Comparison of Bovine and Human Orosomucoids

THE existence of acidic glycoproteins in human[1-3] and bovine[4,5] sera has been well established. A bovine *M*-2 glycoprotein has been isolated in an apparently pure state as described by us earlier[5], and bovine orosomucoid has been purified by Weimer and Winzler[4]. This communication describes the isolation of reasonably homogeneous bovine orosomucoid and discusses its properties in relation to the corresponding human material.

Human orosomucoid was prepared from fraction VI as previously described[3]. Bovine orosomucoid was prepared from a bovine plasma fraction by the carboxymethyl cellulose method[5]. Orosomucoid was the only protein not retained by the carboxymethyl cellulose exchanger, equilibrated with *p*H 4·1 acetate buffer, ionic strength 0·033, when plasma ammonium sulphate fraction *P*-4 (ref. 4) was applied to it at *p*H 4·1 (acetate). Apparent purity of the bovine orosomucoid preparation was determined by filter paper- and cellulose acetate paper-electrophoresis at *p*H 4·5, 7·0 and 8·6. In all cases the orosomucoid preparation showed only one band when paper strips were stained for protein (bromphenol blue and Ponceau *S*) and for carbohydrate (periodate-permanganate[6]). The preparation also appeared homogeneous in the ultracentrifuge at concentrations 3·2 mg/ml.–10 mg/ml. giving a $S_{w,20}$ of 3·2 at infinite dilution. Intrinsic viscosity, determined by plotting relative viscosities versus concentration, was 0·076. Partial specific volume was 0·714, calculated from composition data. Molecular weight of bovine orosomucoid, calculated from the above data, was 49,000. Amino-acid composition, except tryptophan, was determined by the method of Moore *et al.*[7], tryptophan was determined according to Bencze and Schmid[12], and carbohydrates were estimated as described by Winzler[8] and Dische[9]. Sialic acid was determined by the Ehrlich reaction[10] and by the orcinol method[11]. Table 1 summarizes properties of bovine and human orosomucoids.

Only minor differences in the amino-acid, hexose, and hexosamine content of the two proteins are apparent. The sialic acid content in bovine orosomucoid was somewhat higher than in the human preparation, although by paper- and moving boundary-electrophoresis methods bovine orosomucoid has a mobility towards the anode of only 75 per cent that of human orosomucoid at *p*H 4·5. The sialic acid content of bovine orosomucoid, prepared in our laboratories, was also considerably higher than that of purified bovine orosomucoid as reported by Weimer and Winzler[4]. Minor differences in amino-acid composition also exist between human orosomucoid reported herein and orosomucoid isolated by Weimer *et al.*[1]. In the latter case, amino-acids were determined microbiologically.

Table 1. COMPOSITION AND PROPERTIES OF BOVINE AND HUMAN OROSOMUCOIDS

	Bovine (per cent)	Human (per cent)
Nitrogen	11·1	11·1
Lysine	6·10	4·72
Histidine	1·57	1·13
Ammonia	1·51	1·95
Arginine	3·39	3·33
Aspartic acid	5·84	6·08
Threonine	3·37	4·02
Serine	2·87	1·62
Glutamic acid	10·50	10·10
Proline	1·57	2·06
Glycine	1·44	1·22
Alanine	3·11	1·87
½ cystine	1·64	1·08
Valine	2·22	2·55
Methionine	0·74	0·50
Isoleucine	3·30	2·65
Leucine	3·91	4·27
Tyrosine	3·36	4·52
Phenylalanine	8·85	7·12
Tryptophan	0·75	1·70
Hexose	12·0	14·4[3]
Hexosamine	11·8	10·2[3]
Sialic acid		
Ehrlich	16·2	
Orcinol	19·2	12·5[3]
Fucose	0·4	1·5[3]
Uronic acid	0·9	none
$S_{w,20}$	3·2	3·1[13]
Intrinsic viscosity	0·076	0·069[13]
Partial specific vol.	0·714	0·675[13]
Molecular weight	49,000	44,100[13]
$E_{1cm,\ 1\%}$	6·2	8·0[3]

In conclusion, relatively large amounts of apparently homogeneous bovine orosomucoid were conveniently prepared by the ammonium sulphate–carboxymethyl cellulose method. Amino-acid, carbohydrate compositions and the molecular weights of bovine and human orosomucoids were very similar.

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Detection and Metabolism of Hydroxychloroquinsulphate and Hydroxychloroquindiphosphate in Human Beings

FOR the long-term treatment of chronic polyarthritis and collagenosis with hydroxychloroquinsulphate (HS) and hydroxychloroquindiphosphate (HP), accurate adjustment to a definite blood-level would be desirable. Unfortunately, at the present time, there are no specific methods of quantitative determination which can be used by the clinician for both substances. We therefore endeavoured to achieve satisfactory detection of HS and HP by paper chromatography and arrived at the following technique of investigation.

(1) *Qualitative detection of HS and HP.* Schleicher and Schüll filter paper No. 2043 *B*, isopropanol/glacial acetic acid/water (70 : 15 : 15), descending paper chromatography. After drying at room temperature, tint with platinum iodide reagent. (1·1 per cent potassium iodide (Merck) and 0·14 per cent platinum iodide (Merck) added fresh and mixed well in equal parts.) After drying at room temperature, HS and HP are visible on a wine-red background as blue-black specks. R_F values (mean values of 20