent recoiling of the feather barbules of *P. namaqua* is related to the keratinous structure of the feathers. From the X-ray data feather keratin may be considered to be predominantly β-keratin (Edsall & Wyman 1958; Fraser & Suzuki 1965; Rudall 1947). According to Rijke (*loc. cit.*) melting occurs when the barbules make contact with water; the individual long-chain polypeptides coil up, resulting in changes in the dimensional properties of the fibre. This reaction is determined by the degree of axial orientation and the extent of crystallinity and/or cross-linking between cystine groups. In this respect the barbules differ from the keratin of other feather types.

It was the purpose of the present study to examine the structure of the barbules of the feathers of *P. namaqua* experimentally. The barbules were compared with those of some other avian species showing similar barbule conformations. X-ray diffraction and other studies have been done to test Rijke's (1972) results.

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MATERIALS AND METHODS

1. *Microscope slide preparations*

Slide preparations of microtome sections of the feathers were made to determine the internal structure of the barbules. A portion of abdominal feather consisting of barbs and barbules attached to a length of rachis was fixed in methyl benzoate celloidin. The feather portion was then placed in benzene wax at 37°C for 20 min., and then put through three treatments of 52-degree paraffin wax at 60°C for two hours per treatment. The resulting wax block was orientated on a sledge microtome to cut the barbules at right angles. The sections were heated on to slides, dried for 24 hours and stained with haemalum and Congo red. After dehydration, the sections were mounted in Canada balsam.

2. *Comparison of barbules of different species*

Barbules from the feathers of the following 11 species of birds were drawn in the wet and dry conditions: *Podiceps ruficollis, Anhinga rufa, Turnix sylvatica, Charadrius marginatus, Vanellus armatus, Cursorius rufus, Arenaria interpres, Attagis gayi, Thinocorus orbignyianus, Pterocles namaqua,* and *Treron australis.*

3. *Scanning electron micrography*

In order to examine the finer structure of the barbules of *Pterocles namaqua,* barbs and barbules were mounted on a viewing cylinder, coated with a 200-Å layer of carbon particles and examined under the scanning electron microscope.
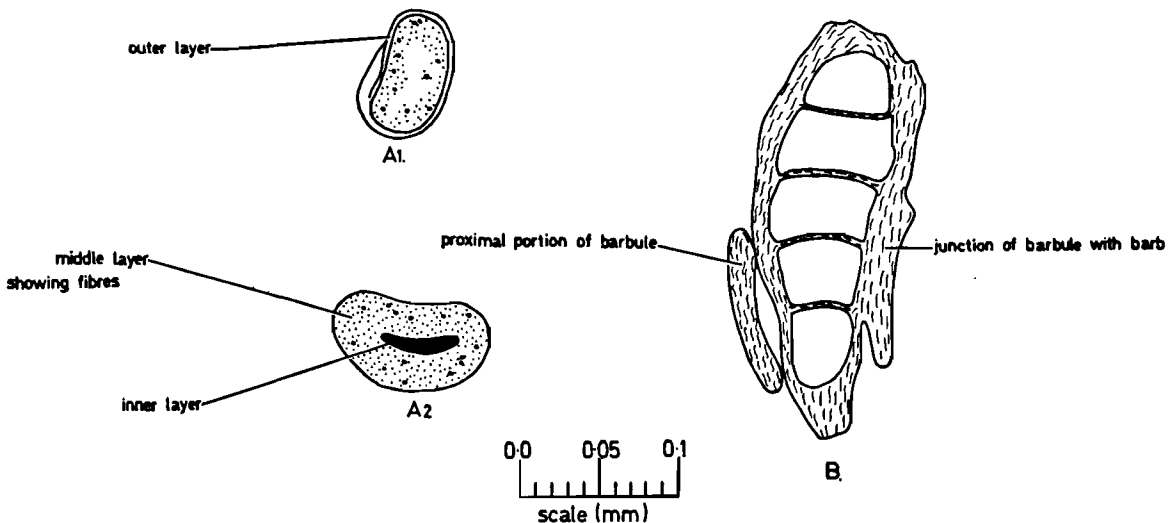

FIGURE 1

Transverse sections of barbules of *Pterocles namaqua* feather. A1 = distal end of barbule; A2 = proximal part of barbule; B = barb with proximal bases of two barbules.
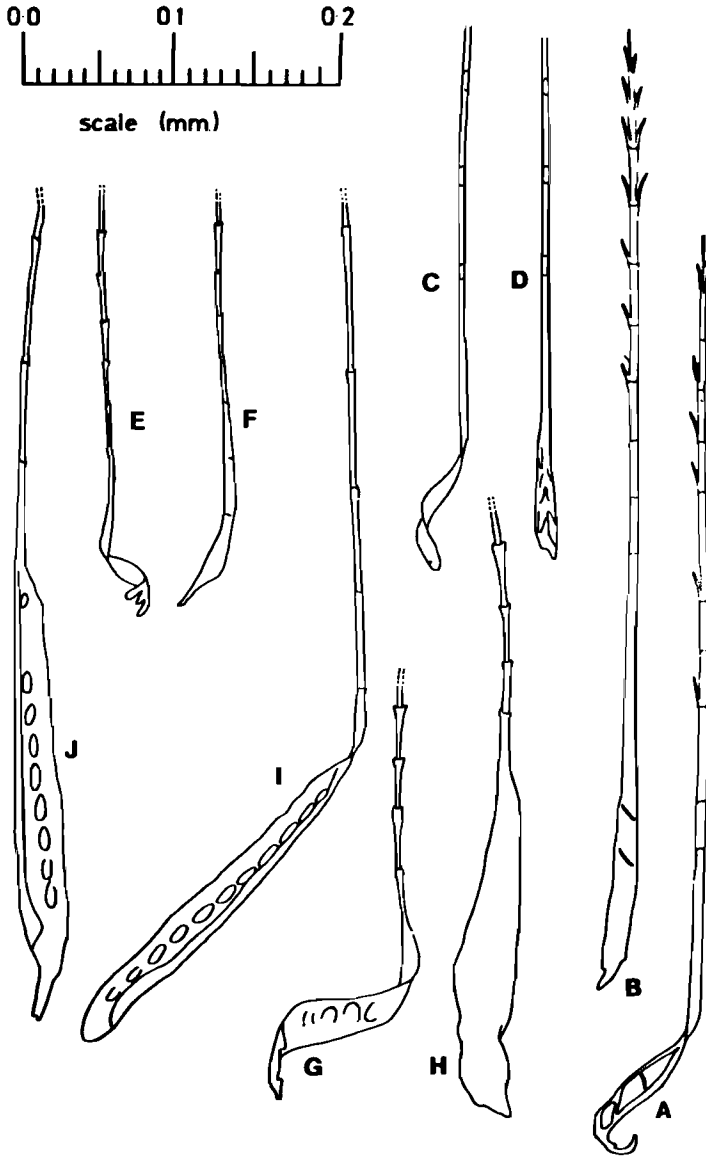
FIGURE 2

Whole barbules from the feathers of various bird species. A = *Podiceps ruficollis* dry, B = *P. ruficollis* wet, C = *Anhinga rufa* dry, D = *A. rufa* wet, E = *Charadrius marginatus* dry, F = *C. marginatus* wet, G = *Attagis gayi* dry, H = *A. gayi* wet, I = *Treron australis* dry, J = *T. australis* wet.

FIGURE 3

Whole barbules from the feathers of various bird species. A = *Turnix sylvatica* dry, B = *T. sylvatica* wet, C= *Vanellus armatus* dry, D = *V. armatus* wet, E = *Arenaria interpres* dry, F = *A. interpres* wet, G = *Pterocles namaqua* dry, H = *P. namaqua* wet, I = *Cursorius rufus* dry, J = *C. rufus* wet, K = *Thinocorus orbignyianus* dry, L = *T. orbignyianus* wet.
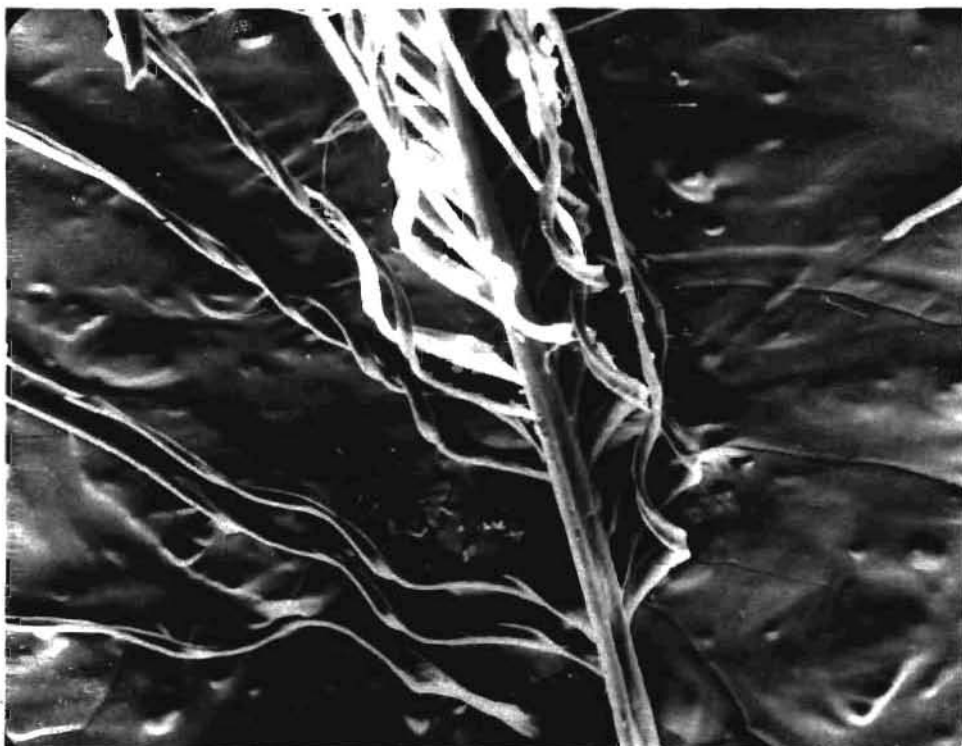
FIGURE 4

Scanning electron micrograph of barb and attached barbules of *Pterocles namaqua*, showing the helical coiling of the barbules ($\times$ 438).
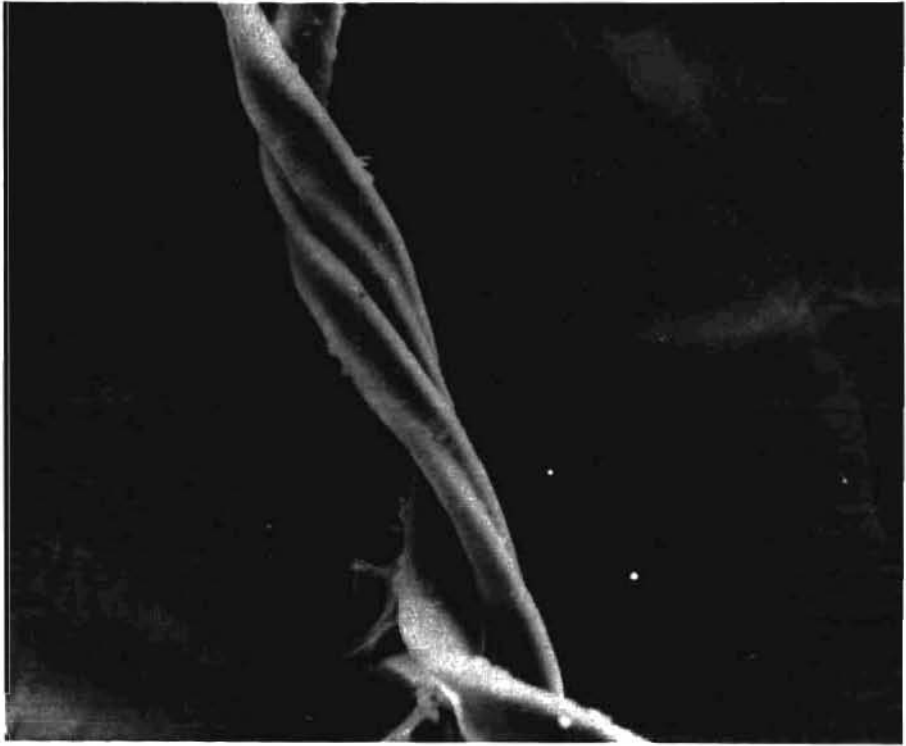
FIGURE 5

Basal portions of two adjacent barbules of *Pterocles namaqua* showing how they coil together in the dry condition (scanning electron micrograph × 875).

FIGURE 6

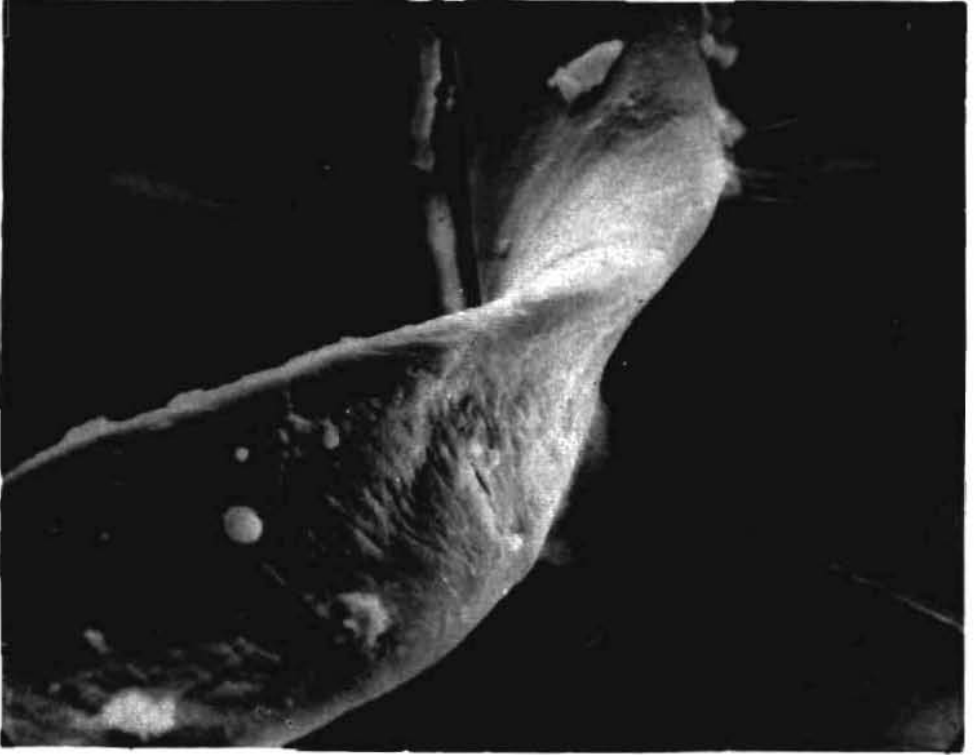Distal end of barbule of *Pterocles namaqua* showing appendages (scanning electron micrograph × 1750).

FIGURE 7

Single helical coil of barbule of *Pterocles namaqua* showing pitting on concave surface and smooth convex surface (scanning electron micrograph × 1750).

4. X-ray diffraction studies

Barbs of *P. namaqua* were pulled off the feather rachis and the downy ends cut off. The remaining barbs and barbules were ground up in a porcelain pestle and mortar. The resulting keratin powder was sedimented on to a microscope slide. The material was investigated with a Philips PW 1051 X-ray Diffractometer with CoKα-radiation. Two runs each were done with the material in the dry and wet conditions. Before the wet run, a single drop of tap water was added to the slide and allowed to disperse through the keratinous material until it looked uniformly damp. The diffraction patterns were determined over an angular range corresponding to $2\theta=3°$ to 51° (dry) and $2\theta = 3°$ to 45° (wet). In both runs the counter was set at 2, with rate meter = 2, time constant = 16, multiplication = 1, chart drive = 20x and a constant speed of 2°θ/min.

A similar technique was employed using barbules only. Diffraction patterns were determined over an angular range corresponding to $2\theta = 6°$ to 33° (dry) and $2\theta = 6°$ to 33° (wet). In both wet and dry runs the counter was set at 2, with rate meter = 1, time constant = 16, multiplication = 1, chart drive = 20x and a constant speed of 2°θ/min.

<div align="center">

RESULTS

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1. Microscope slide preparations

Observations of barbule sections through a light microscope indicate that they may consist of three layers (Fig. 1). There is an inner rectangular layer which looks very dark and tends to become more circular in section toward the distal end of the barbule, a thick middle layer which looks fibrous, and a very thin outer layer. The fibres of the middle layer vary in thickness, the thicker fibres being few in number. The proximal part of the barbule is kidney-shaped in cross section and originates toward the dorsal side of the feather barb.

2. Comparison of barbules of different species

Diagrams of barbules of different species, as well as of *P. namaqua*, are shown in Figs. 2 and 3. The base of the barbules of *P. namaqua* has about three and a half helical coils in the dry condition. Only one basal coil is found in the barbules of *Attagis gayi, Thinocorus orbignyianus, Charadrius marginatus, Vanellus armatus, Cursorius rufus, Arenaria interpres, Turnix sylvatica,* and *Treron australis.* Dry barbules of *Podiceps ruficollis* and *Anhinga rufa* have two helical coils at the base; the more proximal barbules of *Podiceps ruficollis* have only one coil. The result of this coiling is that the barbules of *Pterocles namaqua* lie flat and parallel to the barbs in the dry condition, while the barbules of the other species examined stand almost at right angles to the plane of the feather vane, forming a bed of hairs.

The reverse situation applies to barbules in the wet condition. Those of *Pterocles namaqua* stand at right angles to the plane of the feather vane and have a single coil at the base. Wet barbules of all the other species straighten out and lie in the same plane as the rest of the feather vane, with the result that they tend rather to shed water than to hold it.

3. Scanning electron micrography

Details of the barbules of *P. namaqua* can be seen in the scanning electron micrographs (Figs 4, 5, 6, and 7).

4. *X-ray diffraction studies*

The X-ray crystallographs of the preparations of barbs and barbules of *P. namaqua* appear in Fig. 8a (dry run) and Fig. 8b (wet run). Corresponding graphs for barbules alone appear in Fig. 8c (dry run) and Fig. 8d (wet run). The peaks in these graphs indicate crystallinity in the keratin structure of the feather; the peaks flatten out somewhat in the wet condition, indicating a slightly reduced crystallinity of the keratin.

DISCUSSION

The barbules of *Pterocles namaqua* are not hollow, but are solid, layered and apparently fibrous. The concave/convex cross-section of the basal helically coiled portion of the barbule probably contributes somehow to the mechanism of uncoiling and recoiling, but the method has not been established. On wetting, water may penetrate the barbule to the periphery of the middle layer.

Preliminary studies of water retention suggest that the barbule in its naturally dry state contains a small amount of water which can be eliminated only by prolonged heating at temperatures in excess of those which sandgrouse would experience in the field. It is hoped to extend these investigations in the future.

It is reasonable to suggest that evolutionary processes have selected for such a primary structure of the peptide chains in the feather barbules that contact with or uptake of water at specific sites will cause an immediate change in conformation and this lead to a macrostructure of the barb/barbule meshwork that holds water effectively. The adaptive significance of the water-holding properties of sandgrouse feathers has already been discussed (Cade & Maclean 1967; Maclean 1967, 1968). In the other species examined, the function of the bed of hairs, caused by the orientation of the dry barbules at right angles to the feather vane, is presumably one of insulation; the adaptive value of water-shedding by the wet feather, due to a change in the orientation of the barbules, is obvious, especially in the aquatic *Podiceps* and *Anhinga*.

Cade & Maclean (1967) suggest that water transport in the belly feathers of sandgrouse may have evolved from a transformation of the bathing response common to most birds. However, the use of the belly-feathers for water transport is confined to the sandgrouse and to the order Charadriiformes (Maclean in press). If belly-soaking arose from so common a behaviour pattern as bathing, it is curious that the habit is not more widespread among birds. Since the bases of the barbules of sandgrouse feathers have more helical coils than those of the charadriiforms examined in this study, it may be assumed that sandgrouse feather structure is more highly specialized for water transport, although it might have been derived from the charadriiform type. Maclean (1967) has already suggested a close relationship between the sandgrouse and the Charadrii on the basis of other evidence.

It seems probable that the terminal appendages (reduced barbicels ?) (Fig. 6) of the barbules of *Pterocles namaqua* assist in the water-holding properties of the belly feathers; these appendages spread apart when wet, thereby increasing the surface area. The pitting on the concave surface of the barbule may play some role in the mechanism of coiling and uncoiling, in conjunction with the concave/convex shape of the barbule base.

Grinding the barbs and barbules for the X-ray diffraction studies probably affected the structure of the polypeptide chains of the keratin, and therefore the results. In agreement with
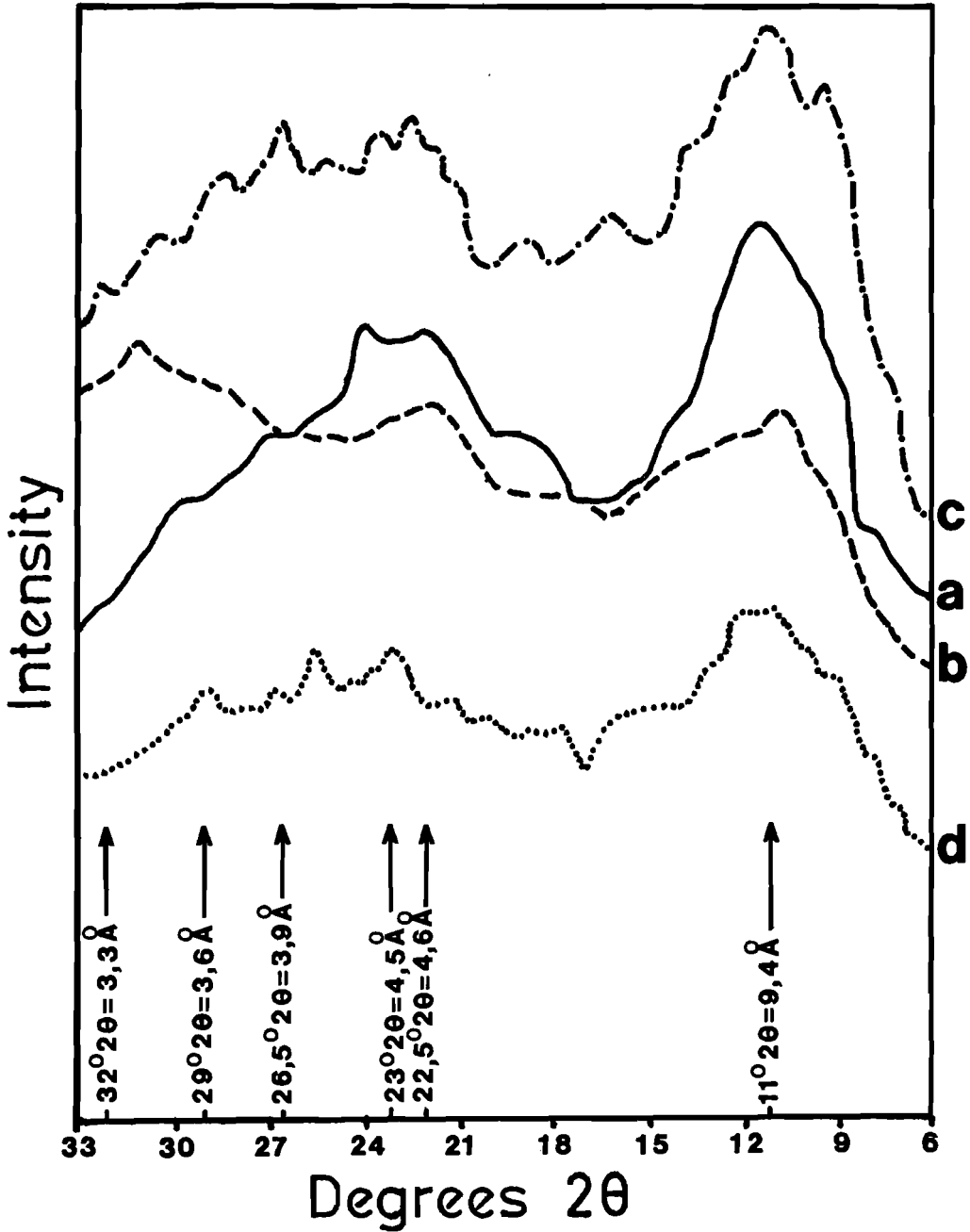
FIGURE 8

X-ray crystallograph showing peaks of crystallinity of dry barbs and barbules (a), wet barbs and barbules (b), dry barbs only (c), and wet barbs only (d) of *Pterocles namaqua* belly feathers.

the findings of Rijke (1972) we also observed peak broadening of the characteristic reflections in the X-ray powder diffraction patterns of wet feathers. There is some kind of molecular transformation of the polypeptide chains on wetting. Edsall & Wyman (1958) suggest that water binds with the charged and polar side chains of the polypeptide units, and that these units expand to accommodate water. This may be the mechanism by which uncoiling occurs. Edsall & Wyman (*loc. cit.*) have shown by X-ray measurements of β-keratin a regular spacing along the fibre axis of 3,33 Å. This distance corresponds to a single amino acid residue in the direction of the chain axis.

Two other well marked X-ray reflections at right angles to this axis occur at 9,7 Å and 4,65 Å. The perpendicular distance of 9,7 Å must represent the distance between adjacent parallel layers of peptide chains. Rudall (1947) showed that the same fundamental X-ray diffraction patterns are given by the keratin of all parts of the feather—barbules, barbs, rachis, calamus, and medulla—all of which consist of β-keratin.

The 9,4 Å and 4,6 Å peaks (Fig. 8) correspond with the X-ray reflection of 9,7 Å and 4,65 Å respectively, obtained by Edsall & Wyman (1958) for β-keratin. The peaks at 3,3 Å (Fig. 8a), 3,6 Å (Fig. 8d), and 3,9 Å (Fig. 8c) probably correspond to the regular spacing of 3,33 Å along the fibre axis of β-keratin (Edsall & Wyman 1958). Slight differences between our results and those of Edsall & Wyman may be the result of mechanical damage to the keratin through grinding. Nevertheless, sandgrouse feather keratin appears not to differ significantly from the β-keratin of other bird feathers.

The loss of crystallinity on wetting the keratin may be due to the expansion of the polypeptide units to accommodate additional water, and thereby causing the barbule to uncoil.

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