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This Week's Citation Classic

Deutsch H F & Morton J I. Dissociation of human serum macroglobulins. *Science* **125**:600-1, 1957. [Department of Physiological Chemistry, University of Wisconsin, Madison, WI]

When gamma globulins with molecular weights of about 1,000,000 are treated with mercaptans at neutral pH they are readily converted into subunits of about one-fifth the size of the parent molecules. Removal of the mercaptan leads to some reformation of the original protein and this reaggregation is blocked by alkylating agents. This demonstrated that the macromolecular type of antibody molecule contains subunits linked by disulfide bonds. [The *SCI*® indicates that this paper has been cited over 480 times since 1961.]

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"My interest in antibodies could be justly attributed to the 'fortunes of war' since my studies in this area were initiated in 1944 at the conclusion of my doctoral work in an unrelated field. I then participated in a project at the University of Wisconsin that was a subcontract of work on blood plasma fractionation at the Harvard Medical School sponsored by the Office of Naval Research. My initial task was to increase the yield of a human gamma globulin fraction that is now known as normal IgG. Concurrent studies at Wisconsin were being made to convert human IgG into smaller molecules by digestion with papain, bromelin, and pepsin. The latter enzyme had been previously employed commercially to diminish adverse effects of horse antitoxic sera. My attention was directed to reports in which cysteine activated papain had been employed to digest human IgG into what are today defined as Fc and Fab fragments. Since no cysteine control had been employed, I reduced IgG with it. Such treatment gave protein with an increased electrophoretic mobility but no significant changes in sedimentation behavior were noted. The heavy and light chain components most likely formed were not detected since dissociating conditions had not been employed.

"These studies were carried out concurrently with the isolation of a new human globulin fraction characterized by a different profile of antibody activities, greater electrophoretic mobility and containing considerable amounts of 19S protein that was relatively insoluble in water, i.e. euglobulin, as compared with the largely water soluble, i.e. pseudoglobulin, IgG fraction. The high molecular weight of the 19S component (approx. 1,000,000) eventually led to its designation as a gamma macroglobulin or IgM. Certain human pathologic sera had been noted to contain large amounts of euglobulin sedimenting at 19S but also containing from 15 to 25% of what appeared to be higher polymers of the 19S material. The electrophoretic homogeneity of one of these molecularly heterogeneous proteins that we had crystallized in 1956 had led us to suggest at this time that even the 19S material might be a polymeric form of a lower molecular weight unit. This was directly probed about one year later and it was found that reduction of both pathological and normal human IgM source proteins readily converted all of the IgM components into protein sedimenting near 7S. These IgM subunits of about 200,000 molecular weight reaggregated reversibly upon removal of the mercaptan and this could be blocked by alkylation of the reduced protein. At this time we also naturally tried the reduction and alkylation of 7S myeloma source Ig proteins. One of these was found to be converted into material sedimenting at 3.5S but this was not the usual result. The later demonstrated effects of detergents and of low pH on breaking non-covalent bonds between antibody subunits was not appreciated at this early date. Thus these experiments are cast in the category of 'ships that pass in the night.' However, our report on the chemical dissociation of IgM proteins essentially ushered in the era of the non-enzymatic production of antibody fragments designated as light and heavy chains and stimulated a great deal of the subsequent studies on the chemical nature of antibody subunits and their linkages. Numerous attempts to effect *in vivo* reductions of IgM components elevated in various pathologic states were also made following our report in *Science* but no significant improvement was noted in these patients."