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.

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A Trial of Tablet Substitutes for the Gregersen Test

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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 "occultest" (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 "occultest" 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

was placed in the centre of the faecal smear and flooded with about 2 drops of tap water, sufficient water being used so as to form a moist circle about $1\frac{1}{2}$ in. in diameter. The time taken for the first appearance of a blue ring to form around the tablet was noted, this ring being best seen in the small islands of white paper showing through the thin faecal smear. Any colour appearing on the tablet itself or on the reverse side of the paper under the tablet was ignored. The blue ring around the "hematest" tablet was usually small and developed slowly, while the ring formed around the "occultest" tablet developed rapidly and was larger.

The Gregersen test was performed by pouring about 1 ml. of Gregersen's solution (prepared by suspending 200 mg. barium peroxide and 25 mg. benzidine in 5 ml. glacial acetic acid) over the faecal smear on the third piece of filter paper. The time taken for the appearance of a blue-green or blue colour was noted. The solution was used within five minutes of preparation and was discarded if it turned green or blue.

Time Standards

The time standards for the Gregersen test were those suggested by Needham and Simpson (1952) and Bannerman (1957) and are set out in Table I. The "hematest" time standard was that suggested by the manufacturer of the tablet, a positive test being the formation of a blue ring within 120 seconds. The time standards for "occultest" and the intermediate ones for "hematest" were obtained by comparing the readings obtained by the tablet tests with those of the Gregersen test, and are presented in Table I.

TABLE I
EQUIVALENT TIME VALUES FOR GREGERSEN TEST, HEMATEST, AND OCCULTEST

Results		Gregersen Test	Hematest	Occultest
Strong positive	++	Blue in 5 sec.	Blue ring in 60 sec.	Blue ring in 35 sec.
Positive	..	 15 ..	Blue ring in 100 sec.	Blue ring in 75 sec.
Weak positive	+ -	 30 ..	Blue ring in 120 sec.	Blue ring in 95 sec.
Negative	..	-	No blue ring in 120 sec.	No blue ring in 95 sec.
		No colour change in 30 sec.		

The agreement between the readings when analysed according to the degrees of positivity as shown in Table I was less than that obtained when the readings were simply classified as positive or negative. These latter results are presented in Tables II, III, and IV.

TABLE II
RESULTS IN 208 PAIRED READINGS OF GREGERSEN TEST AND HEMATEST

Hematest		Gregersen Test	
		0-30 sec. Positive	Over 30 Sec. Negative
0-120 sec. positive	..	153	6
Over 120 sec. negative	..	35	14

In 80% of the paired readings both tests agree, while in 17% the Gregersen test was positive while the "hematest" was negative. In the remaining 3% of the readings the Gregersen test was negative and the "hematest" positive.

TABLE III
RESULTS IN 160 PAIRED READINGS OF GREGERSEN TEST AND "OCCULTEST"

Occultest	Gregersen Test	
	0-30 Sec. Positive	Over 30 Sec. Negative
0-95 sec. positive	133	7
Over 95 sec. negative	12	8

In 88% of the paired readings, both tests agree, while in 7.5% the Gregersen test is positive and the "occultest" negative. In the remaining 4.5% the Gregersen test is negative and the "occultest" positive.

TABLE IV
RESULTS IN 160 PAIRED READINGS OF OCCULTEST AND HEMATEST

Occultest	Hematest	
	0-120 sec. Positive	Over 120 sec. Negative
0-95 sec. positive	106	35
Over 95 sec. negative	7	12

In 74% of the paired readings both tests agree, while in 22% "occultest" is positive and "hematest" negative. In the remaining 4% "occultest" is negative and "hematest" is positive.

Discussion

The agreement between the three tests is of the order which might be expected when using crude tests on faeces, a biological material of variable composition. The results show a similar trend to those of Smith (1958). "Occultest" may be considered to be slightly superior to "hematest" when the following factors are taken into account. "Occultest" is quicker, it gives a clearer end-point, and the readings, based on the criteria of positivity adopted, are in closer agreement with the Gregersen test than those of "hematest."

Summary

The carcinogenic properties of benzidine contained in Gregersen test powders have necessitated a substitute test for faecal occult blood.

Two single tablet tests utilizing orthotolidine as the chromogenic agent have been investigated for this purpose.

Two hundred and eight stools from 24 patients on anticoagulation therapy were tested for occult blood using the Gregersen test and "hematest" tablets. In 160 instances "occultest" tablets were also used.

Tabulating the readings as simply positive or negative there was 88% agreement between the

Gregersen test and "occultest," 80% agreement between the Gregersen test and "hematest," and 74% agreement between "hematest" and "occultest."

"Occultest" appears to be a better substitute for the Gregersen test than "hematest."

"Hematest" and "occultest" reagent tablets were kindly supplied by Ames Company (London) Ltd.

I am grateful to Dr. G. I. C. Ingram and others for their help and encouragement.

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BOOK REVIEWS

A Primer in Medical Technology. By Paul M. Kraemer. (Pp. viii + 338; 33 figures. 58s. 6d.) Oxford: Blackwell Scientific Publications; Springfield, Illinois: Charles C. Thomas. 1958.

This book contains 20 introductory lectures for student technicians. Four are devoted to the kidney and urine analysis, eight to haematology, seven to blood chemistry, and one to the cerebrospinal fluid.

The physiology of the kidney and liver, the pathogenesis of the anaemias, and blood group serology are discussed in an elementary manner; and there is a praiseworthy attempt to explain the intricacies of fluid balance. The coverage, however, is nowhere sufficiently complete to provide an examination text for British students, and some of the procedures suggested would not be acceptable here. Thus, for venepuncture, "the syringes . . . need not be sterile since nothing is injected, only withdrawn" (p. 73).

Much of the information relating to normal and abnormal function would be valuable to any student, but it is conveyed in far too many words. The style is often that of the popular lecture.

The limited scope and high price result in a text not likely to find favour with British students.

A. J. McCALL.

Haematological Technique, 2nd ed. By E. M. Darmady and S. G. T. Davenport. (Pp. viii + 244; 23 figures. 24s.) London: J. and A. Churchill. 1958.

That a second edition has been called for is a measure of the usefulness and demand for this handbook. The authors have obviously taken to heart the criticisms levelled at the first edition; extra sections have been added, and the whole presentation has been greatly improved. However, it is still not quite clear to whom the book is finally addressed; laboratory technicians will find two-thirds of it much to their liking, but such chapters, for example, as those on blood grouping, do not sufficiently fill in the background for them and at the same time are not nearly comprehensive enough for medical students. As a third edition will undoubtedly be needed the authors might well address that, and subsequent editions, wholly to laboratory technicians and at that moment, too, they might look again at the illustrations. The book is well produced and is of a useful size to be propped up on the bench.

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