The amount of protein that was required to prevent the agglutination of the cells depended upon the sol that was used. If a very strong one was employed, human serum diluted 1 : 4 would not prevent the agglutination of washed red cells.

Since it appears that silica (presumably in colloidal solution) can be derived from glass by treatment with water, particularly at a high temperature or at alkaline *p*H, it seemed that a silica sol might be formed under the conditions previously described, and it was concluded that false positive results might be caused in the antiglobulin test and in other serological techniques by the presence of silica sol in the crystalloid solutions that had been prepared for these tests. The physical aspect of this phenomenon is complicated and it is not proposed to discuss it here.

From a practical point of view, therefore, crystalloid solutions which are to be used for the washing of red cells should not be prepared by autoclaving in glass bottles, nor should they be frozen in glass bottles; but if they have to be heated they should be heated in plastic bags, or sterilization achieved by Seitz filtration; alternatively, solutions may be made up freshly without sterilization or freezing. The ease with which citrate solutions become infected makes it desirable, however, that they should be sterilized by some means; and we, ourselves, use Seitz filtration, making up small quantities which are used only once.

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The N-Terminal Amino-Acid of Rabbit Gamma-Globulin and Antibody

Porter[1] found that both the anti-ovalbumin antibody and the non-specific γ-globulin of rabbit serum contained the same single N-terminal amino-acid, namely, alanine. Hughes and Sinex[2] consider this "amazing"; and seeing that the serum component called γ-globulin is a family of proteins that includes diverse antibodies and possibly protein with other functions, and has been shown to be heterogeneous by various physico-chemical criteria, this result is certainly not inevitable.

The N-terminal amino-acid of another rabbit antibody—that against bovine serum albumin—has now been determined together with the end-group of 'inert' rabbit γ-globulin. The method used was the fluorodinitrobenzene method of Sanger[3] with paper chromatography (phthalate *tert.*-amyl alcohol) for identification of the dinitrophenyl amino-acids.

The specific precipitate was found to possess one N-terminal alanine per molecule of 156,000. Aspartic acid, corresponding to one residue per molecule of the bovine serum albumin (mol. weight 69,000) in the precipitate, was also recovered. The non-specific γ-globulin yielded 0·91 mol. of alanine/molecule of 156,000 molecular weight; aspartic acid (corresponding to 0·2/mol.) was also found. Even though no serum albumin could be detected in the original γ-globulin preparation by paper electrophoresis, it is possible that this aspartic acid came from contamination with rabbit serum albumin, in quantities too small to be detected by electrophoresis, that is, less than 5 per cent. Less than 5 per cent of serum albumin in the γ-globulin preparation would suffice to account for the aspartic acid found.

Table 1

γ-Globulin	Asp.	Glu.	Ser.	Ala.	Val.	Thr.	Leu. Ileu.
Human II—1, 2 (ref. 5)	1·06	1·82	0·10				
Human II—3 (ref. 5)	1·01	1·06	0·17				
Human II (ref. 4)	1·0	1·0	0·1–0·2				
Human myeloma (ref. 4)	2·0						
Bovine (ref. 5)	0·13	0·15	0·09	0·09	0·11		
Horse (ref. 5)	+	+	+			+	+
Rabbit non-specific	0·2			1·0 (ref. 1) 0·91			
Rabbit, anti-Ea (ref. 1)				1·0			
Rabbit, anti-bovine serum albumin				1·0			

These findings confirm Porter's. The γ-globulins of all other species investigated so far, differ from that of the rabbit in having more than one terminal amino-acid per molecule and appearing to be more heterogeneous, in that different fractions of the same γ-globulin have different end-groups. In addition to the species listed in Table 1, Porter[6] found that pig γ-globulin contains six free α-amino groups. Smith[7] reports an increase of N-terminal valine in the 'immune globulins' of cattle; these immune globulins, however, are merely fractions containing unknown amounts of antibody, whereas the specific precipitates used by Porter and myself for antibody end-group determination were 90 per cent antibody.

Since antibodies against two different antigens and 'non-specific' γ-globulins (which may include a variety of antibodies) have the same single terminal amino-acid (in the rabbit), it is improbable that this part of the molecule is involved in either of the specific combining groups of the antibody molecule.

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Cup-plate Assay of Serum Fibrinolysin

In recent years, attention has been given to the quantitative estimation of serum fibrinolysin. Study of the plate test of Permin[1] indicated that some modification of this method was necessary to estimate numerous unknown enzyme samples. The cup-plate assay method described by Dingle, Reid and Solomons[2] seemed suitable, since it was designed to estimate large numbers of samples over a wide range of enzyme concentrations. We have found that a combination of the principles of these two methods is applicable to the measurement of serum fibrinolysis.