

The globulin content of the standard solution in the vials is standardized either by a Kjeldahl or a biuret determination. When available Lederle (gamma) globulin diluted 1:200 can be used for this purpose as well. The diluted Lederle standard globulin coagulates on heating and so cannot be used as a sterilized standard.

An interesting phenomenon was observed while preparing the sterilized water-diluted serum. The total protein value determined in the diluted serum before and after sterilization remains constant, but the globulin value determined before and after sterilization showed a marked increase by this denaturation procedure. After renewed concentration as used for the electrophoresis of C.S.F. this solution showed only one globulin fraction and no albumin at all.

Procedure.—(1) Mix 1 ml. standard solution with 1 ml. of ammonium sulphate reagent.

(2) Mix 1 ml. of C.S.F. with 1 ml. of ammonium sulphate reagent.

(3) Mix 1 ml. of saline with 1 ml. ammonium sulphate reagent to be used as blank. Read in the colorimeter with a 420 mm. filter after five minutes.

The comparison of the results of globulin determinations in undiluted sera by the Weichselbaum colorimetric method and the turbidimetric one using diluted sera proved very satisfactory.

Summary

A method is described for the determination of total protein and globulin in biological fluids such as cerebrospinal fluid, urine, etc.

The standard is a sterile aqueous solution of serum which can be used for both the total protein and globulin determinations.

REFERENCES

- Benhold, H., Kylin, E., and St. Ruzsinyák (1938). *Die Eiweisskörper des Blutplasmas*, p. 94. Steinkopff, Dresden.
- Bossak, H. N., Rosenberg, A. A., and Harris, A. (1949). *J. vener. Dis. Inform.*, **30**, 100. See also *Serologic Tests for Syphilis, 1955 Manual*. U.S. Public Health Service, Washington, p. 104 (1955).
- Hiss, P. H. (1904). *J. med. Res.*, **13**, 1.
- King, E. J., and Wootton, I. D. P. (1956). *Micro-Analysis in Medical Biochemistry*, 3rd ed., p. 124. Churchill, London.
- Kingsley, G. R. (1940). *J. biol. Chem.*, **133**, 731.
- Phillips, R. A., Van Slyke, D. P., Emerson, J., Hamilton, P. B., and Archibald, R. M. (1944). *Laboratory Methods of the United States Army*, p. 223. Simmons & Gentskow, Lea & Febiger, Philadelphia.
- Rappaport, F., and Eichorn, F. (1955). *Lab. Digest.*, **18**, 8.
- and Lasowski, E. (1943). *J. Lab. clin. Med.*, **28**, 1640.
- and Loew, M. (1957). *Clin. chim. Acta*, **2**, 126.
- Weichselbaum, T. E. (1946). *Amer. J. clin. Path.*, Tech. Sect., **10**, 40.
- Yeoman, W. B. (1955). *J. clin. Path.*, **8**, 252.

Permanent Sickle Cell Preparations

P. STENTON

From Saint Mary's Hospitals, Manchester 13

(RECEIVED FOR PUBLICATION FEBRUARY 6, 1959)

The usual methods for detecting sickling of red cells consist of the examination of wet preparations of red cells to which bacteria or a reducing agent are added. It would often be an advantage if stained preparations of sickle cells could be obtained for a "permanent" record, for teaching purposes, and for following the development of the sickling phenomenon in the newborn. Very few techniques have been described for the preparation of permanent films. Two methods are described below, the first simple and the second slightly more complex but even so still easy, rapid, and reliable with some advantages over the more simple technique. English (1958) recently described a simple method employing paraformaldehyde which had the disadvantage of liberating noxious formaldehyde fumes into the atmosphere. A technique without unpleasant fumes would be an advantage, and the first method described below is such a technique. Sherman (1940), while investigating techniques for the differentiation of sickle cell trait from sickle cell anaemia, took venous blood under anaerobic conditions and fixed the red cells immediately, taking care to exclude all oxygen until fixation was complete. This method did not reveal sickle cell trait. The basis of the second method, described below, is similar to the method of Sherman, except that the sickling desired is produced artificially by the addition of a reducing substance to a specimen of blood in an anticoagulant collected by venepuncture without any special precautions. Furthermore, using this method sickle cell trait can also be demonstrated.

Methods

Wet Preparations.—Two methods of making wet preparations to demonstrate the presence or absence of sickling were investigated: (1) using sodium dithionate (Itano and Pauling, 1949); (2) using 2% sodium metabisulphite (Lehmann, 1958).

The second technique proved most reliable and consistent, and was adopted for subsequent use.

Method 1.—Red cells were made to sickle in the usual way by mixing one drop of fresh 2% sodium metabisulphite on the centre of a 3 × 1 in. microscope slide with one drop of blood. A $\frac{7}{8}$ in. × $\frac{7}{8}$ in. coverslip was placed over the preparation and the edges were sealed round with paraffin wax/vaseline mixture. The slide was then allowed to stand on the

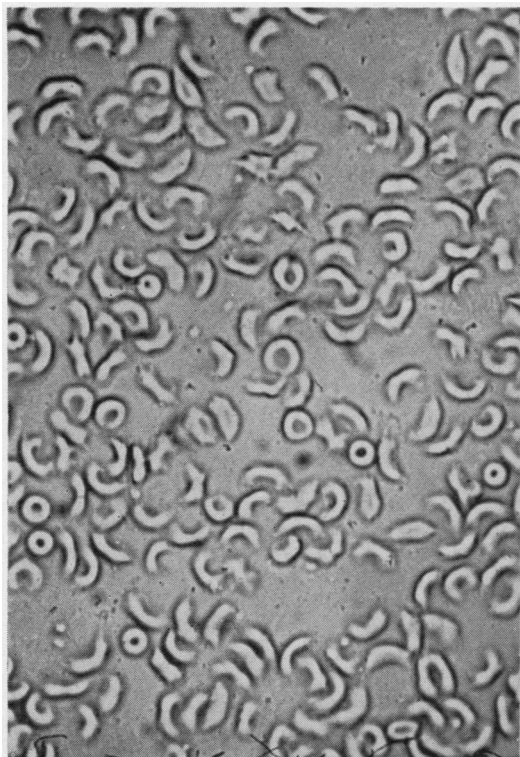


FIG. 1.—Wet preparation; 2% sodium metabisulphite; time taken for cells to sickle 90 min.

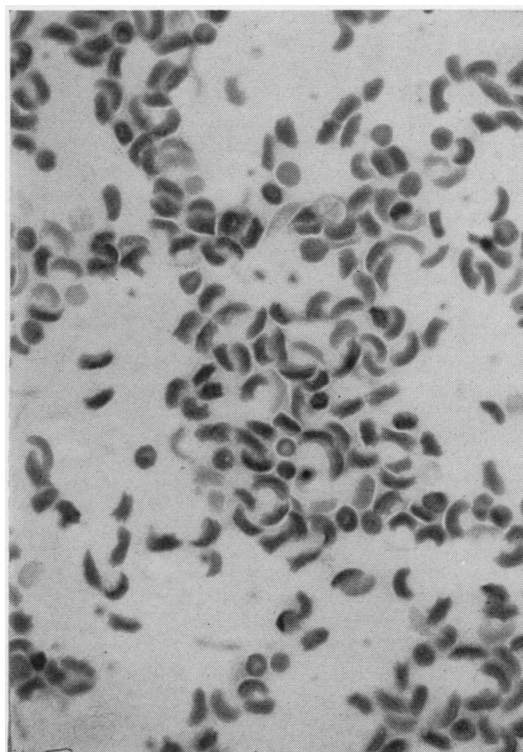


FIG. 2.—Dry stained preparation; tube method; time taken for cells to sickle 90 min.

bench at room temperature, being periodically examined microscopically for the presence of sickling. When sickling was considered to be complete, the coverslip was gently removed in a jet of hydrogen from a cylinder and held in the jet until the film was completely dry. The slide was then placed immediately in a Coplin jar containing acetone-free methyl alcohol and fixed for two minutes. The preparation was then stained in 0.5% alcoholic eosin for two minutes, washed in tap-water, dehydrated through graded alcohols, cleared in xylene, and mounted in "depex."

Method 2.—Into a round-bottomed 10 × 75 mm. tube was placed 0.2 ml. of blood that had been taken into a liquid anticoagulant and 0.4 ml. of fresh 2% sodium metabisulphite, and the suspension mixed. Liquid paraffin was then layered on the surface to a depth of $\frac{1}{4}$ in. This procedure was repeated in 10 tubes which were then allowed to stand on the bench at room temperature. After one hour had elapsed 2 ml. of 10% formal saline was introduced through the layer of liquid paraffin into the red cell/sodium metabisulphite mixture by means of a Pasteur pipette, making quite sure that no air bubbles were allowed to enter. The cells were allowed to fix for 30 minutes. This process was repeated in one tube every half-hour until all the tubes of cells had been fixed. After

a time the cells began to settle in the tubes and it was necessary to keep them in suspension every half-hour by gently rotating the tubes by hand until the cells were once again evenly suspended.

After fixation was complete, the layer of liquid paraffin and a portion of the supernatant were removed by suction. The remaining cells were examined microscopically for sickling. If sickling was present films were made of the red cells on grease-free slides in the usual manner. They were allowed to dry in air and were then stained as described in method 1.

It was found by using the quantities of blood and sodium metabisulphite as described above that the time taken for the cells to sickle was exactly the same as the time taken by the cells in wet preparations and varied in different patients. Fig. 1 shows a wet preparation of cells from a patient with sickle cell trait, and Fig. 2 the same specimen of blood treated as in method 2. The time taken for the cells to sickle in this patient was 90 minutes.

Discussion

Method 1.—Although it is the quicker of the two techniques, method 1 has two disadvantages. The first and most important is that no matter how much

care is exercised in removing the coverslip an uneven film is the inevitable result. The second disadvantage is that if 12 films for teaching purposes are needed then 12 wet preparations have to be set up.

Method 2.—As regards Method 2, however, once the time taken for the cells to sickle has been discovered by the wet preparation, one tube can be set up for the equivalent time, and, using the quantities stated, upwards of 24 films can be prepared, all evenly spread. It follows that if permanent preparations are desired to show the stages of sickling formation this second method is most suitable.

The method of choice for regular, reliable, and most presentable preparations is as follows:

(1) Set up wet preparation and at the same time set up one tube as described in method 2 above.

(2) When the cells in the wet preparation have sickled, add formalin to the tube.

(3) After fixation is complete prepare films and stain as described above.

Summary

Two simple techniques for the permanent preparation of stained sickle cells are described.

In practice it has been found that if the wet preparation and the tube are set up at the same time, the formalin can be added to the tube when sickling is observed in the wet preparation.

I wish to thank Dr. R. F. Jennison for his advice and encouragement in writing up this method and Mr. B. Figg for the photographs.

REFERENCES

- English, H. A. (1958). *J. clin. Path.*, **11**, 457.
 Itano, H. A., and Pauling, L. (1949). *Blood*, **4**, 66.
 Lehmann, H. (1958). Personal communication.
 Sherman, I. J. (1940). *Bull. Johns Hopk. Hosp.*, **67**, 309.

A Trial of Tablet Substitutes for the Gregersen Test

J. E. CULLIS

From St. Thomas's Hospital, London

(RECEIVED FOR PUBLICATION FEBRUARY 2, 1959)

The Gregersen test for faecal occult blood employs benzidine as its chromogenic agent. Benzidine has been implicated as a factor inducing bladder tumours in man (Case, Hosker, McDonald, and Pearson, 1954; Baker, 1953). Supplies of Gregersen's test powders have, therefore, been curtailed and a substitute test has become necessary (Ogilvie, 1957).

Scrutiny of the literature suggested orthotolidine as a substitute (Ruttan and Hardisty, 1912; Kiefer, 1934; Gettler and Kaye, 1943; Hoerr, Bliss, and Kauffman, 1949; Peranio and Bruger, 1951; Hepler, Wong, and Pihl, 1953; Wilcox, 1956; Morgan and Roantree, 1957; Smith, 1958). Orthotolidine, though chemically related to benzidine, has not as yet been shown to have similar carcinogenic properties.

The Gregersen test, as modified by Needham and Simpson (1952), has been used at St. Thomas's Hospital for several years and has also been widely tested and established as clinically valuable in detecting faecal occult blood (Ogilvie, 1927; Meulengracht and Jensen, 1929; Dahl-Iversen and Nissen, 1930; Linn, 1949; Needham and Simpson, 1952; Hughes, 1952; Mendeloff, 1953).

For these reasons the Gregersen test was selected as the standard and the tablet tests, "hematest" and "occullest" (both containing orthotolidine), were selected for comparison. "Hematest" tablets are marketed as suitable for detecting faecal occult blood (Peranio and Bruger, 1951; Wilcox, 1956; Morgan and Roantree, 1957), and "occullest" tablets are marketed as suitable for detecting occult blood in urine (Watson-Williams, 1955).

Experimental

Materials.—Stools from 24 in-patients on a normal ward diet receiving phenindione as an anticoagulant were tested for occult blood. The testing of serial stools from these patients provided an extensive range of Gregersen test results; indeed, in one patient there was a range of Gregersen test results from negative to very strongly positive tests on melaena stools.

Method.—Portions of stool about the size of a walnut were collected in wax cartons and were tested in batches within three days of collection. Thin smears were made from each stool on three pieces of 11 cm. Whatman No. 1 filter paper, one piece being used for each of the three methods. The test tablet