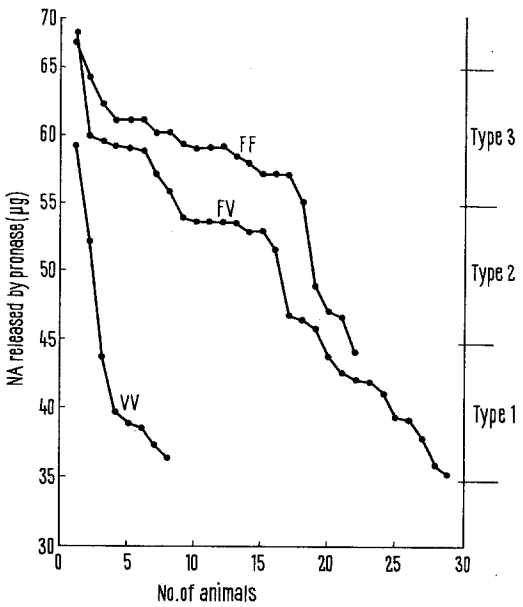
rbc, for instance cat, having the same NA containing glycolipid pattern[7,8].

3. It is known that the NA of bovine rbc consists of 2 forms, N-acetyl-NA and N-glycolyl-NA[1]; therefore a) do different amounts of these 2 NA occur in the F/F, F/V and V/V rbc, in other words: can it be that F/F rbc have only N-acetyl-NA, whereas for instance V/V have only the N-glycolyl compound? b) Do different forms of heterophile cryptantigens[3] appear in F/F and V/V cells after neuraminidase treatment? c) Is there any crossreaction between anti-F and other antibodies or heterophilic agglutinins against NA-containing receptors[5]?

The aim of this investigation was to see whether there is any relationship between the NA containing F-antigen or the F/V-system and the different quantity of the rbc glycoprotein coat, as has been stated earlier[4]. The Figure shows that the pronase-releasable amount of glycoprotein NA demonstrates no direct correlation to the F/F or V/V genetic background, although it is remarkable that F/F cells usually have a high amount of NA (Type 3), whereas in F/V and V/V rbc more examples of the thin glycoprotein layer cells do occur (Type 1). The method of this quantitative NA estimation has been described in full detail earlier[7,9]. Similar results have been obtained by using a combined method with neuraminidase and α-chymotrypsin[7,9] and measuring approximately the total NA.



Different amounts of pronase released glycoprotein NA from F/F, F/V and V/V bovine rbc. The µg NA values correspond to 0.5 ml of packed rbc, Type 1, 2 and 3 show the range of values for the 3 agglutination types according to Coombs[4], corresponding to thin, medium and thick glycoprotein coat.

From these experiments, it may be concluded that the F/V blood group system in bovine rbc obviously does not exclusively govern the expression of the outer NA glycoprotein coat of the cells, but does apparently, as previously stated[6], also influence the stroma (glycolipid?) associated NA, the more as we found some loss of NA glycoprotein during stroma preparation. Therefore the protease-released NA cannot be regarded as a suitable tool for investigating this blood group system, because no direct correlation to the F/V system could be found.

Zusammenfassung. Untersuchungen über den pronaselabilen Anteil der äusseren neuraminsäurehaltigen Glykoproteinschicht verschiedener Rindererythrozyten ergeben bei den einzelnen Tieren zwar signifikante Unterschiede, die jedoch in keiner direkten Beziehung zu den ebenfalls neuraminsäurehaltigen F-Antigenen dieser Zellen stehen.

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Correlation between Electrophoretic Mobility and Heavy Chain Sub-classes of Residual IgG from Patients with Severe Hypogammaglobulinaemia

Previous studies[1] of normal IgG by isoelectric focusing established that fractions of pH near 7.0 were rich in IgG2 and that IgG1 and IgG3 – rich fractions were obtained at pH's higher than 8.0. This result is consistent with the reported fast mobility of IgG2 monoclonal proteins in relation to proteins of other sub-classes[2,3].

In a recent study (results to be published) we have demonstrated abnormalities in the distribution of IgG heavy chain sub-classes in sera from patients with severe hypogammaglobulinaemia.

Although most residual IgGs from hypogammagloublinaemic sera show an electrophoretic mobility similar to that of normal IgG, in some instances proteins showing abnormal electrophoretic mobility have been described[4–6]. In the present study we tried to correlate the electrophoretic mobility of residual IgG with the heavy chain sub-class distribution.

Material and methods. 11 sera containing 80–120 mg/100 ml of IgG were chosen out of a series of 16, previously

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studied (results to be published). Normal human serum was obtained from healthy laboratory personnel of both sexes. Fractions obtained from normal IgG by preparative isoelectric focusing as described in detail elsewhere[1] were used as reference markers. All protein samples were diluted to contain 80–100 mg/100 ml prior to gel electrophoresis. The levels of IgG in all samples were determined by radial immunodiffusion[7].

Titration of IgG sub-classes. Antisera against the 3 main heavy chain sub-classes of IgG (IgG1, IgG2 and IgG3) were raised in sheep and rabbits according to the principles stated by GREY and KUNKEL[8] and TERRY and FAHEY[9]. Reference proteins for each sub-group were kindly supplied by Dr. H. G. KUNKEL.

The assay of the sub-groups was performed by a semi-quantitive immunodiffusion procedure. Samples to be titrated were adjusted to contain 1.0 ($\pm$ 0.2) mg/ml (starting dilution) and a series of double dilutions (up to 1/32) placed in the peripheral wells of an Ouchterlony system, in which the central well was filled with the desired anti sub-class specific serum. After 48 h at room temperature, plates were washed and stained with Amido Black (G. T. Gurr, London) and results expressed as the lowest dilution of serum at which a precipitin line was observed.

The titres obtained for each sub-class are not comparable in their absolute values, since the antibody content was different in each sub-class specific antiserum.

Estimation of electrophoretic mobilities. Immunoelectrophoresis was carried out in 1% agar gel (Oxoid Ionagar), spread on 8 × 11 cm glass plates (15 ml of agar per plate). The gel was dispersed in the chamber buffer (pH 8.6 buffer containing 0.08 *M* boric acid, 0.005 *M* barbitone, 0.025 *M* barbitone sodium and 0.02 *M* NaOH) and electrophoresis was carried out at 40 mA per plate. Control of the length and regularity of the separation was accomplished by incorporating sufficient bromophenol blue into the melted agar to obtain a pale blue agar solution. Since bromophenol blue binds to albumin, both the position of the albumin from different samples, and the path of migration formed by the stain are indicative of the regularity of the run.

Immunoelectrophoretic patterns were developed with sheep anti-Fc (IgG) serum, prepared in the Department of Experimental Pathology, The University of Birmingham. The antiserum was diluted to obtain optimum results. Plates were incubated for 24 h at 4 °C, and then washed, dried and stained with amidoblack (G.T. GURR).

We considered as the average electrophoretical mobility for a given fraction the point at which its precipitin arc met a tangent parallel to the antiserum trough. The distances between these points and the serum wells were determined for all fractions, and were expressed as a percent of a minimal possible migration of IgG obtained by measuring the distance between the serum well and the cathodical end of the precipitin arc of normal IgG.

Results. Although the method used for the determination of the electrophoretic mobilities is only approximate, it was possible to correlate the mobilities of the isoelectric focusing fractions and their respective pH's giving a linear relationship (Figure 1). A reasonable degree of reproducibility may be obtained as shown in Table I, where results from several different runs of both IgG fractions obtained by isoelectric focusing and hypogammaglobulinaemic sera are noted.

Figure 1 also shows the distribution of mobilities of IgG from the 11 hypogammaglobulinaemic sera, and the basis for the classification as fast, medium or slow. IgG's showing a more anodal electrophoretic mobility than that expected from an isoelectric focusing fraction with pH 7.5 were considered fast moving. When the electrophoretic mobility was more cathodal than that expected from an isoelectric focusing fraction of pH 8.0, IgG's were classified as slow moving. IgG's in intermediate positions were considered as of medium mobility.

In Table II we present the correlation between electrophoretic mobility and the distribution of IgG1, IgG2 and IgG3 in the 11 hypogammaglobulinaemic sera studied. Figure 2 expresses the same correlation graphically; residual IgG's containing relatively large amounts of IgG2 have a faster electrophoretic mobility than those residual IgG's in which this sub-class was not detected.

Only one case departed from the general pattern. Serum h 7 contained the residual IgG with faster mobility

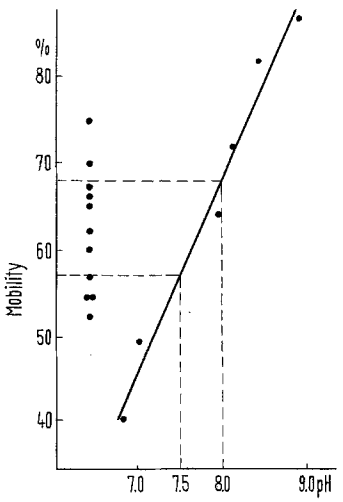

Fig. 1. Linear correlation between pH and electrophoretical mobility of isoelectric focusing fractions. Fast moving fractions of low pH correspond to low percents of the minimal possible mobility for normal IgG and vice-versa. The vertically arranged dots on the left represent the mobilities of hypogammaglobulinaemic sera's IgG's. The broken lines illustrate how those IgG were divided in fast (dots below the lower broken line), slow (dots above the upper broken line) and medium-moving (dots between the broken lines).

Table I. Electrophoretic mobilities of some isoelectric focusing fractions of normal IgG, and IgG from some hypogammaglobulinaemic sera. Results are expressed in % of a minimal possible mobility (see text), and each figure corresponds to an individual determination

Isoelectric focusing fractions				
pH	Mobility in agar gel (%)			
6.8	40.9	39.1	41.7	
7.0	43.5	50.0	52.1	
8.45	76.1	88.3		
8.95	84.8	91.7		
Hypogammaglobulinaemic sera				
Serum	Mobility in agar gel (%)			
h 6	73.9	75.8	78.1	
h 7	52.1	52.2		
h 14	60.9	62.1	62.5	65.6
h 15	52.2	56.2	58.2	
h 16	62.5	65.2	65.5	68.8
h 4	63.0	65.5	70.3	

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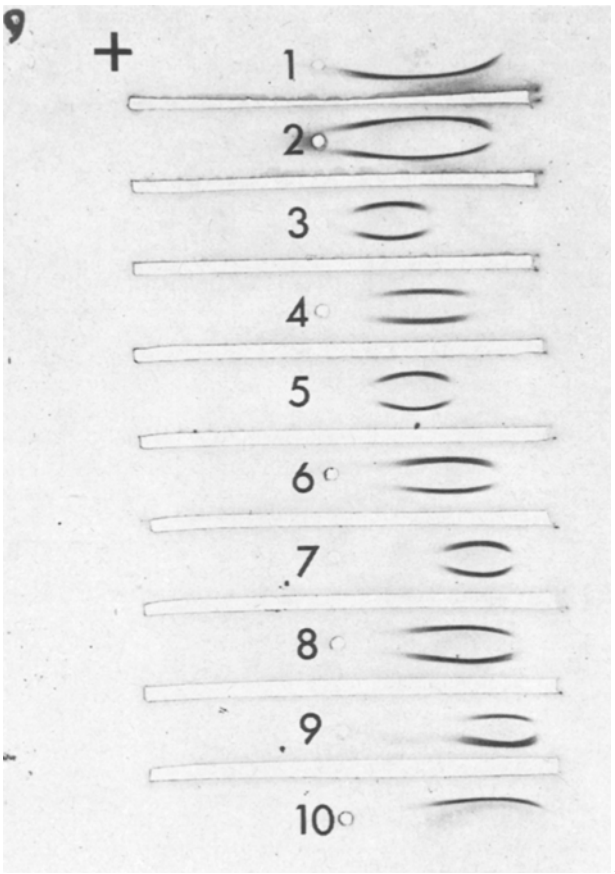

Fig. 2. Comparison of the electrophoretical mobilities of some hypogammaglobulinaemic sera and some isoelectric focusing fractions of IgG. The mobilities for each serum and IgG fraction, in this particular study, are given in bracketts. (1) Normal IgG; (2) serum h 5 (54%); (3) IgG fraction, ph 6.8 (40.9%); (4) serum h 15 (52%); (5) IgG fraction pH 7.0 (43.5%); (6) serum h 4 (63%); (7) IgG fraction pH 8.45 (76.1%); (8) serum h 10 (70%); (9) IgG fraction pH 8.95 (84.8%); (10) serum h 6 (74%). Anti Fc (IgG) serum was used to fill the troughs.

but had less IgG2 than expected. Additional studies proved that residual IgG had 'spontaneously' split into Fab and Fc-like pieces, and the recorded mobility corresponded to the Fc fragment.

Discussion and conclusions. Our results seem to prove that in hypogammaglobulinaemia, the abnormal electrophoretic mobilities of the residual IgG (previously reported by GOLEBIOWSKA and ROWE[6]) depend in the majority of cases on the distribution of IgG sub-classes.

Large amounts of IgG2, and possibly IgG4, due to the fast electrophoretic mobilities of these sub-classes[1,2,9] will lead to an overall fast mobility of the residual IgG. In the absence of IgG2, the mobility will reflect that of IgG1 and IgG3, both slow moving sub-classes[1,9].

Table II. Correlation between electrophoretical mobility and IgG sub-class distribution in 11 hypogammaglobulinaemic sera

	Titres Serum	Mobility	IgG1	IgG2	IgG3
Fast	h 7	52%	1/16	1/2	0
	h 5	54%	1/16	1/16	0
	h 9	54%	1/8	1/16	0
	h 15	57%	1/8	1/16	0
Medium	h 13	60%	1/16	1/8	1/4
	h 14	62%	1/32	1/8	0
	h 16	65%	1/32	1/16	1/4
	h 4	66%	1/6	1/4	1/2
	h 8	67%	1/8	1/4	1/2
Slow	h 10	70%	1/16	1/1	1/4
	h 6	75%	1/16	0	1/1
Normal IgG (Start. Dil. 1 mg/ml)			1/16	1/8	1/1

Summary. The correlation between the electrophoretic mobility of residual IgG in hypogammaglobulinaemic sera and the distribution of IgG sub-classes was studied. It was found that IgG from sera with raised levels of IgG2 show fast electrophoretic mobility, and sera with very low or absent IgG2 show a slow moving IgG. IgG3 was absent or just detectable in fast moving IgGs, and present in higher titers in some slow IgGs.

Résumé. La correlation entre la mobilité electrophorétique de l'IgG résiduelle des sérums hypogammaglobulinémiques et la distribution des sous-classes de l'IgG est l'objet de cet étude. Les IgG résiduelles de mobilité rapide ont des titres élevés d'IgG2 et celles de mobilité lente ne présentent que des traces d'IgG2.

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Toxicity of Rabbit Anti-Dog Lymphocyte Serum to Human Cell Cultures

In previous work[1], rabbit anti-dog lymphocyte serum plus rabbit complement was found to have a rapid cytocidal effect on blood lymphocytes of patients with chronic lymphocytic leukemia but had little or no effect on normal human lymphocytes. In this study, the antiserum was tested against cells in long term cultures which had been derived from blood of normal persons and of patients with acute leukemia and from BURKITT's lymphoma. The method of cell culture is described by MOORE et al.[2].

The cells suspended in RPMI 1640 were incubated for 90 min at 37 °C with fresh rabbit serum (10%) and with a rabbit anti-dog lymphocyte serum in varying dilutions. The number of viable cells was counted by means of a