were carried out using the lithium and lead salts in acetone and ethanol respectively. Their compositions were studied by the ester-fractionation procedure.

2. The fishes rohu and nain belong to different genera, and significant differences have been found in the proportions and degree of unsaturation of their C_{16}, C_{18}, C_{20} and C_{22} groups of unsaturated acids, and also in the amounts of total saturated acids and of stearic acid.

3. The high saturated acid content of the fats is remarkable, especially in fats from rohu. The increase in the percentage of stearic acid seems to have been at the cost of unsaturated C_{18} acids, and thus may indicate bio-hydrogenation. Small differences in the composition of the fats from body and viscera of the same fish have been observed in both species.

4. The fat content of the viscera of these fish is about 45%.

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The Organic Constituents of Mammalian Compact Bone

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The inorganic matter which characterizes bone as a tissue has been studied by a large number of workers (Neuman & Neuman, 1953). On the other hand, the material composing the so-called 'organic matrix' has received very little attention.

Collagen, the principal organic constituent of bone, has for a long time been assumed to be similar to the collagen of the softer connective tissues (Huggins, 1937), although hardly anything was known about it, except that under mild hydrolytic conditions, it gave rise to gelatin. Quite recently Neuman (1949), using microbiological methods of analysis, has shown that the amino acid composition of collagen from bone does not differ significantly from that of collagen in other tissues. The same conclusion has been reached by Rogers, Weidmann & Parkinson (1952). Randall, Fraser, Jackson, Martin & North (1952) have examined collagens from a number of tissues, including bone, with the electron microscope, and have found typical non-branching striated fibrils in all specimens.

Hawk & Gies (1901) isolated a mucopolysaccharide complex which they termed 'osseomucoid' from ox bone, and Seifert & Gies (1904) showed that it was invariably present, as a minor constituent, in the bones of a number of mammalian species. Hisamura (1938) separated osseomucoid into two components, one of which contained a hexosamine and a hexuronic acid and the other a hexosamine and galactose. Two hexosamines, glucosamine and galactosamine, were found in osseomucoid by Masamune, Yosizawa & Maki (1951), who separated them by paper chromatography. Rogers (1949) reported the presence of 0·1–0·25% of hexosamines and 2–4% of reducing substances in human bone and later (Rogers, 1951) isolated a substance containing equimolar weights of a hexosamine, a hexuronic acid and the hydrogen sulphate group.

The presence in bone of two proteins, one soluble in cold water, and similar in composition to collagen, and a second which resists solution in hot water, has been shown by Rogers *et al.* (1952).

The citrate content of bone has been extensively examined by Dickens (1941). This ion is generally considered to be intimately associated with the inorganic crystal lattice (Neuman & Neuman, 1953).

The object of the present study was to obtain more information about the composition of compact bone tissue. Ox-femur bone, which was readily freed from extraneous tissues, was powdered by a method involving negligible chemical degradation. It was then separated into its known constituents by a variety of methods. Prolonged extraction with water at 100° dissolved the principal organic constituent, collagen, by converting it into gelatin. The residue from this operation consisted of inorganic matter with a small amount of a protein which resisted solution in boiling water. A little of the inorganic matter dissolved during the hot-water extraction. Treatment of bone powder with dilute hydrochloric acid dissolved the inorganic matter leaving the organic matrix. A mucopolysaccharide-protein complex was separated from this organic portion by extraction with lime water. The residue, which consisted mainly of protein, was extracted with hot water to dissolve the collagen and leave the water-resistant protein. By carrying out these processes quantitatively a chemical 'balance sheet' was drawn up to show how far the entire composition of bone was accounted for in terms of its known constituents.

In the second part of this investigation the detailed composition of the organic constituents of bone was studied using analytical techniques. In particular, the amino acid composition of the protein involved in the mucopolysaccharide complex was determined quantitatively, and the identity of the components of the polysaccharide portion tentatively established.

EXPERIMENTAL

Materials

Compact bone tissue. The epiphyses of ox femora were sawn off leaving the straight centre portions of the diaphyses about 10 cm. in length. The fatty marrow was completely scooped out and small areas of spongy bone, inside the tube of compact bone, were scraped away with a scalpel. The periosteum was similarly scraped off while wet and any areas of discoloured bone, where ligaments had been attached, were removed. The remaining tissue consisted entirely of compact bone which was completely white, dense and hard.

Bone powder. The tubes of compact bone were turned in a lathe using a light cut. The light, friable turnings were collected on a clean cloth and reduced to a powder in a high speed disintegrator. The powder, which passed through a 100-mesh test sieve, was spread out in a thin layer and exposed to the air for 48 hr. to allow the moisture content to reach an equilibrium value.

Bone collagen (containing water-resistant protein). Sulphur dioxide was passed into a mechanically stirred suspension of 50 g. of bone powder in 1 l. of water until pH 2·5 was reached. The suspension was stirred for 30 min., allowed to settle and the supernatant liquid removed by suction. More water was added and the treatment with sulphur dioxide repeated. The residue was washed twice with water and treated with 10% NaCl solution at pH 7, overnight, to remove albumins and globulins. After further washing, the residue was treated overnight with 10% $CaCl_2$ at pH 9 to remove the mucopolysaccharide fraction (Partridge, 1948*a*). The residue from this operation was washed successively with water and acetone and dried in a vacuum desiccator. The protein, which was a finely divided white powder, was exposed to the air in a thin layer to allow the moisture content to reach equilibrium. (Moisture content, 15·25; ash, 0·08; total N, 18·26% on dry, ash-free basis.)

Mucopolysaccharide-protein complex. 'Osseomucoid' was prepared from ox femora using the lime water extraction method of Hawk & Gies (1901). The preliminary demineralization of the bone was carried out in a refrigerator at 2° to minimize possible degradation. Excess of $Ca(OH)_2$, above the amount required to prepare half-saturated lime water was added to allow for the buffering capacity of the protein. It was found necessary to add a slight excess of acid to the filtered lime-water extract to obtain the mucopolysaccharide-protein complex in a form which could be readily filtered. Filtration was carried out rapidly, the precipitate being washed successively with acetone and ether, since exposure of the white powder to the air, while moist, resulted in its conversion into a brown gum.

Osseomucoid was also prepared from commercially demineralized bone (ossein).

Water-resistant protein. Shavings of demineralized bone were autoclaved with a tenfold excess of water for 3 hr. at 15 lb./in.^{2} pressure. The residue was filtered from the brown supernatant liquid and again autoclaved for 2 hr., after more water had been added. The residue was filtered off, washed successively with hot water, acetone and ether and dried in a vacuum desiccator.

Analytical methods

Moisture. The loss of weight on drying a thinly spread layer of the powdered material at 105° for 24 hr. was used to calculate the moisture content.

Ash. The material was gently ignited in a platinum dish, then heated for 2 hr. at 550° and the residue weighed.

Total nitrogen. This was determined by the micro-Kjeldahl method, using a modification of the procedure of Chibnall, Rees & Williams (1943). The $CuSO_4$ in the catalyst mixture was replaced by an equal weight of $HgSO_4$, which reduced the digestion time necessary for maximum recovery of nitrogen, from 8 to 2 hr. A portion of the diluted solution was made alkaline with 30% NaOH, 5% $Na_2S_2O_3$ solution and the ammonia was steam distilled into 1% boric acid solution (Yuen & Pollard, 1953). This solution was titrated with 0·01 N-HCl using an ethanolic solution of 0·025% methyl red and 0·016% methylene blue as indicator.

Analysis of whole bone tissue. The oven-dry bone was extracted with ether and then with a large excess of water at 100° for 24 hr. The residue, consisting of inorganic matter, and water-resistant protein, was filtered on a sintered glass crucible, washed, dried at 105° and weighed. The total N content of the dry residue was determined and used to calculate the weight of resistant protein present, assuming that it contained 16·0% N. The water extract was evaporated to dryness and the residue, which consisted mainly of degraded gelatin, was dried at 105°, weighed and finally ashed to obtain the weight of soluble inorganic matter.

Sulphate sulphur. This was determined by the method of Bowes & Cave (personal communication) in HCl hydrolysates of the mucopolysaccharides.

Hexosamines. The combined glucosamine and galactosamine content was determined by the method of Elson & Morgan (1933) as modified by Bowes & Cave (personal communication), except that the heating with acetylacetone was carried out in stoppered flasks for 50 min. instead of in open test tubes for 20 min. to improve the colour yield.

Later, glucosamine and galactosamine were independently determined after chromatographic separation, by the ninhydrin method of Moore & Stein (1948).

Uronic acid. The modification of Tollen's test, due to Maughan, Evelyn & Browne (1938) was used.

Total reducing sugars. These were determined by the modification, worked out by Heidt, Southam, Benedict & Smith (1948) of the micro-volumetric method of Somogyi (1937).

Hydrolysis. Materials to be examined for amino acid composition were heated for 24 hr. with a 100-fold quantity of 20% (w/w) HCl in sealed glass ampoules (Macpherson, 1946). The HCl was subsequently removed by evaporation in a vacuum desiccator containing NaOH and P_2O_5.

Where hexosamines were determined by the method of Elson & Morgan (1933), the time of heating was reduced to 8 hr. and the acid concentration to 4 N.

The hydrolysates for examination of the sugar residues by paper chromatography were prepared by heating with N-H_2SO_4 at 100° for 5 hr. (Yosizawa, 1950).

Paper chromatography. The separation of both amino acids and sugars was carried out on Whatman no. 1 filter paper (Brimley & Barrett, 1953).

The solvents used for separation of amino acids were phenol:water (ammonia) and *n*-butanol:acetic acid:water (4:1:5, v/v) (Partridge, 1948*b*). Since the R_F values of the amino acids are low in the latter solvent, increased resolution of the slower-moving ones was obtained by returning the paper to the tank for further development, after drying the paper in the usual way at the end of the first development. Up to four successive developments have been given by this means, at the end of which the spots remained sharp. Ethanolic ninhydrin (0·1%) was used as spray reagent.

For the separation of sugars and substituted sugars the miscible solvent mixtures *n*-butanol:pyridine:water (5:3:2, v/v) of Chargaff, Levine & Green (1948) and *n*-butyl acetate:acetic acid:water (3:2:1, v/v) of Yosizawa (1950) were employed. The latter solvent was developed by Yosizawa for the separation of glucosamine and galactosamine, but is found not to be entirely satisfactory since the separation of these spots is small even when the solvent front is allowed to move much further than the distance specified. Since the hexosamine spots frequently tended to tail, an elongated, diffuse spot resulted instead of two distinct spots, when both amino sugars were present. Aniline hydrogen phthalate (Partridge, 1949) was found to be the most useful of a number of spray reagents examined. Hexosamines gave no colour after 5 min. heating and a chocolate brown after 30 min. heating. Separate spraying with alkaline acetylacetone solution and *p*-dimethylaminobenzaldehyde in HCl:ethanol (Partridge, 1948*b*) was used to confirm the position of the hexosamine spots.

Quantitative amino acid analysis. This was carried out as by Moore & Stein (1951), the amino acids being separated on columns of Dowex 50 resin, 200 to 400-mesh, 12% cross-linking. A specially constructed drop-counting fraction collector was used for collecting 1 ml. (20 drop) fractions.

Difficulty was at first experienced in purifying samples of thiodiglycol, which was incorporated in some of the buffers to prevent oxidation of methionine (Moore & Stein, 1951). Even after distillation *in vacuo*, the thiodiglycol gave rise to a yellow colour on heating with the ninhydrin reagent. This colour was found to be produced by an unidentified impurity present in the more volatile fractions. This difficulty was overcome by distilling over the first 30% of the once-distilled thiodiglycol and retaining the remaining 70% left in the distilling flask, for use in the buffers. The purified material was a pale yellow liquid, b.p. 142° at 2 mm. Hg pressure and n_D^{12} 1·5200.

The volumes of standard NaOH and HCl solutions quoted by Moore & Stein (1951) for adjusting certain buffer solutions to pH 5·0 were found to be incorrect. The following volumes were found necessary for neutralization of 1 ml. of buffer: pH 3·42, 0·1 M citrate, 0·12 ml. of N-NaOH; pH 4·25, 0·1 M citrate, 0·063 ml. of N-NaOH; pH 6·7, 0·1 M citrate, 0·042 ml. of 2 N-HCl; pH 6·8, 0·1 M phosphate, 0·09 ml. of 0·5 N-HCl; pH 6·5, 0·2 M citrate, 0·075 ml. of 2 N-HCl.

Glucosamine and galactosamine were separated from each other and from amino acids on a 15 cm. Dowex 50 column (Eastoe, 1954) and were estimated by the ninhydrin method.

RESULTS

Analysis of whole bone tissue

Extraction with cold water. Powdered bone (10 g.) was extracted with cold water by continuous leaching. The bone powder was finally washed and the filtrate and washings made up to 200 ml. The solution showed a small negative optical rotation and had a solids content of 0·98% (dried at 105°) of the weight of bone. The residue, after ashing, was 0·41% of the weight of the bone. No reducing sugars could be detected in the solution. A hydrolysate of the soluble material was examined by paper chromatography and the amino acid pattern was found to be indistinguishable from that of bone collagen. This confirmed the suggestion that the water-soluble organic matter consisted mainly of a gelatin or procollagen type protein or its degradation products (Rogers *et al.* 1952).

Reducing sugars. Hydrolysates of powdered bone were prepared using different concentrations of acid with various heating times at 100°. In none of the hydrolysates did the reducing sugar content exceed 0·2% of the bone tissue. When allowance was made for the interfering effects of amino acids on the method of estimation it was considered that not more than 0·05% of reducing sugar residues were present.

Chemical composition of compact bone. The results of the gravimetric analysis of the air-dried bone powder are shown in Table 1. The organic matter, soluble in hot water, was considered to be mainly degraded gelatin, derived from the collagen. The weight of collagen was assumed to be the weight of

Table 1. *Composition of air-dried compact bone tissue (ox femur diaphysis)*

	% by wt.
Inorganic matter, insoluble in hot water (probably including up to 1% of citrate)	69·66
Inorganic matter, soluble in water	1·25
Collagen	18·64
Mucopolysaccharide-protein complex	0·24
Resistant protein material	1·02
Fat	0·00
Sugars, other than in mucopolysaccharide	0·00
Water (lost below 105°)	8·18
Total	98·99

Table 2. *Inorganic matter in compact bone tissue (ox femur diaphysis)*

	% by wt.
Total inorganic matter (from Table 1)	70·91
Bone, ashed at 450°	70·1
Bone, ashed at 500°	68·7
Bone, ashed at 550°	67·9

the soluble organic matter minus the weight of mucopolysaccharide-protein complex obtained by lime-water extraction. This complex would probably slowly break down and dissolve in hot water.

Almost 99% of the weight of bone was thus accounted for. The remaining 1% was attributed to small accumulated errors in the gravimetric analysis. The difficulty of allowing for the different weights of water retained at 105° by bone powder, the inorganic residue and the organic matter, respectively, is not readily overcome. All of the values in the table were obtained by direct weighing except for the comparatively small weight of resistant protein, which was calculated from the total nitrogen content of the insoluble residue.

The combined weight of the soluble and insoluble inorganic matter (Table 1) agrees well with the values for the residue from bone, ashed at various temperatures (Table 2). The first value quoted is high, due to inclusion of citrate and water retained at 105°. The ash value at 450° probably tends to be high also, owing to the difficulty of burning off carbon at this temperature, while the values for 500 and 550° may be low owing to loss of carbon dioxide from the carbonate.

Total nitrogen content of bone. The air-dried bone, the composition of which is given in Table 1, was found to contain 3·62% N. Assuming that demineralized bone contains 18·1% N, this value corresponds to 20·0% of total organic matter in the bone. This is in good agreement with the value of 19·9% for the total of collagen, resistant protein and mucopolysaccharide complex (Table 1).

Collagen

The total nitrogen content of demineralized bone shavings was found to be 18·10%, which was appreciably lower than the generally accepted value for hide collagen of 18·6% (Bowes & Kenten, 1948). This was at first attributed to decomposition during demineralization, contamination with mucopolysaccharide or both. A number of samples were prepared in which finely powdered bone was rapidly demineralized with sulphurous acid, a comparatively mild treatment, and the mucopolysaccharide was later removed by calcium chloride extraction (see Materials). All of these preparations had a nitrogen content close to 18·26%, only slightly higher than the earlier value. These low values are possibly attributable to the presence of a protein resistant to hot water, which had a nitrogen content of only 14·6%. No method has been found for removing this impurity from collagen, without converting the collagen into gelatin.

Paper chromatograms of bone collagen showed an amino acid composition which could not be distinguished from that of hide collagen. Quantitative studies of the amino acid composition of bone collagen are in progress.

Resistant protein

This residue consisted partly of a stringy elastic mass, resembling 'cartilage tubes' (Le Gros Clark, 1945) and partly of an amorphous powder. The dry, ash-free material contained 14·62% N. This value was probably low compared with that of the main protein constituent of the residue, due to the presence of impurities unavoidably concentrated in a product of a residual nature. The amino acid picture, obtained from the examination of paper chromatograms, was quite unlike those of collagen and elastin (from ligamentum nuchae). There was a closer resemblance to blood albumin, although several slight differences were noted. The protein had a particularly high tyrosine content. The amino acid composition appeared to be closely similar to that reported for the reticular tissue of lymph nodes (Bowes & Kenten, 1949).

As the resistant protein was not homogeneous, no quantitative study of the amino acid composition was made. It is probable that the composition varies with the time of autoclaving, since the resistant protein passes gradually into solution.

Mucopolysaccharide-protein complex

The complex, as isolated, was a white amorphous powder, soluble in dilute alkali and reprecipitated by addition of acid. The alkaline solution was noticeably viscous. The air-dried material contained 9·18% moisture and no ash. The dry material contained C, 46·5; H, 6·5; N, 11·3; S, 1·83; SO_4S, 1·63%. Pure chondroitin sulphate $(C_{14}H_{21}O_{14}NS)_n$, which may be regarded as typical of the sulphur-containing mucopolysaccharides, would contain N, 3·05 and S, 6·97%. The low sulphur content of the isolated

material suggested that only a small part of it could be a sulphated polysaccharide, while the high nitrogen content indicated the possible presence of a protein. Most of the sulphur present was in the form of sulphate, the remaining 0·2% being compatible with the sulphide sulphur of a protein. After 8 hr. hydrolysis with 4N hydrochloric acid at 100°, the average value for the hexosamines present was 9·46%. During the heating, browning of the hydrolysate took place and also some decomposition of the hexosamines. Experiments in which known amounts of glucosamine hydrochloride were added to gelatin before hydrolysis, indicated that some 20% of the hexosamine decomposed. The corrected value for total hexosamines was therefore 12·1%.

An attempted determination of uronic acids failed, owing to the decomposition of a substantial proportion of these sugar derivatives during hydrolysis (4 hr. at 100° with 4N hydrochloric acid). The results obtained for uronic acid liberated and remaining undecomposed under these conditions indicated a minimum content of 3·0%. The true value probably much exceeded this. The estimation of the total reducing sugars present in hydrolysates also failed, owing to interference by amino acids. The results for the hexosamine and uronic acid determinations, like those for the total nitrogen and sulphur, suggested that only a part of the complex was a sulphated polysaccharide.

The sugar residues present in hydrolysates were tentatively identified by paper chromatography. A chromatogram developed with *n*-butyl acetate: acetic acid:water and sprayed first with alkaline acetylacetone and then with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde), showed two pink spots of low R_F value, merging into one another, in the positions corresponding to galactosamine (chondrosamine) and glucosamine (chitosamine). A similar chromatogram sprayed with aniline hydrogen phthalate showed three further spots which were identified as mannose, either galactose or glucose and either galacturonic acid or glucuronic acid. In addition there was a faint pink spot of higher R_F value in the position corresponding to the spots for the pentose sugars, xylose and arabinose. Further chromatograms were developed with *n*-butanol:pyridine:water which separated galactose from glucose; only the former sugar was found to be present in the osseomucoid hydrolysate. In addition, it was shown that the pentose present was xylose and not arabinose. The authors at first considered that xylose arose from decarboxylation of glucuronic acid during the acid hydrolysis. It would appear, however, that all reported attempts to demonstrate this reaction have failed. On the other hand, our evidence is not sufficient to prove the existence of xylose, which commonly occurs in plants, in an animal polysaccharide. The sugar residues in the complex were thus provisionally identified as galactosamine, glucosamine, and hexuronic acid, galactose and a small amount of mannose.

One-dimensional amino acid chromatograms were developed with phenol:water and *n*-butanol: acetic acid:water (4:1:5) systems. The protein portion of the complex was found to be entirely free from hydroxyproline and contained a large amount of tyrosine. When compared with a chromatogram of blood albumin (British Drug Houses Ltd.) a marked resemblance was noticed.

A quantitative amino acid analysis was carried out on the mucopolysaccharide-protein complex by the method of Moore & Stein (1951), the results of which are summarized in Table 3. The amino acid composition was quite unlike that of collagen and elastin, being similar to that of the blood plasma proteins, although differing in some details (Tristram, 1953). The amino acid and hexosamine

Table 3. *Amino acid and hexosamine composition of the mucopolysaccharide-protein complex*

	Weight basis (g./100 g. dry, ash-free material)	Molar basis (moles/10^6 g.)
Alanine	3·70	41·5
Glycine	2·65	35·3
Valine	4·50	38·4
Leucine	7·27	55·4
Isoleucine	3·65	27·8
Proline	4·24	36·9
Phenylalanine	2·86	17·3
Tyrosine	1·98	10·9
Tryptophan	—	—
*Serine	3·61	34·4
*Threonine	4·13	34·7
Cystine	1·13	9·4
Methionine	0·84	5·6
Arginine	3·87	19·4
Histidine	2·65	17·1
Lysine	4·26	29·2
Aspartic acid	9·66	72·6
Glutamic acid	11·67	79·4
†Amide N	1·07	76·4
Hydroxyproline	0·00	0·0
Hydroxylysine	0·00	0·0
Glucosamine	1·23	6·8
Galactosamine	7·67	42·8
Methionine (separated as sulphoxide)	0·25	1·7
Total	81·82	616·6
Average residue weight		114·6
Z‡		115·7
% recovery (wt.)		70·7
% recovery (N)		100·5

* The values for serine and threonine have been corrected for decomposition during hydrolysis by multiplying by factors of 100/89·5 and 100/94·7, respectively (Rees, 1946).

† Not included in totals.

‡ Chibnall (1942).

analysis accounted for all the nitrogen and 70 % of the weight of the complex. The remaining 30 % was attributed to the non-nitrogenous sugar residues.

DISCUSSION

The analysis accounted for 99 % of the weight of the bone in terms of the inorganic matter, collagen, mucopolysaccharide fraction and resistant protein. The complete absence from compact bone of fat and carbohydrates, other than the mucopolysaccharide, is significant. This is not in agreement with the work of Rogers (1949), who found 2–4 % of reducing substances in human femora.

The presence of a protein, resisting solution in hot water, in bone confirms the findings of Rogers *et al.* (1952) and is in agreement with the literature results for other collagenous tissues, e.g. hide (Bowes & Kenten, 1949), and dentine (Stack, 1951). These residual proteins are very difficult to characterize because they undergo gradual decomposition during preparation and are also likely to be contaminated with impurities.

In the present study, a hydrolysate of the entire mucopolysaccharide-protein complex, was examined to discover what residues were present and in what quantities. Previous workers have attempted to isolate purified polysaccharides from the complex. The present findings of the occurrence of galactosamine, hexuronic acid and sulphate sulphur are in agreement with the isolation of a polysaccharide, probably chondroitin sulphate, by earlier workers (Hisamura, 1938; Rogers, 1951). The presence of hexosamine and galactose are also consistent with the composition of another polysaccharide, isolated by Hisamura (1938), which contained these residues, together with a high proportion of protein. The residues detected in our study, with the exception of mannose, were identical with those shown to be present by Masamune *et al.* (1951).

The protein constituent of the complex was shown to be neither collagen nor degraded collagen since it had an entirely different amino acid composition. This finding, together with the work of Hisamura (1938), which showed that the protein was quite firmly combined in the fraction containing hexosamine and galactose, suggested that the protein was not an accidental contaminant, precipitated with the polysaccharides but rather that the protein and some of the carbohydrate residues were intimately associated in the intact bone, possibly as a mucoprotein. The simultaneous precipitation of chondroitin sulphate, the mucoprotein containing hexosamine and galactose, and possibly other polysaccharides would thus result in the production of the mucopolysaccharide-protein complex as a mixture of variable composition.

It would appear that the contemporary picture of connective tissue as made up of collagen with chondroitin sulphate and hyaluronic acid as the only mucopolysaccharides is over-simplified. These latter would account for only galactosamine, glucosamine and glucuronic acid residues whereas galactose and mannose also occur in bone. Consden (1953) has reported the occurrence of glucosamine, galactose, mannose and a trace of glucose in subcutaneous connective tissue.

SUMMARY

1. At least 99 % of the weight of compact mammalian bone (ox femur diaphysis) is accounted for by inorganic matter, collagen, a mucopolysaccharide-protein complex and a protein which resists solution in hot water.

2. Compact bone from ox femora contains no fat and no carbohydrate other than that of the mucopolysaccharide complex.

3. No differences have been found between bone collagen and collagens from other tissues.

4. The protein that resists solution in hot water is heterogeneous, one portion resembling cartilage tubes in appearance and the other being an amorphous powder.

5. The mucopolysaccharide-protein complex contains the sugar residues galactosamine, glucosamine, hexuronic acid, galactose and mannose. Xylose, of unknown origin, was also found in the hydrolysate. The whole of the nitrogen content has been accounted for in a quantitative determination of the amino acids and hexosamines. The amino acid composition of the protein is unlike that of collagen, but bears some similarity to the blood serum proteins.

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Animal Fats

3. THE COMPONENT ACIDS OF OSTRICH FAT*

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In continuation of our study of animal fats (Gunstone & Paton, 1953*a*, *b*) we now report the composition of ostrich fat, which has not been examined previously.

EXPERIMENTAL

The fat used in this investigation was obtained from an adult male ostrich (*Struthio camelus*) which had been in captivity in Edinburgh Zoo. Its diet consisted of pasture supplemented with whole maize. In its natural state the ostrich eats small mammals, birds, snakes, lizards and insects, as well as grass, leaves, fruits, berries and seeds. 'A post-mortem revealed a knotted mass of grass filling the proventriculus and gizzard. A varied bacterial flora was isolated from the bone marrow and there was severe congestion of the duodenum and a fatty infiltration of the liver.' (Information kindly supplied by Mr E. C. Appleby.)

The crude fatty tissue (830 g.) was autoclaved at 120° and broken up in a homogenizer before extraction with light petroleum (b.p. 40–60°). The fat (705 g.) was obtained as a white low-melting solid of iodine value 80·4, saponification equivalent 282·8, and free acid 2·1% (as oleic acid), whilst the mixed acids obtained on hydrolysis had iodine value 83·4 and equivalent 273·3.

The mixed acids (197·6 g.) were divided into three fractions by crystallization from methanol (10 ml. per g.) at −40° overnight and by recrystallization of the insoluble portion at −20° from methanol (10 ml. per g.). The results are shown in Table 1.

Each fraction was methylated by treatment with methanol and either HCl at room temperature (*B* and *C*) or conc. H_2SO_4 at the reflux temperature of the solution (*A*) and the resulting esters distilled through an electrically heated and packed column (Towers, Widnes T. 117) under reduced pressure. From the iodine value and saponification equivalent of each fraction and the spectrographic examination, after alkali isomerization, of selected fractions the composition of the mixed acids was calculated by the methods previously described (Gunstone & Paton, 1953*b*). The results are shown in Table 2.

* Paper 2 of this series: Gunstone, F. D. & Paton, R. P. (1953*b*), *Biochem. J.* **54**, 621.

Table 1. *Low-temperature crystallization of fatty acids from ostrich fat*

	Acids			Esters	
Fraction	Wt. (g.)	(%, w/w)	Iodine value	Iodine value	Sap. equiv.
A. Insoluble at −20°	77·5	39·2	21·1	20·3	280·2
B. Soluble at −20°, insoluble at −40°	60·0	30·4	94·4	88·8	292·5
C. Soluble at −40°	60·1	30·4	154·9	146·6	284·2