In spite of the roughness of the approach, equation (6′) describes satisfactorily the viscous behaviour of the steam. Certainly, the foregoing statements cannot be used to test a general relation describing very accurately the temperature-dependence of the gas viscosity; but they can, however, be regarded as a further confirmation of the validity of equation (1′), which expresses the viscosity of a liquid as a function of temperature.

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BIOLOGICAL SCIENCES

Characterization of the Fc′ Fragment of Immunoglobulin G in Normal Human Urine

A FRAGMENT in normal human urine related specifically to the heavy chains of immunoglobulin G (IgG) was found[1] by gel diffusion and immunoelectrophoretic analysis to be antigenically similar to the Fc′ fragment produced by papain digestion of the IgG molecule. The presence of a fragment of this kind in urine has been confirmed[2,3], but no attempt has yet been made to purify and chemically characterize it. It has been proposed[4] (with experimental support[5]) that the Fc′ fragment obtained by papain digestion of human IgG is a dimeric subunit extending from near residue 14 to near residue 105 (numbering from the C-terminal end) of the γ-chain. We now report the isolation of urinary Fc′ fragment, together with physicochemical data confirming its close similarity to enzymatic Fc′ fragment.

The isolation of the Fc′ fragment from urine was difficult, and complicated by the presence of various urinary substances with similar charge and size characteristics. Pooled urine (110 l.; 8·9 g protein) from normal adult males was concentrated and subjected to zone electrophoresis in 0·1 M barbital buffer, *p*H 8·6, as previously described[6]. Concentrated material of α_2-mobility (2·7 g protein) was applied to a column of 'Sephadex *G*-100' equilibrated in 0·1 M *tris*-hydrochloric acid, *p*H 8·0, containing 1 M sodium chloride. The eluted Fc′ fragment and free light chains were identified with specific antisera and the Fc′ fragment was found to be eluted later than monomers of free light chains[7]. After pooling and concentrating, the material containing Fc′ fragment (146 mg protein) was again fractionated on 'Sephadex *G*-100'. The protein recovered at this stage (53 mg) was applied to a column of CM-cellulose[8] equilibrated in 0·03 M acetate buffer, *p*H 5·4. Most of the proteins, including the Fc′ fragment, did not bind at this *p*H, and 41 mg was recovered. Zone electrophoresis of this material in borate buffer[6], *p*H 8·9, gave 14 mg of protein containing Fc′ fragment. Finally, the fragment was applied to a column of DEAE-cellulose and eluted with a linear gradient from 0·04 to 0·2 M sodium phosphate buffer[8], *p*H 7·8. The Fc′ fragment emerged as a separate fraction with 0·08 to 0·12 M buffer; a large fraction of contaminating protein was eluted later. Approximately 2·7 mg of Fc′ fragment was recovered after dialysis against distilled water and lyophilization. This material was analysed by previously described methods[4,6].

Ouchterlony gel-diffusion analyses of the isolated urinary Fc′ fragment gave the following results. Antisera to κ and λ light chains failed to react with the preparation of urinary Fc′ fragment but an antiserum raised against enzymatic Fc′ fragment reacted with the urinary preparation even when the latter was diluted to a concentration of 0·03 mg/ml. Further, the urinary Fc′ fragment showed a reaction of complete identity with the enzymatic Fc′ fragment using an antiserum to pFc′ fragment (a related but larger fragment produced by peptic digestion[4]). Immunoelectrophoretic analysis of the urinary preparation with the antiserum to pFc′ fragment gave an immunoprecipitin arc with an anodic mobility similar to that of the enzymatic Fc′ fragment (Fig. 1). (In contrast, the larger pFc′ fragment migrates cathodically.) Antisera

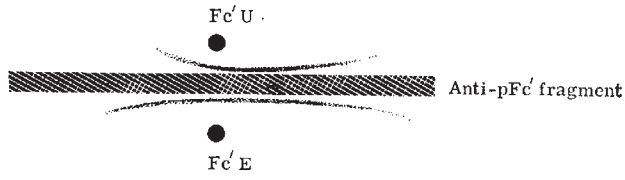

Fig. 1. Immunoelectrophoretic analysis of urinary Fc′ fragment (Fc′U) and enzymatic Fc′ fragment (Fc′E) using a rabbit antiserum raised against pFc′ fragment (a larger fragment including the Fc′ fragment within its structure). Anode to the right.

to whole serum proteins and whole urine proteins[6] failed to detect any other component. A further indication of purity was obtained by polyacrylamide disc electrophoresis[9] (Fig. 2). Urinary Fc′ fragment and enzymatic Fc′ fragment migrated rapidly to similar positions in the gel—there was no evidence of slowly migrating contaminants.

Sedimentation analyses in the ultracentrifuge of the urinary Fc′ fragment gave a symmetrical peak with a sedimentation coefficient ($s^0_{20,w}$) of 1·9 *S*. This value is greater than the figure of 1·3 *S* reported by Bienenstock[3] for a urinary fraction obtained by gel chromatography and known to contain Fc′ fragment. The coefficient is somewhat lower than some published data for enzymatic Fc′ fragment (2·35 *S* and 2·1 *S*; refs. 4 and 8 respectively) but within the range of coefficients for enzymatic Fc′ fragments reported in one recent study (1·8–2·4 *S*)[10]. The Stokes's radius of the urinary Fc′ fragment was found to be 19 Å by analytical gel chromatography on 'Sephadex *G*-100' (ref. 11). A molecular weight of 18,000 for urinary Fc′ fragment obtained from the Stokes's radius, the sedimentation coefficient and the partial specific volume[12] (assumed[4] to be 0·75) was the same[13] as or somewhat lower[4,10] than figures reported for enzymatic Fc′ fragment.

The amino-acid compositions of urinary and enzymatic Fc′ fragments are compared in Table 1. The amino-acid content of both preparations has been calculated on the basis of a total number of 180 residues per molecule (excluding tryptophan)[4]. Values are also given to the nearest even integer because the Fc′ fragment is a dimeric subunit composed of two symmetrical portions of the heavy (γ) chains. There is a good degree of overall similarity between the two types of preparation. Eight amino-acids are present in the same amounts, six amino-

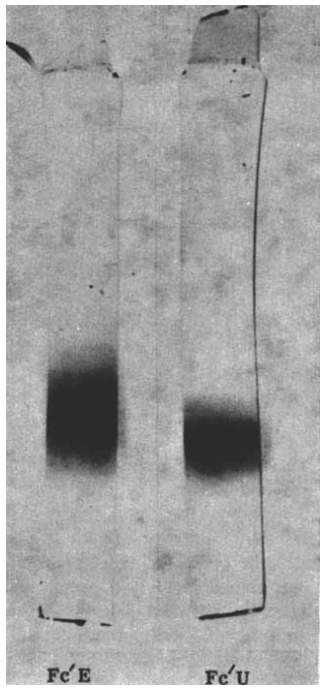

Fig. 2. Polyacrylamide disc electrophoresis of urinary Fc′ fragment (Fc′U) and enzymatic Fc′ fragment (Fc′E).

acids differ by only two residues (one residue per γ-chain) and only three amino-acids—aspartic acid, cysteine and tyrosine—show a difference greater than two residues.

Table 1. AMINO-ACID COMPOSITION OF URINARY Fc′ FRAGMENT (Fc′U) AND ENZYMATIC Fc′ FRAGMENT (Fc′E)

	Residues per 180 residues		Nearest even integer	
	Fc′U	Fc′E	Fc′U	Fc′E
Lys	12·9	11·3	12	12
His	2·2	2·8	2	2
Arg	5·6	5·6	6	6
Asp	19·1	15·8	20	16
Thr	12·1	12·2	12	12
Ser	19·7	21·9	20	22
Glu	25·4	25·0	26	26
Pro	18·1	17·5	18	18
Gly	11·4	9·2	12	10
Ala	6·3	4·0	6	4
1/2 Cys	8·6	3·6	8	4
Val	12·2	14·9	12	14
Met	1·6	2·9	2	2
Ile	2·5	2·3	2	2
Leu	11·1	13·9	12	14
Tyr	4·7	8·2	4	8
Phe	6·5	8·9	6	8
Total	180·0	180·0	180	180

Results of 24 h hydrolyses. Values for threonine and serine have been corrected by division with the factors 0·95 and 0·89, respectively. Tryptophan was not determined.

These observed amino-acid differences might be partly explained by the fact that the urinary Fc′ fragment arises from a limited number of the four IgG subclasses which are known to differ slightly in their amino-acid sequence. It seems more probable, however, that the differences were mainly caused by the presence of a small glycopeptide impurity in the urinary Fc′ preparation. This was indicated by the amino-acid analysis of the Fc′U preparation, which revealed the presence of ten residues of hexosamine per 180 residues of amino-acids (Fc′E is known to lack carbohydrate[4]). The higher cysteine content observed in Fc′U also raises the possibility that the reducing environment of urine had permitted a disulphide interchange between the two intrachain S–S bridges of the Fc′U fragment and a cysteine-rich glycopeptide. If such a reaction occurred regularly it would not normally be possible to isolate Fc′U without the glycopeptide contaminant.

Table 1 also shows that Fc′E and Fc′U have a similar histidine content. There are known to be six histidine residues in the carboxy-terminal half of the Fc region (the pFc′ fragment) but only two such residues occur in the Fc′ fragment[4]. The presence of two histidine residues in Fc′U thus not only indicates a similarity with Fc′E but also rules out the possibility that Fc′U is more closely related to the larger pFc′ fragment[4].

Although Vaughan *et al.*[2] demonstrated Fc-like material in normal urine, they did not distinguish between Fc and Fc′ fragments, both of which are now known to be present[3,14]. Bienenstock[3] used a combination of gel filtration on 'Sephadex *G*-200' and 'Biogel *P*60' to obtain fractions enriched with Fc fragment and Fc′ fragment respectively. He noted the low sedimentation coefficient of the fraction containing Fc′ fragment (1·3 *S*) compared with enzymatic Fc′ fragment (2·1 *S*) and also commented that the slopes of the boundaries suggested heterogeneity. From this study it was thus not clear whether urinary Fc′ fragment was indeed identical to the enzymatic product. The author suggested that variable Fc′ fragments might result from different sites of cleavage although each fragment would retain its fast mobility and the common Fc′ antigenic marker.

Our results show that urinary Fc′ fragment is significantly similar in amino-acid composition, sedimentation coefficient and molecular weight to enzymatic Fc′ fragment. We have not investigated the possible origin of urinary Fc′ fragment, but other work[2] seems to indicate that urinary "Fc-like fragments" derive from the catabolism of plasma IgG. Bienenstock[3] has shown that urinary enzymes *in vitro* break down Fc fragment to Fc′ fragment but are not able to degrade intact IgG. (The stability of urinary IgG has also been observed by other investigators (compare ref. 3).) In urine there may be an enzyme (or enzymes), with a specificity similar to that of papain and esterase[15], which cleaves the γ-chains of Fc fragments originating from plasma[14] near residues 14 and 105 (numbered from the C-terminus). Fc′ fragment might also be excreted into urine from plasma and be a catabolic product of IgG metabolism. If, as seems likely, the urinary Fc′ fragment arises by one or both of these catabolic processes, our data are further evidence for a region of general enzymic susceptibility near the mid-point of the Fc fragment.

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