criteria[10–12]. Nevertheless, the incipient wasting disease might have some effect on the myoneural junction, a defect not evident clinically but reflected in the extreme vulnerability to curare. This is difficult to rule out entirely.

Pharmacological studies[15–18] on the distribution and excretion of ^{14}C-labelled *d*-tubocurarine dimethyl ether and other forms of curare in normal animals indicate that 90 per cent of an intravenous dose disappears from the blood within 2 min. Within 24 h up to 91 per cent of the material can be recovered in the urine. Most of the excreted curare in the urine is chemically unchanged and still biologically active. Although paralysis may last but 15 min, within the same 15-min period only 10 per cent of the material is found in the urine. The conclusion drawn from these investigations is that curare is rapidly complexed in the body but slowly released and excreted by the kidneys. Observations of the clearance and excretion of radioactively labelled curare by normal and neonatally thymectomized mice are being made to test the hypothesis of a defect in the complexing of curare in the thymectomized animals.

As our investigations relate to the pathogenesis of myasthenia gravis, the initial hypothesis has gained support. In the human baby most evidence suggests that, although the thymic system as measured by its immune function is qualitatively intact, it is quantitatively deficient[19]. The occurrence of transitory myasthenia in babies of myasthenic mothers may depend, at least in part, on this quantitative deficiency of the thymic component of the lymphoid system and the consequent deficient ability of the baby to complex a postulated humoral substance which has crossed the placental barrier from the mother. The alternative, of course, is the more popular view that the mother produces an auto-antibody which damages the myoneural junction or interferes with its function.

In conclusion, these data demonstrate that neonatally thymectomized mice are more sensitive to curare than control mice when assayed by the loss of righting reflex. It is suggested that the thymus and its thymus-dependent lymphoid tissue may function as a system which, in addition to its immune function, acts in detoxification of certain naturally occurring toxins.

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A NATURALLY OCCURRING FRAGMENT RELATED TO THE HEAVY CHAINS OF IMMUNOGLOBULIN G IN NORMAL HUMAN URINE

By DR. M. W. TURNER and DR. D. S. ROWE

Department of Experimental Pathology, University of Birmingham

IN recent years there has been considerable progress in the elucidation of structural, metabolic, and antibody features of the immunoglobulins. It is now known that these proteins are comprised of two varieties of polypeptide chains called heavy and light chains[1], and that the heavy chains differ in the four main classes of immunoglobulins—IgG, IgA, IgM and IgD (refs. 2–4). There are two types of light chains, type *K* and type *L*. Both types are common to all classes of immunoglobulins.

Previous investigations on normal human urine have demonstrated free light chains of both types[5,6] as well as intact IgG and IgA[6,7]. The presence of light chains, and their pathological counterparts, the Bence Jones proteins, is important to considerations of immunoglobulin biosynthesis and metabolism, and a demonstration of other fragments would be of similar interest. This article describes the finding of another immunoglobulin component in normal human urine. This component is related to the heavy polypeptide chains of IgG.

Large volumes of urine were collected from two healthy adult males and stored at 4° C in the presence of 0·1 per cent sodium azide. The urine samples were dialysed overnight against running tap-water, centrifuged to remove cellular debris and then lyophilized. The lyophilized products were reconstituted in 0·2 M *tris*-hydrochloric acid buffer (*p*H 8·0) to 1/200 and 1/120 of their original volumes respectively. Insoluble material was removed by high-speed centrifugation. In the analyses to be described both samples yielded similar results.

The reconstituted urine samples were fractionated by gel filtration on columns of 'Sephadex *G*-200' (ref. 8), using 0·2 M *tris*-hydrochloric acid + 0·2 M sodium chloride (*p*H 8·0) buffer for the elution. A representative elution pattern is shown in Fig. 1. Proteins were eluted from fraction 20 to fraction 60. Albumin was eluted in the region of fraction 40. Appropriate fractions were concentrated four-fold by ultra-filtration, and Ouchterlony gel-diffusion analyses[9] were carried out using antisera specific for the polypeptide chains indicated. IgM and IgD were not detected. IgA was detected in several of the early fractions. Fractions from two regions reacted with the antiserum to the γ-heavy chains of IgG. The activity in fractions 30–36 was due to the presence of intact IgG molecules as has been previously demonstrated[7].

These fractions gave a reaction of identity on Ouchterlony analysis with serum IgG. Fractions 51–58 also precipitated with the antiserum. These fractions showed a reaction of partial identity with serum IgG. The antiserum used in these tests was prepared by immunizing rabbits with the heavy chains of IgG isolated by the method of Franěk and Zikán[10]. On Ouchterlony analysis this antiserum reacted strongly with heavy (γ) chain preparations and also weakly with light chain preparations. Before it was used, the antiserum had been absorbed with the Fab fragments of IgG. No reactivity with light chains could then be detected.

Fig. 1 also shows the distribution of the two types of light chains in the separation. It will be noted that both type *K* and type *L* chains were detected in fractions from the same region as the material reacting with the antiserum apparently specific to the γ-heavy chains. Thus it was of importance to establish whether the reactivity with this antiserum was, in fact, due to heavy chain determinants in these fractions or to some hitherto undetected anti-light chain antibodies in the antiserum.

It is clear from the observations of Nachman and Engle[11] that light chains separated from normal pooled human gamma globulin contain antigenic determinants not detectable in the intact native molecule. These hidden determinants can also be demonstrated in Bence Jones proteins. Antibodies to such determinants could have been present in the anti-heavy chain antiserum used in this investigation, and might not have been completely removed by absorption with Fab. To eliminate this possibility, two further absorptions of the antiserum were carried out. The original antiserum was absorbed to slight antigen excess by a light chain preparation from normal IgG, and by a mixture of type *K* and type *L* Bence Jones proteins. Neither of these absorptions affected the reactivity of the antiserum with the urinary proteins. It is still precipitated with fractions 30–36 and 51–58. The protein in these latter fractions was again found to be antigenically deficient as compared with intact IgG (Fig. 2).

These findings imply that the low molecular weight protein in fractions 51–58 was antigenically deficient not only with respect to intact IgG, but also with respect to the γ-heavy chains of IgG. Ouchterlony analysis (Fig. 3)

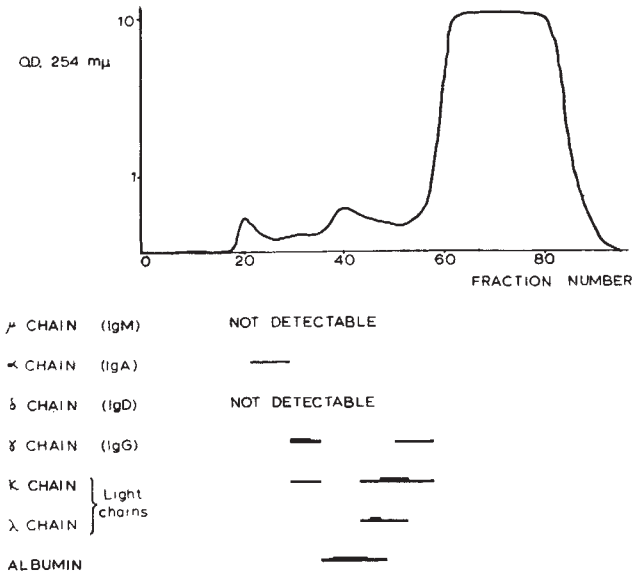

Fig. 1. Elution pattern of gel-filtration of a normal urine concentrate on 'Sephadex *G*-200'. Optical densities were measured at 254 mμ by a recording spectrophotometer. The final large peak did not contain protein. Shown below as horizontal bars are the distribution ranges of fractions precipitating on Ouchterlony analysis with antisera specific to the indicated immunoglobulin polypeptide chains, and to serum albumin

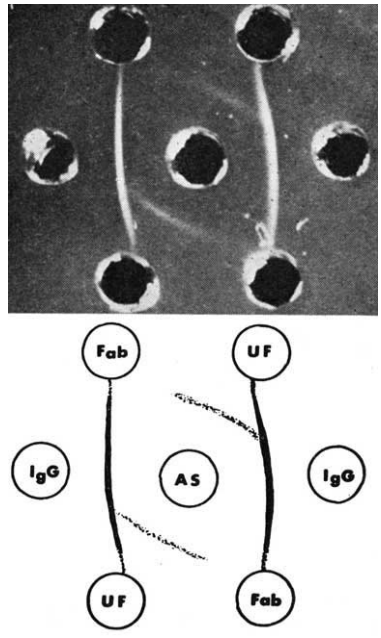

Fig. 2. Ouchterlony analysis showing reaction of partial identity between the low molecular weight urine fractions 51–59 (*UF*) and IgG (1 mg/ml.). The antiserum (*AS*) was specific for γ-heavy chains and failed to react 4 mg/ml., chromium concentration 0·1 mg/ml

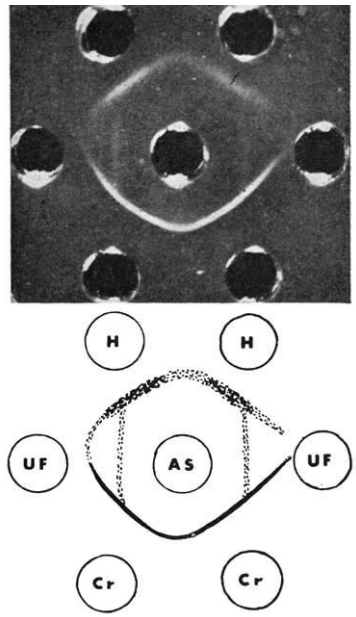

Fig. 3. Ouchterlony analysis showing reaction of partial identity between the low molecular weight urine fractions 51–58 (*UF*) and heavy chains (*H*) prepared from serum IgG. A similar relationship is seen to the abnormal protein (*Cr*) from a patient with heavy-chain disease. The antiserum (*AS*) was specific to γ-heavy chains. Heavy-chain concentration 4 mg/ml., chromium concentration 0·1 mg/ml.

showed that this was the case; a γ-chain preparation formed a clear spur over the low molecular weight urine protein.

Franklin, Lowenstein, Bigelow and Meltzer[12] have described a plasma cell dyscrasia in which there was an excessive production of protein related to γ-heavy chains. It has been shown[13] that this material is not the complete heavy chain, but is similar to the Fc fragment produced by papain digestion of IgG. It was possible that the low molecular weight urine protein was a normal counterpart of this pathological protein. However, the Ouchterlony analysis shown in Fig. 3 excludes this possibility. Using

the antiserum specific to heavy chains, the low molecular weight urine protein was antigenically deficient with respect to the pathological 'heavy chain' protein (Cr) (kindly supplied by Dr. Franklin).

The low molecular weight urine protein was then considered in relation to the fragments of IgG produced by papain digestion. Papain in the presence of cysteine yields two fragments, Fab and Fc, distinguishable by immunoelectrophoresis (Fig. 4). The relationship of these fragments to the peptide chains of the molecule has been elucidated by Cohen[14]. Fab contains the light chains and part of the γ-heavy chains; Fc contains the remainder of the γ-chains, and its antigenic determinants are specific to the γ-chains. Thus, antisera which are Fc specific are also γ-chain specific. The previous results suggest that the low molecular weight urine protein is related but antigenically deficient with respect to Fc.

Using appropriate antisera papain digests of IgG can be shown to contain an additional component which is antigenically deficient with respect to Fc. This fragment has been previously termed F′ (ref. 15) but will here be referred to as Fc′ being consistent with current immunoglobulin nomenclature[3]. Fc′ is distinguished by its anodal mobility on immunoelectrophoresis (Fig. 4).

The relationship between the low molecular weight urinary protein and Fc′ has been determined by comparative immunoelectrophoresis (Fig. 4). These two components appear to be antigenically identical. They are also identical with respect to electrophoretic mobility (Fig. 5). This figure shows that the urinary light chains, although eluted in the same 'Sephadex' fractions, are clearly distinct from Fc′ by their electrophoretic mobility as well as by their antigenic determinants. This indicates that the Fc′ determinants and the light chain determinants are present on different molecules in the urine. No fragments comparable in their electrophoretic mobility and antigenicity with Fab or Fc components were detected in any urine fraction.

Various low molecular weight γ-globulins in plasma and urine have recently been described[16–18]. These proteins, which migrate in the γ-globulin electrophoretic region, are clearly distinct from Fc′ which is of α-globulin mobility. In addition fragments antigenically related to Fc- and

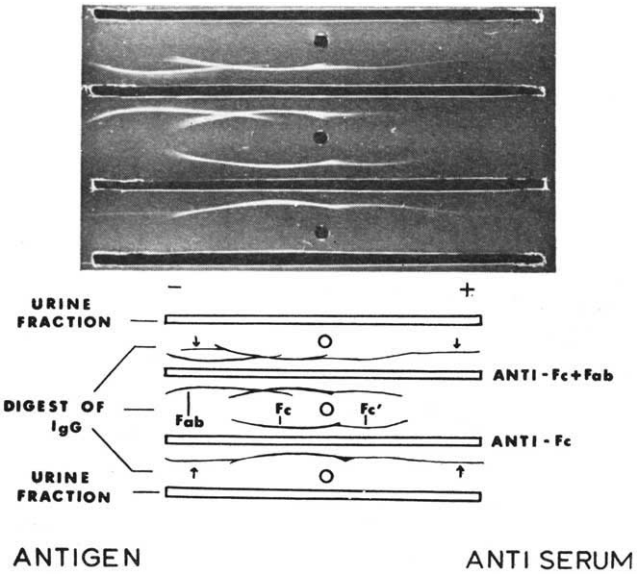

Fig. 4. Comparative immunoelectrophoresis. The three centre wells were filled with papain digest of IgG and electrophoresis was performed. Antisera were then placed in the centre troughs and the low molecular weight urine fractions 51–58 in the outer troughs. The antisera reacted with the urine fractions to produce straight lines indicated by the arrows. These lines fuse with the Fc′ line of the papain digest (antigenic identity), partially fuse with the Fc line (partial identity) and are independent of the Fab line (non-identity)

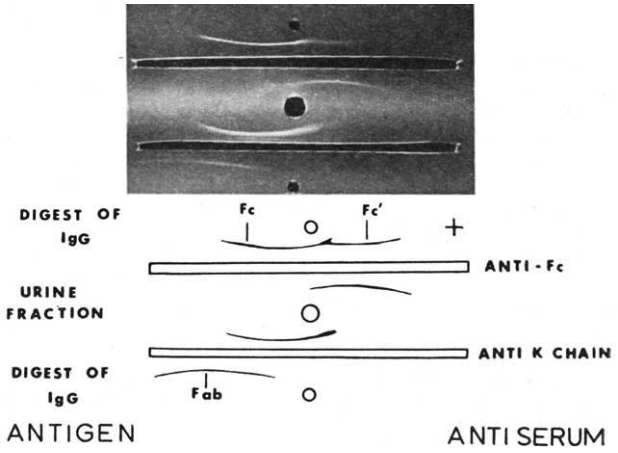

Fig. 5. Immunoelectrophoretic analyses showing that the electrophoretic mobilities of Fc′ from urine and Fc′ in a papain digest of IgG are identical. However, Fc′ differs in its electrophoretic mobility from urinary light chains in the same urine fractions as revealed by the antiserum to kappa light chains. There is no evidence of material with the antigenic and electrophoretic properties of Fc or Fab in the urine fraction

γ-chains have been described[6], but these were not identified as Fc′.

In a study of 'heavy chain disease' Takatsuki and Osserman[19] demonstrated Fc′ as a constituent of the abnormal proteins from four cases, but Fc′ was not detected in the fifth. In a study of myeloma proteins the same authors had similar findings, G-myeloma proteins of class *C* showed Fc′ after treatment with papain and cysteine, whereas in G-myeloma proteins of class *Z* Fc′ were not detectable. Fc′ may therefore be part of some IgG molecules, but not of others.

The Fc′ of normal urine may represent an independently synthesized chain, comparable with light chains. Such material might be present in the plasma and readily excreted in the urine on account of its low molecular weight; it might also be found in larger amounts in serum and urine of patients with certain disorders of immunoglobulin synthesis. Alternatively, Fc′ may be the result of enzymatic degradation of some IgG molecules present in urine. Poulik and Schuster[20] have shown that Fc′ is not produced from IgG by digestion by papain alone, but requires in addition the presence of cysteine. Reducing agents which may be present in urine might, in conjunction with proteolytic enzymes, lead to the separation of Fc′. However, it was notable that other fragments of enzymatic degradation, such as Fc and Fab, were not detected in those studies.

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