Technical methods

Simple method for the cytological examination of cerebrospinal fluid

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The cytological examination of cerebrospinal fluid (CSF) is important in the diagnosis of central nervous system leukaemia and other diseases. However, it is not easy to prepare a good smear because the cells are easily lysed after a short period and also are easily damaged by centrifugation, fixation, and concentration (Sörnäs, 1967). The sedimentation method of Sörnäs appears to be a much better method for both cell recovery and morphology (Sörnäs, 1967; Aronson *et al.*, 1974). We have de-

veloped a simpler and more practical sedimentation method which is reported below.

Material and methods

A thick circle, about 1.5 cm in diameter, was made on a clean fat-free glass slide with a laboratory wax pencil. Fresh CSF was then applied dropwise within the circle; 0.2-0.3 ml of CSF was retained in the circle without difficulty. The slide was then placed in a Petri dish with water at the bottom to provide saturated humidity. After sedimentation for 15-30 minutes the fluid was absorbed with the tip of a piece of blotting paper and dried with an air jet. Slides were then stained with Wright stain and mounted. The area within the circle was searched with a low-power lens to locate the suspicious cells which were then examined under higher power.

Annoying debris from wax at the edge of the circle can be reduced by briefly flaming the slide beforehand, and wax will adhere to the glass better. The circle can also be easily made by Permount (histological mounting medium, Fisher Scientific Co, Fair Lawn, New Jersey). We apply Permount to a clean cut edge of rubber tube and then compress the edge of the tubing to the glass.

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Fig. 1

Fig. 2

Fig. 3

Fig. 1 Cyto-centrifuge preparation of CSF from a patient with Chediak-Higashi's syndrome showing spreading of cytoplasm and an obscure granule.

Fig. 2 Sedimentation preparation of CSF from a patient with Chediak-Higashi's syndrome showing better morphology and a giant granule.

Fig. 3 Leukaemic cell in sedimentation preparation containing only a few cells per μ l of CSF.

Results and discussion

After evaluation and comparison of many different methods, Sörnäs developed and recommended the sedimentation method for cytology of CSF (Sörnäs, 1967; Aronson *et al.*, 1974). The cytocentrifuge method has also been recommended recently by a few investigators (Drewinko *et al.*, 1973; Evans *et al.*, 1974). We have found much cell distortion with the cytocentrifuge method and very poor yields, as has been reported by others (Addiego and Woodruff, 1973). The distortion of cell morphology, especially the spreading of the cytoplasm, makes interpretation difficult (Fig. 1). Reduced distortion of cell morphology was well demonstrated in CSF from a patient with Chediak-Higashi's syndrome. Giant granules in the cytoplasm of lymphocytes were seldom seen in cytocentrifuge preparations but were well demonstrated in the preparations by our sedimentation method (Fig. 2).

The method described here is also simpler and more practical. It is difficult to scrape off completely the petroleum jelly in the method of Sörnäs (1967), and this method has also been criticised as time-consuming (Castleberry *et al.*, 1975). Our method not only eliminates the procedure of applying and scraping off the petroleum jelly but also shortens the time considerably. Since the height of fluid within the circle was no more than 3 mm, the time required for cells to settle at the rate of 0.1 mm/min will be

less than 30 minutes. The cells, of course, are less concentrated in our method than in that of Sörnäs. However, the same amount of CSF will be adequate to make more slides. By using low-power lens for scanning the suspicious cells and oil lens for detailed morphology, we probably examine as many cells as in Sörnäs' method. In a few patients whose CSF contained few cells per microlitre the smear revealed a few definitely leukaemic cells (Fig. 3).

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A new method of quality control for the Coulter Model S Counter

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Conventional methods of quality control depend on the use of standards for machine calibration and the use of control samples to detect drift. The methods applied to haematology require the use of expensive whole blood reference preparations. Control samples, although they can be prepared daily or slightly less frequently (Hamilton and Davidson, 1973), must be interposed at frequent intervals between test samples. The control value can be affected by carry-over from the previous specimen (Brittin

et al., 1969) and therefore must be used twice and the second value taken as the more accurate. Control specimens may thus occupy a significant proportion of machine time. The use of a method independent of whole blood standards and controls offers a considerable saving in expense and time.

The method described depends on the stability of the modal values of the population passing through the laboratory. The mode is chosen because—unlike the mean value (Cavill, 1971)—it is not affected by moderate numbers of highly abnormal results which inevitably form an unrepresentatively large proportion of the workload.

Method

PREPARATION OF THE MACHINE

Recalibration of the machine is rarely necessary provided that routine cleaning is carried out. Overnight the counting orifices and diluting chambers are