thesizing L-ascorbic acid is quite large. Therefore, this incapability may not be fortuitous. It would rather appear that the incapable Passeres are more evolved than other birds of the same branch of the phylogenetic tree.

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26 November 1968; revised 26 February 1969 ■

Serum C'3 Lytic System in Patients with Glomerulonephritis

Abstract. *The serums of patients with hypocomplementemic glomerulonephritis contain a substance that combines with a normal serum cofactor in the presence of magnesium ion to specifically cleave the third component of complement. This lysis of C'3 is 80 to 90 percent complete in 20 minutes at 37°C and* p*H 7. Neither the nephritic factor nor its cofactor is identifiable with the complement system.*

It has always been assumed that the reduction in serum complement seen in several forms of glomerulonephritis is secondary to an ongoing immune reaction in the kidney. Recently, however, Pickering, Gewurz, and Good (*1*) demonstrated an inactivator of guinea pig complement in the serums of a number of these patients. They determined that this "anticomplementary substance" probably acted on one or more of the terminal six components of complement and theorized that the inactivating factor or factors may be partially responsible for the low levels of complement seen in such patients.

This report shows that a factor is present in these serums which, when combined with a cofactor present in normal human serum, specifically cleaves the third component of human complement (C'3 or β_{1C}-globulin) into at least two breakdown products, β_{1A} and α_{2D}. We have designated the factor found in these nephritic serums the C'3 nephritic factor (C'3NeF) to distinguish it from other nonspecific inactivators of complement (*2*) and, more specifically, from the C'3 inactivator described by Tamura and Nelson (*3*).

To demonstrate the nephritic factor and its cofactor, monospecific antiserums directed against each of the three major antigenic determinants of C'3, previously designated A, B, and D (*4*), have been utilized. As C'3 is broken down, the B antigen that is associated only with the intact native molecule disappears; ultimately, β_{1A}, containing only the A antigen, and α_{2D}, containing only the D antigen, are formed. In this study, therefore, the rate of C'3 breakdown has been quantitated by measuring the disappearance of the B antigen by use of the immunoelectrophoretic - precipitin method (*5*).

In a mixture of equal volumes of normal human serum and serum from a patient with persistent hypocomplementemic glomerulonephritis (*6*), there is a rapid decline in the concentration of the B antigen with concurrent formation of the β_{1C} breakdown products, β_{1A} and α_{2D}. The kinetics of this reaction are such that 80 to 90 percent of the B antigen disappears within 20 minutes after mixing. The rate of disappearance is the same in such a mixture even if the volume of the nephritic serum is reduced by 50 percent. Further characterization of this reaction shows that there is full activity at a temperature of 37°C over a broad *p*H range of 6 to 9. Below *p*H 6 or above *p*H 9 and at temperatures from 0° to 25°C, however, there is a marked reduction in the rate of breakdown.

Several lines of evidence make it clear that the cleavage of C'3 in the serum mixture is dependent not only on the nephritic factor but also on one or more cofactors present in fresh human serum. First, the presence of sodium fluoride or disodium ethylenediaminetetraacetic acid at the time the serums are mixed completely inhibits breakdown of C'3. However, addition of these reagents after the reaction has started, even if the interval is as short as 30 seconds, does not alter the reaction rate. Both Na_2EDTA and NaF exert their effect in this system by specifically removing Mg^{++}; Ca^{++} does not appear to be involved. Second, as shown in Table 1, nephritic serum causes only slight breakdown of the β_{1C}-globulin in a euglobulin fraction of normal serum. Breakdown at approximately the normal rate does occur, however, when normal pseudoglobulin is added to the mixture. It would appear, therefore, that at least

Table 1. Localization of C'3NeF in the pseudoglobulin fraction of nephritic serum; requirement of a cofactor for lysis of C'3.

Reactants	B antigen (unit/ml)		
	Time 0*	Time (20 minutes)†	Decrease (%)
Nephritic serum and normal serum	23.8	3.2	87
Nephritic serum and normal euglobulin	14.3	12.5	13
Nephritic serum, normal pseudoglobulin, normal euglobulin	12.9	2.2	83
Nephritic pseudoglobulin, normal pseudoglobulin, normal euglobulin	16.1	3.8	77
Nephritic euglobulin, normal pseudoglobulin, normal euglobulin	17.1	16.7	2

* Reactants mixed at 0°C without incubation. † Reactants mixed at 37°C and incubated for 20 minutes.

two separate and distinct reactions are necessary for C'3 lysis. The first is a combination of C'3NeF found in nephritic pseudoglobulin (Table 1) with a cofactor in normal pseudoglobulin which occurs rapidly and is dependent on the presence of Mg^{++}. Separately, neither the C'3NeF nor the cofactor exert any discernible effect on the C'3 molecule; their product, however, causes lysis of C'3. This combination of C'3NeF and its cofactor, therefore, will be referred to as the C'3 lytic nephritic factor (C'3 LyNeF). These reactions are summarized below:

$$\text{C'3NeF} + \text{cofactor} \xrightarrow{Mg^{++}} \text{C'3LyNeF} \quad (1)$$

$$\text{C'3LyNeF} + \text{C'3} \longrightarrow \beta_{1A} + \alpha_{2D} \quad (2)$$

A similar reaction sequence has recently been described by Müller-Eberhard as occurring between a factor in cobra venom and a β-globulin normally present in human serum (*7*). In the presence of bivalent cations, this reaction yields a C'3 inactivator complex which converts C'3 into C'3i. As with the active principles in the cobra venom reaction, neither the nephritic factor nor its cofactor are identifiable as components or products of the complement system. It has not been possible to identify C'3NeF as convertase enzyme (C'4,2a complexes) (*8*) for the reasons that (i) convertase present in nephritic serum would not require a cofactor for its activation and (ii) differing from convertase, C'3NeF is extremely stable, retaining its activity after incubation of nephritic serum for 7 days at 37°C.

There is also evidence that C'3NeF is not aggregated gamma globulin, antigen-antibody complexes, or antibody-complement complexes of the form AbC'1a,4. To ascribe C'3NeF activity to these complexes, the assumption must be made that some or all of the first three reacting components of complement are absent from the nephritic serum. In this event, the addition of normal pseudoglobulin to the nephritic serum would supply the missing component or components and a bivalent cation-dependent reaction would occur to form convertase. The convertase, like C'3LyNeF, would then break down C'3 in the absence of bivalent cations. Such an assumption, however, seems untenable. Although C'1, C'2, and C'4 have not been directly measured in the specific nephritic serum in these studies, these components have previously been shown (*9*, *10*) to be present in adequate concentrations in the serums of other patients with hypocomplementemic persistent nephritis. Further evidence against identification of C'3LyNeF with convertase is provided by the observations that (i) treatment of normal pseudoglobulin with iodine to form oxy-C'2 (*11*) before addition of nephritic pseudoglobulin caused no enhancement of the rate of the breakdown of C'3 and (ii) whereas the half-life of convertase is only 12 minutes at 32°C (*8*), C'3LyNeF retained full activity when incubated for 40 minutes at 37°C as a mixture of nephritic and normal pseudoglobulin. The C'3LyNeF also differs from the C'3 inactivator described by Tamura and Nelson (*3*) in the C'3LyNeF causes cleavage of fluid-phase C'3.

The role of this C'3 lytic system in the pathogenesis of either the reduced β_{1C} levels or the glomerulonephritis itself is not known at present. Conceivably, this system, if present in the glomerulus, could initiate the renal inflammation by activating the last six complement components. Requirements for an immune complex and convertase enzyme would, therefore, be bypassed. On the other hand, C'3NeF may be part of a protective mechanism; the destruction of serum C'3 would reduce the amount of this component deposited on the glomerulus, thus partially preventing activation of subsequent components. The elucidation of the precise function of this system may be an important step in understanding the biological role of complement in glomerulonephritis.

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16 December 1968

Tricyclic Antidepressants: Evidence for an Intraneuronal Site of Action

Abstract. *Desipramine, a tricyclic antidepressant drug, almost completely prevents the accumulation of tritiated norepinephrine by sympathetic neurons of the rat heart after the injection of a tracer dose of the labeled amine. However, desipramine does not alter the accumulation of norepinephrine after the injection of a large dose of the neurohormone. Despite the failure of desipramine to block the neuronal uptake of norepinephrine, it still prevents exogenous norepinephrine from displacing the endogenous neurohormone (previously labeled with H^3-norepinephrine) from intraneuronal storage sites.*

Biochemical and histochemical evidence indicates that adrenergic neurons concentrate norepinephrine (NE) by two mechanisms; one, at the neuronal membrane, takes up NE from the circulation and from sympathetic receptors; another within the neuron incorporates the amine into storage vesicles (*1*). The ability to inhibit the uptake mechanism in the neuronal membrane is a characteristic of tricyclic drugs, such as imipramine, desipramine, and amitriptyline, which are widely used agents in therapy of mental depression (*2*). Since this mechanism is mainly responsible for the termination of the effects of NE, the tricyclic drugs are believed to act by prolonging the association of the neurohormone with peripheral receptor sites after nerve stim-