patibility problems, but also to the secondary effects of excessive iron storage. In general, blood should be used sparingly, with the object of allowing other therapeutic measures to have their effect, or to counter acute haemorrhage or permit surgery.

Conclusions

Anaemia is a common accompaniment to the reticuloses, and, according to its severity and rate of development, it may be well or poorly tolerated, often proving a significant factor contributing to a fatal outcome. It arises from a variety of causes: in a few cases it is truly incidental, while in others it arises as a sequel to recognizable disorders resulting from the primary disease, such as haemorrhage or gastrointestinal infiltration. Least understood are the intrinsic anaemias, the pathogenesis of which remains largely obscure. These may be due to increased red-cell destruction, to marrow depression, or, perhaps most commonly, to a combination of both, and there is evidence that more than one mechanism may be responsible for each of these factors.

Apart from infections and the effects of tissue infiltration, anaemia is the commonest terminal condition encountered, and it is clear that its careful management can contribute much to the life and comfort of patients with these disorders, for which as yet there is no definitive treatment to be offered. The assessment of the individual case can be assisted by tracer procedures where these are practicable, and may suggest the most useful course to be followed within the relatively limited range of therapy at present available. As with other aspects of the treatment of these cases, the results of treating the anaemia are at best essentially temporary and the degree of improvement is often limited. Nevertheless, this constitutes an important facet of the total management of the patient with a reticulosis for which at the present time a more fundamental approach is not possible.

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ISOLATION OF ANTIBODY-LIKE GAMMA-GLOBULIN FROM LUPUS GLOMERULI

BY

P. FREEDMAN, M.D., M.R.C.P.
St. George's Hospital, London

AND

A. S. MARKOWITZ, Ph.D.
Hektoen Institute for Medical Research, Chicago, U.S.A.

Gamma-globulin and complement have been demonstrated in the renal lesions of glomerulonephritis and systemic lupus (Freedman, 1960; Freedman and Markowitz, 1962). This report deals with an immunological property of globulins, eluted from a glomerulonephritic and a lupus kidney, which in the case of the lupus eluate manifested activity strongly suggesting that it was an antibody. A preliminary report of these experiments has already been made (Freedman and Markowitz, 1959).

Materials and Methods

Three kidneys were used: one normal (free from renal disease), one glomerulonephritic, and one from a patient with lupus nephritis. The kidneys were obtained within a few hours of death, packed in cold physiological saline, and stored at −20° C. until processed. By both clinical and histological data the designated category assigned to each kidney was felt to be correct.

Isolation and Elution of Glomeruli.—The glomeruli from approximately one whole kidney from each of the three necropsies were isolated by Greenspon's method (Greenspon and Krakower, 1950). The isolated glomeruli were suspended in 0.02 M citrate buffer, *p*H 3.2, and gently agitated by means of a magnetic stirrer for two hours at room temperature. At the end of this period the glomeruli were removed by cold centrifugation and the three eluates were neutralized. Evaluation of these eluates was conducted by filter-paper electrophoresis as well as immunologically with a rabbit antihuman gamma-globulin serum.

Demonstration of Biological Activity of Eluates.—An immunofluorescent procedure (Freedman *et al.*, 1960) was employed to demonstrate tissue affinity between the eluates containing presumed gamma-globulin and both kidney and liver tissue. Frozen sections of tissue were stained for gamma-globulin by means of a fluorescein conjugated anti-human gamma-globulin serum. Companion sections were covered with eluate at room temperature for varying periods, each preparation being covered with a Petri dish containing moistened filter-paper to prevent drying of the tissue. In practice the length of the reaction time was limited by degeneration of the tissue architecture. The time generally used was four hours. After the appropriate interval the sections were repeatedly washed in buffered saline and stained for gamma-globulin with the fluorescent conjugate. Any gamma-globulin take-up by the tissues would be identified by the increased fluorescence. In addition, frozen sections from the parent kidney were first eluted in the citric acid buffer solution for 90 minutes prior to treatment with the glomerular eluate, lest all the available antigenic sites be occluded with antibody deposited *in vivo*.

Results

In order to rule out any possibility of the eluates being composed of non-specifically adsorbed gamma-globulin, the freshly isolated glomeruli were suspended in buffered saline, *p*H 7.2, for a period of several hours, and timed aliquots of the suspending fluid were immunologically evaluated by a precipitin test. No gamma-globulin was found from the three specimens under such conditions. However, when the isolated glomeruli were subjected to the acid-elution procedure, strongly positive precipitin tests were obtained on the eluates from the lupus and glomerulonephritic glomeruli, which were confirmed electrophoretically as gamma-globulin. Similarly, precipitin tests for albumin were negative on the three eluates.

Biological Activity of Eluates against Kidney Tissue

The eluate from the suspension of normal glomeruli, containing no serologically detectable gamma-globulin, was without effect when tested against normal kidney. Prior evaluation by the immunofluorescent procedure had demonstrated no globulin in the glomeruli of the parent kidney.

The glomerulonephritic eluate was also without apparent effect when tested against normal kidney sections as well as sections of kidney from which the glomeruli had been obtained. This negative finding occurred in both the uneluted and the eluted sections. The glomerulonephritic kidney when examined immunohistologically displayed well-marked specific fluorescence indicative of gamma-globulin throughout the glomerular capillary wall. The glomerular crescents, in contrast to the capillary tufts, were non-fluorescent.

The eluate derived from the lupus glomeruli, when layered on sections of normal kidney and also eluted sections of lupus kidney, appeared in some to increase the specific fluorescence for gamma-globulin in the glomerular capillary wall and in Bowman's capsule, but these changes were not constantly found. The lupus kidney had been studied previously by biopsy (Freedman *et al.*, 1960, SLE Case No. 8B). Examination of the frozen sections shortly after death confirmed the previous finding of glomerular-localized gamma-globulin.

In view of previous studies, which had suggested that complement played a significant part in the pathogenesis of glomerulonephritis (Peters and Freedman, 1959; Lange *et al.*, 1960) the foregoing experiments with the eluates were repeated, this time with the addition of a drop of fresh normal human serum to each preparation. This in no way altered the lack of biological activity of the eluate from the normal glomeruli. Also the glomerulonephritic eluate could not be induced to attach to any part of the kidney tissue from either normal or its own eluted kidney.

In the case of the lupus eluate, however, a marked difference in biological activity was demonstrated in the presence of fresh normal human serum. Under these circumstances a marked affinity of the gamma-globulin of the lupus eluate for all cell nuclei became apparent. Intense specific nuclear fluorescence was visible, indicative of gamma-globulin, in the nuclei of the glomerulus, tubules, and apparently in the interstitial tissue in sections of normal kidney. This contrasted sharply with the absence of nuclear fluorescence when the eluate alone was added to the sections. When the lupus eluate was added to eluted sections of the parent kidney in the presence of fresh normal serum, nuclear fluorescence was again visible, although this tended to be less intense than the fluorescence seen in normal-kidney tissue. There also appeared to be some increase in specific fluorescence in the glomerular capillary wall, when compared with control eluted sections stained with the anti-gamma-globulin conjugate.

Biological Activity of Eluates against Liver Tissue

The biological activity of the three eluates was also tested against sections of liver tissue. This was obtained from a patient with chronic hepatitis. Histology of the liver had shown diffuse fibrosis with focal necrosis and bile staining. Control studies of frozen sections revealed no gamma-globulin in the tissue. Layering on the normal fresh sera used in these studies did not induce any gamma-globulin take-up by the tissues. The normal eluate and the glomerulonephritic eluate, both alone and together with normal fresh serum, had no demonstrable activity. The lupus eluate applied alone led to minimal fluorescence of some nuclei. Although this was of low order, it did appear to represent some change from the control sections. The addition of normal fresh serum to the lupus eluate produced intense specific fluorescence of nuclei reminiscent of the result obtained with kidney sections.

Characterization of Serum Component Facilitating the Attachment of Lupus Gamma-globulin to Tissue

A wide variety of normal fresh sera were used to demonstrate this activity. Since sections of tissue vary slightly, no very close comparison was possible between the capacities of the different sera to enhance the attachment of the lupus gamma-globulin. Within the limitations of the fluorescent antibody technique, no significant differences in activity were observed between any of the normal sera employed.

Effect of Heat.—The effect of heating the fresh normal serum to 60° C. for three minutes, a procedure known to inactivate serum complement, was tried. It was found that this preliminary heating of the normal serum effectively prevented the serum from facilitating the attachment of the lupus globulin to kidney tissue.

The result observed after adding the heated normal serum to the lupus eluate covering the kidney tissue was the same as that observed by adding the eluate alone.

Guinea-pig Complement.—A single experiment was conducted in which guinea-pig complement, used as a source of complement for the haemolytic system in the Wassermann reaction, was added to the lupus eluate on kidney tissue. No nuclear fluorescence was observed and the result was comparable with the experiment in which lupus eluate alone or lupus eluate plus heat-inactivated normal fresh human serum was employed.

Normal Gamma-globulin Control.—Some control studies were carried out with concentrated pooled normal gamma-globulin on kidney tissue. With the most concentrated solution available (containing 165 ± 15 mg./ml.), which was thick and viscous, some attachment of gamma-globulin to the tissue occurred, but this was apparent over the whole section, including the nuclei, and no localized specific fluorescence was observed. There appeared to be no change in the tissue fluorescence for gamma-globulin when this concentrated solution was layered over the sections in the presence of normal fresh human serum. When this concentrated gamma-globulin solution was diluted 1:10, which was still very considerably more concentrated than the kidney eluates described previously, and then applied to kidney sections, a much lower general increase in fluorescence was observed, and this also was not influenced by the presence of normal fresh serum.

Complement Studies

A detailed account of the preparation and properties of an anti-human complement serum and its use in the study of renal disease is described elsewhere (Freedman and Markowitz, 1962). Towards the end of the investigations here described an anti-human complement serum was prepared and was used in a small number of experiments on kidney tissue. The anti-complement serum was employed both by the direct technique, conjugating the gamma-globulin fraction with fluorescein isothiocyanate, and by the more sensitive indirect technique. In the latter instance, since the antiserum was prepared in the rabbit, the fluorescent marker used was sheep anti-rabbit gamma-globulin conjugated with fluorescein isothiocyanate. The specificity tests were as described elsewhere (Freedman and Markowitz, 1962).

The results of this group of experiments may be briefly summarized as follows. No specific fluorescence indicative of human complement was found in the normal kidney sections alone, nor after they had been in contact with normal fresh human serum, normal pooled gamma-globulin solutions, or the various eluates alone. With the lupus eluate plus fresh normal human serum, specific fluorescence was observed in cell nuclei generally. No other specific fluorescence was seen. No specific fluorescence was observed in the tissue with this conjugate when the sections were covered with the normal or glomerulonephritic eluate plus normal serum.

Discussion

The results described follow a sequence of observations which began with the early studies on the production of nephrotoxic nephritis some 60 years ago (Lindemann, 1900). This was the first experimental demonstration of the production of glomerulonephritis by immunological means. Since then glomerulonephritis has been studied in diverse ways. A unifying concept, at least in some of these experimental diseases, was afforded by the fluorescent antibody studies of Mellors *et al.* (1955) and of Ortega and Mellors (1956) in which the animal's own gamma-globulin was localized in the glomerular lesions produced after an interval both by anti-kidney serum produced in another animal and by foreign protein. The subsequent antibody response to this foreign protein led to the interaction of antigen and antibody in the glomerular capillary wall with resulting tissue damage. The nephrotoxic antibody also produced tissue damage directly by combining with the glomerular basement membrane, the degree of damage being related to the potency of the antiserum. There is evidence suggesting that it also persisted and reacted later as a foreign protein (Ortega and Mellors, 1956).

More recent studies, at first on necropsy material (Mellors and Ortega, 1956; Mellors *et al.*, 1957) and later on renal biopsy tissue (Taft *et al.*, 1958; Freedman *et al.*, 1960), have confirmed that gamma-globulin is present in a high proportion of the renal lesions in glomerulonephritis and in some, at least, of the collagen diseases. Theoretical arguments were advanced in favour of this gamma-globulin representing antibody (Peters and Freedman, 1959).

Further experimental studies on the human kidney have supported this concept. By analogy with the behaviour of other antigen-antibody complexes, many of which are known to dissociate when the *p*H of the medium is lowered to 3.2–3.5, frozen sections of kidney tissue were so treated and dissociation of gamma-globulin was demonstrated (Freedman and Markowitz, 1959; Freedman *et al.*, 1960). Later studies have suggested that complement is present together with the gamma-globulin in the renal lesions in glomerulonephritis and in disseminated lupus erythematosus (Freedman, 1960; Freedman and Markowitz, 1962).

The studies here described provide further data for the characterization of the tissue-bound gamma-globulin in this group of diseases. Glomeruli were isolated from normal, glomerulonephritic, and lupus kidneys. Gamma-globulin was dissociated from the glomerulonephritic and from the lupus glomeruli and not from the normal glomeruli. Dissociation, although readily demonstrable at *p*H 3.2, was not apparent at *p*H 7.

The demonstration of biological activity of this dissociated gamma-globulin is of great interest, both in assessing the role of the globulin and in considering the pathogenesis of this group of diseases. Certain factors militate against such an *in-vitro* demonstration. Under the conditions of the experiments here described an unknown degree of damage is likely to have been incurred by the reacting proteins during the elution procedures. Also, the conditions provided for the recombination of these possibly damaged proteins *in vitro* were by comparison with the situation *in vivo* both crude and highly artificial. Despite these obvious disadvantages, a consistent antinuclear activity was demonstrated for the gamma-globulin eluted from the lupus glomeruli. This activity corresponds closely with that of the gamma-globulin present in the serum of patients with disseminated lupus erythematosus (Friou *et al.*, 1957). Also the experiments with the eluted gamma-globulin, normal fresh serum, and anti-human complement serum suggested that complement was also bound by the gamma-globulin and the tissue nuclei. This would further support the concept that the gamma-

globulin dissociated from the lupus glomeruli was acting as an antibody.

The failure to demonstrate biological activity of an antibody nature in the case of the gamma-globulin from glomerulonephritic glomeruli was an interesting feature of these studies. The glomerulonephritic and lupus kidneys from which the glomeruli were isolated shared several common features. Both were found to contain glomerular-bound gamma-globulin, and in both instances complement was also present at the sites of the lesions (Freedman, 1960; Freedman and Markowitz, 1962). Gamma-globulin was dissociated with equal facility from both glomerular suspensions at *p*H 3.2; in fact, more gamma-globulin was eluted from the glomerulonephritic glomeruli. An important difference was the presence of a circulating antibody in lupus, which contrasted with the failure to demonstrate such an antibody in glomerulonephritis at any stage of the disease, using the existing techniques. It may well be that an antibody directed against the glomerular basement membrane, either in its normal state or in some way altered as a result of disease, is more sensitive to damage during the elution procedures, or that the antigenic groups on the basement membrane of the diseased kidney itself are in some way altered by the elution technique employed.

Another variable and possibly significant factor concerns the actual *in-vitro* conditions which were provided for recombination to occur. Chance experimentation in the case of the lupus glomerular eluate established conditions favourable for the attachment of the globulin to tissue nuclei. It would be equally possible that different conditions need to be established for the *in-vitro* demonstration of biological activity of the glomerulonephritic gamma-globulin.

Since the glomerular suspensions employed were obtained from only one kidney in each instance, the general applicability of these findings may well be questioned. This is a valid criticism, especially with regard to the negative findings with the glomerulonephritic gamma-globulin. The general problem of the pathogenesis of glomerulonephritis has been discussed elsewhere (Peters and Freedman, 1959), and at the present time there would seem to be evidence to suggest that the glomerulonephritic reaction may be produced by a variety of antigenic stimuli, most of which are at present unrecognized. Similar considerations may apply to disseminated lupus erythematosus, although patients with this disease process share many more clinical and pathological features than do patients with the diverse forms of glomerulonephritis. Nevertheless, there remains the distinct possibility that disseminated lupus erythematosus represents a heterogeneous mixture of pathogenic mechanisms. In this case the general concept of an immunological mechanism would remain unchallenged in view of the common findings of gamma-globulin and complement in the glomerular lesions. The presence of antinuclear antibody in the tissue-bound gamma-globulin, even if present in a small proportion of these cases, would still be of interest in shedding light on possible mechanisms of pathogenesis. The role of the antinuclear antibody in the development of the renal lesion remains to be determined.

Summary

Observations are reported on glomeruli isolated from normal, glomerulonephritic, and lupus kidneys. Frozen sections of these kidneys had shown glomerular-localized gamma-globulin in the glomerulonephritic and in the lupus kidney but not in the normal kidney. Gamma-globulin was dissociated with the isolated glomerulonephritic and lupus glomeruli at *p*H 3.2 but not at *p*H 7. No gamma-globulin could be detected in the eluate from the normal glomeruli.

The eluates from the glomerulonephritic and lupus glomeruli, containing gamma-globulin, were tested for biological activity on eluted sections of the parent kidney as well as on sections of normal kidneys. No evidence of tissue affinity was observed under these conditions for the gamma-globulin derived from the glomerulonephritic glomeruli.

The gamma-globulin from the lupus glomeruli was found, in the presence of small amounts of fresh normal human serum, to attach itself to cell nuclei generally. With eluted sections of the parent kidney there was some evidence of uptake of globulin by the glomerular capillary wall and Bowman's capsule, as well as by the nuclei. There was evidence that complement as well as gamma-globulin was bound to the nuclei. The factor present in fresh serum which facilitated the attachment of the lupus globulin to cell nuclei was inactivated by heating to 60° C. for three minutes.

These studies suggest that, in disseminated lupus erythematosus, at least part of the gamma-globulin eluted from the glomeruli may be identical with the circulating antinuclear factor.

Part of this work was undertaken while one of us (P. F.) was the Bilton Pollard Fellow of University College Hospital Medical School, at the Department of Medicine, Research and Educational Hospital, University of Illinois, in the department of Professor R. M. Kark, whose help we gratefully acknowledge. We should also like to thank Professor M. L. Rosenheim and Professor A. C. Dornhorst for their valuable help, encouragement, and criticism.

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In a report to the London County Council the Council's health committee says that for some months home teaching for a few mentally ill patients has been carried out as an experiment in two health divisions. The scheme has been welcomed by hospital doctors and general practitioners, and the committee recommends a gradual expansion of the arrangements. As a start it is proposed to make increased part-time use of the services of occupational therapists who would normally be working with tuberculous patients; the scheme will be reviewed after one year's working. (L.C.C. Meeting, April 3.)