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Cryoglobulinemia

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Cryoglobulinemia is a common condition associated with an elevated serum globulin fraction. Relatively large amounts are found in patients with kala-azar, systemic lupus erythematosus, rheumatoid arthritis, periarteritis nodosa, etc. Cryoglobulins are present as a secondary phenomenon in specific disorders. Cryoglobulins may be considered as homogeneous proteins produced most probably by groups of plasma cells or lymphocytes in response to the antigenic stimulus of infection or inflammation, or they may arise as a result of spontaneous benign or malignant proliferation of these groups of cells as in myeloma and leukemia.

The particular characteristics of cryoglobulin is to precipitate or gel (or both) at serum temperatures below 37°C and to reverse these effects on being re-warmed to 37°C.

The spontaneous gelling of blood immediately on or during withdrawal into a syringe has at times been observed in leprosy patients with hypergammaglobulinema. (These observations were made in the sera of leprosy patients by the writer while on duty at the National Leprosarium, Carville, Louisiana, U.S.A.) The gelling is independent of clotting and the serum may form a precipitate on cooling. The name 'cryoglobulin' has been given to this cold-precipitable serum protein which, when first described, was shown to have the mobility and ultra-violet spectral characteristics of a gamma globulin and crystallized on cooling. In some instances of cryoglobulinemia gelling and precipitation occur in the syringe while the needle is still in the patient's vein. In these cases, warm the syringe to 38°C and take the blood in a warm room. More often the cryoglobulins precipitate at temperatures varying from 0° to 37°C over a wide variation of time. Cryoglobulins may be present as a scarcely visible turbidity, a marked flocculation or a viscid gel. Antigenically this protein appears to be related to normal gamma globulin.

Precipitation of globulins in serum by cold must be differentiated from precipitation of protein in plasma, especially heparinized plasma. Fibrinogen readily separates from heparinized plasma when present in excess as in the case in most inflammatory disorders.

Commonly, in an erythrocyte sedimentation test performed when cryoglobulin is present, no sedimentation is found at 0°C, whereas at 37°C the sedimentation is very rapid—exactly the reverse of the sequence in the presence of cold agglutinins. The cryoglobulin precipitates at a low temperature and plugs the sedimentation tube so that the red corpuscles cannot form a sediment. This has sometimes led to the conclusion that the blood in these patients 'clots' instantaneously—an erroneous interpretation of a correct observation. There is no true coagulation since the pseudo-clot is easily soluble on warming the plasma or serum. The presence of cryoglobulin is certain when the pseudo coagulation is reversible with changes in temperature.

Cryoglobulins are only one type of blood protein with unusual physical properties and thermal characteristics. For example, C-reactive protein is cold-precipitable, pyroglobulins precipitate at 56°C; Bence Jones Protein precipitates at 45-50°C and the protein of amyloidosis has a high carbohydrate content[4].

The precipitation of this globulin in the small vessels of the skin on cooling causes 3 types of lesions: (*a*) Raynaud-like syndromes with bluish discoloration of ears, nose and fingers, (*b*) purpura with oedema, and (*c*) the most severe type—ulcers and necrosis of the skin with little tendency to heal.[9]

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Mathews and Trautman[2] observed that many leprosy patients at Carville were intolerant to cold and led to the recognition of sensitivity to sudden temperature changes—but not necessarily cold temperatures, in a number of other leprosy patients. Exposures to such environmental changes have led to a variety of cutaneous manifestations including acrocyanosis, purpuric eruptions and ischemic necrosis. These workers observed that subsequent tests revealed that significant levels of cryoproteins were present in the sera of most patients with active lepromatous leprosy and dimorphous (borderline) leprosy. This protein was not observed in the sera of patients with tuberculoid leprosy.

For reasons which are unknown but which may be climatic, racial or related to differences in pathogenicity of *M. leprae*, leprosy presents clinical variations in different regions. Wade[10] has stressed these differences. In the Philippines, for example, the lepromatous type comprises 40 to 50% of all known patients, a very much higher proportion than has been reported in Africa, China and India.

Spotted or diffuse leprosy of Lucio is a syndrome described by Lucio and Alvarado in Mexico in 1852 and later by Latapí[1]. It is a diffuse lepromatosis with outbreaks of multiple necrotizing vascularities. At each site there occur frank necrosis of the capillaries and a secondary dermeopiderma necrosis.

According to most workers (Guinto *et al.*, Mauze, Mayama and our own work at Carville)[4 3 5 7] the total proteins are normal or increased in the majority of leprosy sera. The quantity of albumen is usually moderately reduced, and in the writer's experiences at Carville, in one case complicated by amyloidosis, albumen was undetectable by ordinary chemical methods and poorly demonstrated by paper electrophoresis. There is a moderate hypergammaglobulinemia in many of the patients and obvious reversal of the albumen-globulin ratio.

There is less unanimity of opinion in regard to the quantity of the various globulin fractions in leprosy. The globulin increase may be a total increase of all of the globulin moities, or may be of a single fraction, or any combination of fractions. The most common occurence is the elevation in the gamma globulin region in active lepromatous patients. There is a definite tendency for the gamma globulin levels to increase with severity of the disease and with bacillary content of smears from the skin and mucous membrane. The next most common is a beta-gamma increase, then other smaller combinations occur in smaller numbers.[4]

It is known that the gamma globulin fraction is markedly elevated during Erythema Nodosum Leprosum reactions, more so than during non-reaction periods. This finding is usually attributed to a non-specific response to tissue damage although part of the gamma globulin fraction might represent antibody formation. As the erythema disappears the globulin fraction is lowered. In this reversible inflammatory state cryoglobulinemia subsides as the patient improves and the general protein pattern approaches the normal level.

Waldenström[9] describes a number of conditions in which hyperglobulinemia is an important finding, such as viral infections, sarcoidosis, subacute bacterial endocarditis, etc. In the 'rheumatic' maladies or 'collagenosis' an increase in the gamma fraction is a common occurrence. Mathews and Trautman[2] state in substance that the notable resemblance of reactive episodes in leprosy to manifestations of collagen diseases may be more than coincidence. This resemblance does not end with the clinical manifestations. The presence in a significant number of leprosy patients of what the authors term cryoprotein, of rheumatoid factors, circulating thyroglobulin-antibody and biologically false positive serological tests for syphilis are additional factors pointing to a close relationship. However, of great importance is the fact that not only is leprosy a disease associated with many manifestations normally found in collagen disease but it is a disease for which an aetiologic agent is known.

Cryglobulins and purpura: A subject of frequent discussion is the association of purpura with the presence of serum cryoglobulin. This condition was first described by Lehmann and

Fleming (cited by Waldenström[9]). Lerner *et al.* have investigated this condition and coined the name 'purpura cryoglobulinemica'. They consider it a sub-group of Waldenström's purpura Hyperglobulinemica.

ANALYSIS OF CRYOGLOBULINS

For the qualitative and quantitative analysis of cryoglobulins centrifuge the refrigerated sample at 4°C for one half to one hour, and pour off the supernatant serum. The lipid fractions float to the top. Wash the precipitate in normal saline, redissolve and precipitate overnight at 4°C. Usually 3 washings are required for an electrophoretically pure sample, otherwise other protein fractions remain absorbed in the precipitated protein. Washing leads to a loss of some cryoglobulin so that the exact quantitative determination may be difficult. Mathews and Trautman, in their fractionation of cryoprotein washed their precipitate with 10 washings of cold normal saline. This probably accounts for their albumen-like electrophoretic mobility of the cryoprotein since washing leads to a loss of cryoglobulin. Determine the mobility of the cryoglobulin by simultaneous electrophoresis (using a barbital buffer pH 8.6) of the whole serum, the washed cryoglobulin precipitate that has been redissolved in normal saline and the decanted supernatant serum. The mobility of the abnormal protein may be anywhere in between the beta and gamma bands or, exceptionally, in the alpha$_2$ region.

Most cryoglobulins have physical characteristics similar to normal gamma globulins. The sedimentation constant S_{20} lies between 6.0 and 7.8; the molecular weight is 160 200,000. Antigenically, these proteins appear to be related to normal gamma globulin. Some cryoglobulins have been isolated in crystalline needle, rhombic or cubic forms, suggesting that they have a greater degree of homogeneity and purity than normal gamma globulin. Normal gamma globulin has not been crystallized. Most cryoglobulins redissolve partly or completely on warming to 38°C and are soluble in normal saline.

Other than the report of Mathews and Trautman[2] this interesting field of cryoglobulins has not been studied in leprosy as far as the writer is aware. The currently available immunochemical and physiochemical data indicate that in the adult there are 3 major circulating immune globulins, Gamma-2, Gamma$_1$ A, and Gamma$_1$ M. Gamma-2 is the principal constituent of Cohn Fraction 11 and constitutes approximately two-thirds of the protein within the electrophoretically defined gamma area of serum. This boundary also extends through the beta and into the alpha$_2$ mobility region. It has been demonstrated by immunoelectrophoresis that all 3 immunological fractions are increased in the disease states associated with diffuse hypergammaglobulinemia, e.g., chronic infections, sarcoidosis and the collagen disease.[6]

The following information on the ultracentrifugation of serum proteins appears highly technical but is recommended.[8] It was thought desirable to include it here for the benefit of those workers who may be interested in knowing that there is such a procedure.

For both clinical and research purposes the required differentiation of the protein macromolecular spectrum can be secured by the procedures of ultracentrifugation because they can be adapted to the physical characteristics of proteins of interest. Analytical ultracentrifugal procedures permit differentiation, characterization and quantitation of the serum protein fraction 'gamma globulins' (in the nomenclature of paper electrophoresis) into 3 different distinct protein classes:—

(i) the class of 'true' gamma globulins with sedimentation constant of 7S;
(ii) the class of macroglobulins with sedimentation constants S15 and higher (further characterized and quantitated by specific sedimentation constants found —15S, 21S, 24S, etc.);
(iii) the class of proteins with sedimentation constants between S7 (gamma globulins) and S15 (macroglobulins) for which there is yet no class name.

Apparently cryoglobulinemia is a common condition with a raised serum globulin fraction,

but it must be properly investigated or it will be overlooked.

It would be of interest, at least qualitatively, to observe if this gelling phenomenon occurs on different populations with leprosy. If it is noted, perhaps some of the well equipped Research Centres for Leprosy could make a scientific study of this type of sera.

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