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Rapid Detection and Titration of Antihuman Serum Albumin Antibody

HUMAN serum albumin (HSA) is an antigen frequently used for the examination of the synthesis of antibody in various species. The techniques usually utilized are variants of either precipitin or haemagglutinating methods. Although these procedures are extremely sensitive, both suffer from the disadvantage of requiring a time-interval between the addition of reagents and final reading of the results. The haemagglutinating techniques, furthermore, require absorption, frequently multiple times, of the sera with the red cells which are used in the testing method. The following technique, a slight modification of a method described¹, has proved to be reproducible, rapid, and to have the sensitivity of tanned red cell haemagglutination. Ten ml. of bentonite stock solution prepared according to the method of Bozicevich *et al.*¹ is centrifuged at 1,800 r.p.m. for 15 min. The pellet is re-suspended in 1 ml. of distilled water and an equal volume of a 1 per cent saline solution of human serum albumin is then added. The mixture is shaken gently and permitted to incubate at room temperature for 15 min. One ml. of 0.1 per cent aqueous methylene blue and 15 ml. of distilled water is added, the suspension shaken, and after 10 min recentrifuged for 10 min. The pellet is re-suspended and washed twice in 10 ml. of distilled water. The final suspension is made up with 5 ml. with distilled water. A suspension so prepared and stored at 4° C is shaken gently before use. The flocculating characteristics are retained for periods of two weeks with no change in titre.

The agglutination procedure is performed by adding one drop of the test serum to one drop of the bentonite suspension and centrifuging for 2 min in a 'Sero-fuge'. A positive test is observed as varying degrees of clumping after gently flicking the tube, and is most easily read over a fluorescent white lamp. Titrations may be carried out using a calibrated loop containing as little as 0.025 ml. with serial transfer of the serum to be tested into 10 × 75 ml. test-tubes containing saline. A fixed volume of bentonite, for example, 0.025 ml., is added and the centrifugation carried out as previously noted. Inhibition investigations with 1 per cent albumin added first to the serum followed by the bentonite have been successful. The serum

may be tested for the presence of macroglobulin and the titre of 19 and 7S antibody estimated by the addition of 2-mercaptoethanol followed by incubation for 30 min at 37° C before adding the bentonite.

Equivalent titres of antibody are obtained when the same dilutions of antisera are compared utilizing the foregoing technique and the tanned red cell haemagglutination procedure with a microtitrator².

The technique is recommended for its simplicity and rapidity of results. Once the bentonite has been sensitized the entire titration may be performed and results obtained within a matter of minutes, thus avoiding the tedious and technical difficulties of precipitin and haemagglutination procedures. Both rat and rabbit serum have been tested and yielded satisfactory results. Other substances such as bovine and human γ -globulins have not resulted in satisfactory specific agglutination patterns. It is thus likely that the success of this simplified procedure results from propitious physico-chemical properties of human serum albumin.

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PATHOLOGY

Virus-like Particles in Sub-acute Sclerosing Encephalitis

SUB-ACUTE sclerosing encephalitis (van Bogaert) is a fatal disease of the central nervous system of uncertain origin, usually affecting children and adolescents. The pathological features of this disease are diffuse inflammatory cell infiltrates in the white and grey matter, gliosis, and demyelination¹. Frequently, Type A intranuclear inclusion bodies have been observed in neurones of cases running a short clinical course (Dawson's inclusion body encephalitis) while in the more chronic cases these bodies are less frequently found. Although often a viral aetiology has been suggested, the cause of this disease remains obscure, since animal inoculations of brain tissue have been unsuccessful in transmitting the disease¹.

To our knowledge, ultrastructural investigations in this disease have not been made. This electron microscopic examination of a cortical biopsy from a case of sub-acute sclerosing encephalitis has revealed a large number of virus-like particles in the cytoplasm of astrocytes in the white matter; similar virus-like particles in the cytoplasm of neurones in the grey matter were not seen.

The patient was a nine-year-old Negro female with a fifteen-month history of progressive mental deterioration, difficulty in holding objects with her hands, difficulty with gait, slurring of speech, fine tremors, enuresis, a twelve-month history of generalized seizures and a ten-month history of precocious puberty. These were followed by progressive motor and mental deterioration and transitory migratory seizures involving various extremities and facial muscles. Her history had been that of progressive deterioration with double incontinence, inability to recognize her surroundings, and inability to control arms and hands. On physical examination she was unresponsive to all but painful stimuli. There was gross cachexia, an in-dwelling Foley catheter, and a naso-gastric feeding tube in place. Gross postural contractures of all four extremities were present. The head was normal size, and there was increased rigidity of the neck with no adenopathy. The pupils were equal, but did not react to light. There was