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THE INTERACTION BETWEEN SODIUM DODECYLSULPHATE SOLUBILIZED HUMAN GHOSTS AND ANTISERA TO HUMAN SERUM LIPOPROTEINS: A NON-IMMUNE PRECIPITATION EFFECT OF SODIUM DODECYLSULPHATE

JONATHAN R. GREEN [a], MICHAEL J. DUNN [b] and ALUN H. MADDY [b]

[a] *Department of Immunology, A.R.C. Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ, and* [b] *Department of Zoology, University of Edinburgh, Edinburgh EH9 3JT, Scotland (U.K.)*

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Summary

It has been demonstrated that the presence of sodium dodecylsulphate in gel double diffusion plates can give rise to non-immune precipitin lines. Consequently immunological data obtained in the presence of the detergent cannot be interpreted unequivocally.

A recent paper reporting serum lipoprotein apoproteins as major protein constituents of the human erythrocyte membrane [1] has aroused considerable interest, not only because of its immediate relevance to membrane structure and synthesis but also because of its possible significance in vascular disease. We have performed immunological experiments similar to those of Langdon and confirm his major observations, but further investigation has produced results which do not support his conclusions. We have used the Crowle modification of the Ouchterlony double diffusion test to study the precipitation reaction between sodium dodecylsulphate solubilized proteins and antisera against human serum lipoproteins. The precipitation reaction observed between sodium dodecylsulphate solubilized human ghosts and antisera to human serum α- and β-lipoproteins appears to be the result of a non-immune precipitation effect of sodium dodecylsulphate. Using antisera against human serum lipoproteins it is possible to demonstrate an apparent 'line of identity' between ghosts dissolved in 0.1% sodium dodecylsulphate and several unrelated sodium dodecylsulphate solubilized proteins of plant, bacterial and fungal origin. Precipitin-like lines have also been observed between non-immunized rabbit sera and a solution of 0.1% sodium dodecylsulphate alone.

Human ghosts were prepared and extracted with 0.5 mM EDTA, pH 7.5, as previously described [2]. Human serum α-lipoprotein and β-lipoprotein were donated by Dr D. Pepper, Blood Transfusion Service, Edinburgh. *Escherichia coli* ribosomes were prepared by the method of Munro et al. [3]. Sodium dodecylsulphate solutions of human ghosts, EDTA extract of ghosts, α-lipoprotein, β-lipoprotein, *Escherichia coli* ribosomes, barley β-amylase (Sigma Chemical Co. Ltd) and *Schizosaccharomyces pombe* were prepared by making an initial solution in 2% sodium dodecylsulphate followed by dialysis for 36 h against a solution of 0.1% sodium dodecylsulphate.

Antisera were either purchased or raised in rabbits in our own laboratories as follows. Goat antisera to human serum α-lipoprotein and β-lipoprotein were obtained from Miles Laboratories Ltd. Rabbit antisera to human α-lipoprotein and β-lipoprotein were obtained from Behringwerke A.G. We produced our own rabbit antisera to human ghosts, EDTA extract of ghosts, serum α-lipoprotein and serum β-lipoprotein by intramuscular injection in Freund's complete adjuvant followed by a subcutaneous injection of Pertussis vaccine (Burroughs Wellcome Ltd). Antisera specificity was examined by immunoelectrophoresis against normal human serum, human serum α-lipoprotein and β-lipoprotein. Gel double-diffusion was performed by the Crowle modification [4] of the Ouchterlony technique using 1% agar in 0.15 M NaCl, 5 mM phosphate pH 7.4, with 0.1% sodium azide. Since the commercial plates used by Langdon are unavailable to us, we were unable to repeat precisely his original experiments but we have no reason to believe that our results are in any way a peculiarity of the Crowle method.

Analysis of the antisera by immunoelectrophoresis confirmed the monospecificity of all the commercial antisera and that of our own against β-lipoprotein. The antiserum produced in our own laboratories against α-lipoprotein reacted weakly with several serum components and absorption with a small

TABLE I

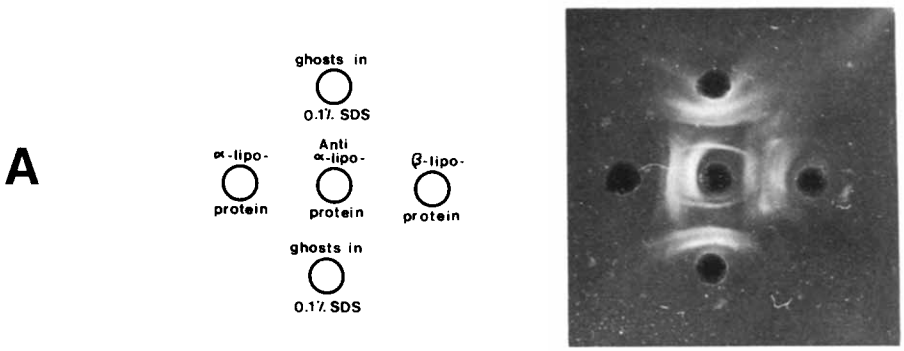
SUMMARY OF THE PRECIPITIN REACTIONS OBSERVED BETWEEN ANTISERA TO SERUM LIPOPROTEINS, GHOSTS, AND EDTA EXTRACT OF GHOSTS REACTING WITH RELATED PROTEINS IN THE PRESENCE AND ABSENCE OF 0.1% SODIUM DODECYLSULPHATE (SDS)

Test Protein	Antisera raised against:				Normal sera from non-immunized rabbits
	α-Lipoprotein	β-Lipoprotein	Ghosts	EDTA extract	
Human serum	+	+	—	—	—
α-Lipoprotein	+	+	—	—	—
α-Lipoprotein in SDS	+	+	+	+	+
β-Lipoprotein	+	+	—	—	—
β-Lipoprotein in SDS	+	+	+	+	+
Ghosts in SDS	+	+	+	+	+
EDTA extract	—	—	+	+	—
EDTA extract in SDS	+	+	+	+	+

+ = precipitin lines observed.
— = precipitin lines not observed.

amount of human serum was necessary to render it monospecific. However, when tested by double diffusion all the antisera to lipoproteins had minor cross-reacting components. No reaction could be detected between human serum and the antisera raised against ghosts or EDTA extract. Table I summarises the precipitin reactions observed between the antisera and various

related protein solutions. It will be seen that cross-reactions between lipoproteins and ghosts are only observed when sodium dodecylsulphate is present in one of the reactants. Although anti-lipoprotein sera react with ghosts dissolved in sodium dodecylsulphate as reported by Langdon and repeated here (Fig. 1A), antisera to ghosts or EDTA extract do not react with lipoproteins



Figs 1A—1D. The effects of sodium dodecylsulphate on the precipitin lines formed in gel double diffusion plates. The double diffusion test was performed by the Crowle modification of the Ouchterlony technique; 1% agar in 0.15 M NaCl, 5 mM phosphate buffer (pH 7.4) with 0.1% sodium azide; gel thickness 0.36 mm: separation between central and peripheral wells 5 mm: plates incubated for 3 days at 20°C: 20 μl of protein were used in all wells except those indicated in Fig. 1D.

unless sodium dodecylsulphate is present. The following arguments and further evidence lead us to the conclusion that the "cross-reaction" between ghosts and lipoproteins may not indicate any immunological relationship between the proteins of the membrane and serum lipoproteins but is an artefact caused by the presence of sodium dodecylsulphate.

(i) The failure of antisera against ghosts or EDTA extract to react with lipoproteins in the absence of sodium dodecylsulphate is not due to the inability of the lipoproteins to diffuse in the agar because normal precipitin lines were formed between lipoproteins and anti-lipoprotein sera in the absence of detergent.

(ii) It is conceivable that the cross-reaction can only be detected in the presence of sodium dodecylsulphate because the common determinants are only exposed after detergent treatment. However, the reaction of all the antisera with sodium dodecylsulphate solutions of β-amylase, *E. coli* ribosomes

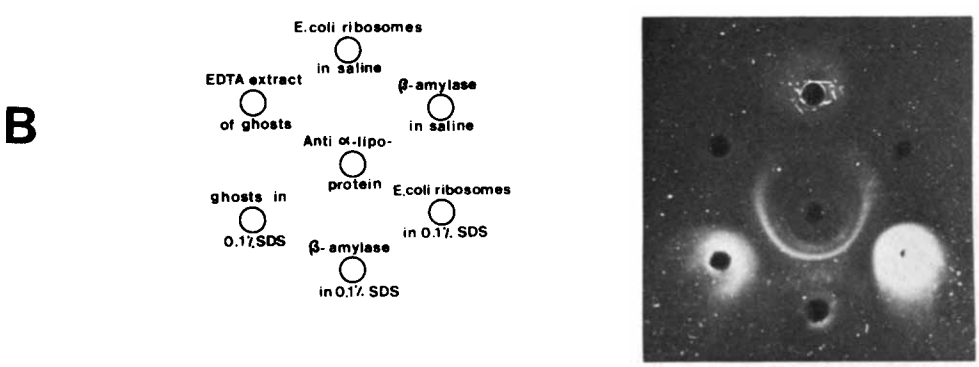


Fig. 1B. See Fig. 1A for legend.

and *S. pombe* (Table II), even showing 'lines of identity' (Fig. 1B), reduces this possibility to the point where such cross-reactions become meaningless.

TABLE II

SUMMARY OF THE PRECIPITIN REACTIONS OBSERVED BETWEEN ANTISERA AGAINST SERUM LIPOPROTEINS, GHOSTS AND EDTA EXTRACT OF GHOSTS REACTING WITH UNRELATED PROTEINS DISPERSED IN SALINE OR 0.1% SODIUM DODECYLSULPHATE (SDS)

Test Protein	Antisera raised against:				Normal sera from non-immunized rabbits
	α-Lipoprotein	β-Lipoprotein	Ghosts	EDTA extract	
β-Amylase in saline	—	—	—	—	—
β-Amylase in SDS	+	+	+	+	+
E. coli ribosomes in saline	—	—	—	—	—
E coli ribosomes in SDS	+	+	+	+	+
S. pombe in saline	—	—	—	—	—
S. pombe in SDS	+	+	+	+	+
0.1 % SDS alone	+	+	+	+	+

+ = precipitin lines observed.
— = precipitin lines not observed.

(iii) The absence of any interaction between sera from non-immunized rabbits (the normal rabbit sera of Figs 1C and 1D) and sodium dodecylsulphate solubilized ghosts is a crucial element in Langdon's argument. We have regularly observed that a solution of sodium dodecylsulphate solubilized ghosts or even 0.1% sodium dodecylsulphate alone will form precipitin lines with all the antisera and sera from non-immunized rabbits (Figs 1C and 1D).

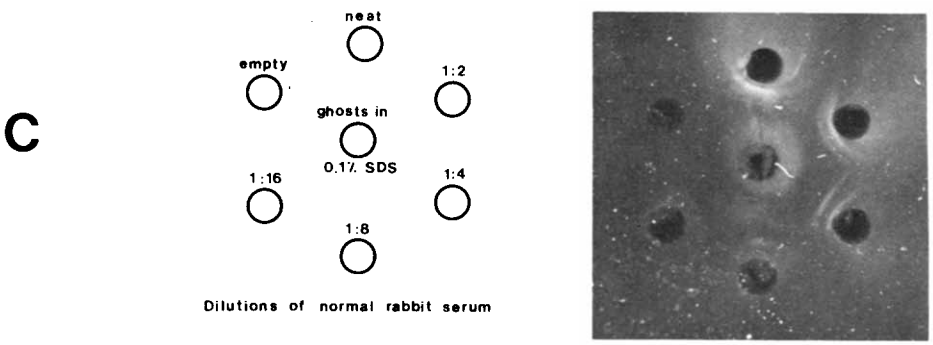


Fig. 1C. See Fig. 1A for legend.

Our results do not necessarily contradict Langdon's observation, for this reaction is only apparent when optimal amounts of detergent and serum protein diffuse towards each other. It will also be noted that the lines only form when sodium dodecylsulphate diffuses towards a protein · · hence Langdon's observation that precipitin lines did not form complete circles around peripheral wells.

Although there are several other examples in the literature of non-immune precipitation reactions occurring in gel double diffusion tests [5—8] we have been unable to trace any reference which adequately explains the non-immune precipitation effect of sodium dodecylsulphate. Formation of the insoluble potassium salt of sodium dodecylsulphate might be predicted as a possible artefact but we were unable to demonstrate any such precipitate in agar

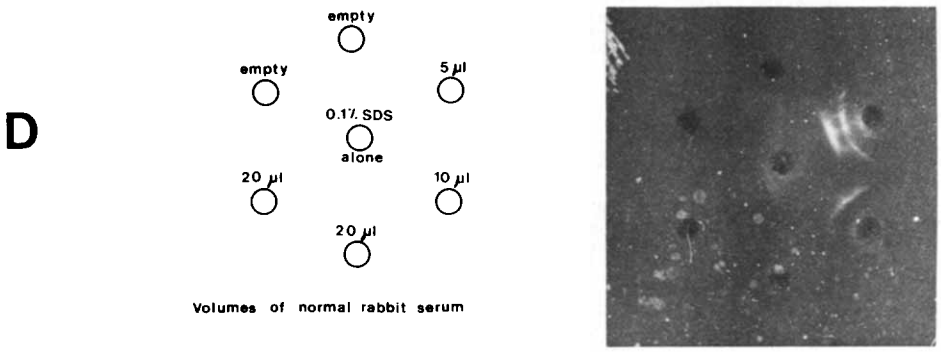


Fig. 1D. See Fig. 1A for legend.

between solutions of sodium dodecylsulphate and a solution containing potassium ions at a concentration within the normal serum range. The results are not changed by the addition of 100 mM phosphate buffer, pH 7.2, to the samples or replacement of the phosphate buffer in the agar with borate, pH 8.5, as is used in the commercial plates obtained from Miles Laboratories.

It would be remarkable if sodium dodecylsulphate does not cause artefacts in the precipitin reaction. If, as Reynolds and Tanford suggest [9] the detergent encases the polypeptide chain, the layer of detergent would be expected to mask the natural antigenicity of the protein: if the detergent diffuses away from the protein the protein's innate insolubility would cause its precipitation: if the detergent remained associated with the protein without masking the natural antigens it would be expected to solubilize the antigen-antibody complex.

In view of these findings the apparent precipitin reaction between sodium dodecylsulphate solubilized ghosts and anti-lipoprotein sera cannot be accepted as evidence for the presence of serum lipoprotein apoprotein in human ghost membranes unless the possibility of artefacts such as we have described above is positively eliminated. The possibility of lipoprotein apoprotein being incorporated into the erythrocyte membrane remains open and, although we do not call into dispute Langdon's chemical evidence for homology, we conclude that the immunological evidence requires careful re-examination.

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