Method for the detection and measurement of antibodies to streptokinase in cerebrospinal fluid

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One of the difficulties encountered in intravenous therapy with streptokinase as a plasminogen activator is the early development of antibodies during treatment which, in addition to neutralizing the activator, may also cause sensitivity reactions. The presence of pre-formed antibodies markedly influences the dose of activator necessary to achieve a satisfactory thrombolytic state in plasma. These factors are also of importance if intrathecal streptokinase is used to induce a fibrinolytic state in the cerebrospinal fluid (Stewart, 1964). A method has been devised, therefore, for the detection and measurement of such antibodies, by measuring their effect on streptokinase in a semi-purified system where the lysis time under controlled conditions is known. A standardized bovine fibrin clot is made incorporating a known amount of streptokinase and human plasminogen, the lysis time of which is predictable. Addition of cerebrospinal fluid to the mixture before clotting will lengthen the lysis time if antibodies to streptokinase are present. Two difficulties have to be overcome. First, there must be no streptokinase or other activator in the cerebrospinal fluid as this will alter the lysis time of the system. Secondly, the amount of plasminogen in the system might be altered by the addition of a plasminogen-rich cerebrospinal fluid. This is overcome by the enriching of the system with excess plasminogen so that the largest amount likely to be added in any cerebrospinal fluid will be insignificant. The effect of varying the plasminogen content is shown in Figure 1. It will be seen that little change in lysis time is produced by an increase in plasminogen from 1 to 2

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Fig. 3. Section from a pleural aspirate containing neoplastic cells.

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Sherry units. The largest amount of plasminogen likely to be added in any cerebrospinal fluid will be very small compared with this change and can therefore be ignored. A concentration of 1 Sherry unit of plasminogen in the system appears to be suitable.

Figure 2 shows the effect of varying the streptokinase concentration over a wide range while keeping plasminogen constant. It will be seen that if a concentration of 0.5 units of streptokinase is used this will give a convenient lysis time of about 20 min., while permitting a large increase of lysis time with small reductions in streptokinase. A small amount of added plasminogen therefore produces no change in lysis time, while a small inhibitory effect on the streptokinase will produce a large change.

![Graph showing the relationship between streptokinase units and lysis time.]

FIG. 2.

The reagents used are as follows:

- Bovine fibrinogen (Armour) 0.0.8% in phosphate buffer
- Phosphate buffer .......................... M/15 pH 7.4
- Purified human plasminogen 10 sherry units/ml in 0.85% saline
- Streptokinase (Wellcome) .......... 5 u. /ml. in water
- Thrombin (Parke-Davis) ............. 50 u./ml. in saline

The test is carried out in 5 in. x ½ in. test tubes and the reagents are used in the following quantities:

- Fibrinogen .......................... 1 ml.
- Buffer .......................... 1 ml.
- Plasminogen ......................... 0.1 ml.
- Cerebrospinal fluid .................. 0.1 ml.
- Streptokinase ......................... 0.1 ml.
- Thrombin .......................... 0.1 ml.

The tubes are placed in a transparent water-bath at 37°C as soon as the clots have formed and the lysis times are read visually or by means of a photoelectric instrument (Newman, 1964). Inhibition of streptokinase can then be estimated from a standard curve (Fig. 2). Lysis times are normally about 20 minutes, but a control tube without cerebrospinal fluid should always be included as the plasminogen loses potency slightly after several hours at room temperature.

A model of human karyotype for teaching

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Lecturing on human chromosomes using the standard aids of blackboard and slides presents several difficulties. The slide, though admirable for magnifying real chromosomes, demonstrates one defect at a time. The blackboard is unsatisfactory for illustrating the whole karyotype and, if gamete formation or translocation are being shown, constant rubbing out and redrawing are necessary. It was therefore decided to produce a background on which could be placed chromosome models illustrating the whole Denver system.

The background on which the chromosomes are mounted is a board 18 inches by 28 inches covered on both sides by a white plastic material. The chromosomes are cut from a sheet of black plastic. The cost of the board and plastic sheet is 22s 6d. and may be purchased under the trade name Cellograph. It is normally sold for making visual aids for teaching children.

To obtain the outlines of chromosomes, a slide of the Denver system of chromosome arrangement is projected on to the plastic. A tracing is then made and the chromosomes cut out. They can then be arranged as required anywhere on the board and are easily detached and replaced as long as the board is kept clean. The set is now being used for teaching purposes.

Miller, Hyde, and Selden (1963) advocated the use of a felt board and chromosomes. For our purposes, however, it was decided that a plastic teaching set had advantages which were: (1) a rigid board which could be stood up or hung as desired; (2) it provides a background which can be drawn on with a felt pencil, especially useful when

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