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A new single-drop technique for the observation of dynamic blood coagulation

by BRIAN J. FORD, *Science Unit, Mill Park House,* 57 *Westville Road, Cardiff, Glamorgan*

SUMMARY

A single-drop method is described in which blood specimens may be observed in dynamic coagulation, in an artificially produced cellular environment. The method is eminently suitable for rapid, repeatable observations of hæmostatic phenomena on a routine basis.

INTRODUCTION

The bulk of light microscopic studies carried out on coagulating blood have been on static specimens. Yet coagulating blood under normal *in vivo* conditions is typically in a state of flow, and the mechanisms of coagulation under such hæmodynamic conditions are significantly different from those when the blood is at rest. This disparity has been known for 90 years (Bizzozero, 1882). The inadequacy of the static preparation has been referred to in recent years (Ford, 1965a; Dintenfass, 1971).

To date the only alternative methods of study *in vitro* have been the relatively cumbersome ring-circuit techniques such as those described by Chandler (1958) and Poole (1959). A far simpler method of observation, using in-specimen flow induced by peripheral pressure on the preparation coverslip, has been described (Ford, 1965b) in which dynamic aspects of coagulation can be observed. However, there remained a need for a rapid, reliable means of observation that was adaptable to routine use in research. This paper describes a suitable single-drop technique which has been found to give excellent results on a routine basis. No special apparatus is required.

METHODS

The most recently described techniques for examining blood flow *in vitro* (Bull *et al.*, 1967) are complex and introduce a degree of artificiality into the behaviour of the specimen that may materially alter its condition from that found in life. Clearly, the less interference with the specimen the better. For this reason the experiments in the present programme of research have centred on a single drop of freshly-drawn blood obtained by fingerprick. The technique that has been derived relies on the capillarity exerted between the slide and the preparation coverslip. With care it is possible to induce the formation of pseudocapillaries through the consolidating coagulum which provides a suitable source of dynamically coagulating blood for microscopy. Drops of fresh blood are collected on

cleaned 3 × 1 in. slides and allowed to stand for a period sufficient for peripherae drying to occur and also for coagulation to become initiated. In the present trials a time of 7 min has been considered optimal. At the end of this time the coagulating blood is surrounded by a gelled 'ring' of blood, and the addition of a coverslip exerts sufficient centrifugal pressure to cause progressive breakdown of this peripheral region, with the formation of one or more undulating channels that may later divide to form pseudocapillaries. The specimen can be transferred to the stage of a microscope with ease, and may be examined by any conventional means of optical microscopy except oil-immersion (which disturbs the delicate balance of the preparation). Routine observation of certain coagulation abnormalities is facilitated (Ford, 1970).

The element of artificiality in this technique is not great and may be significantly less than in the more elaborate alternative methods available. This technique provides a unique opportunity to examine blood flow:

(a) within minutes of obtaining the sample;
(b) without any sophisticated apparatus;
(c) in an *in vitro* cellular situation.

The last factor is perhaps the most interesting of all. *No other technique provides a cellular micro-environment for the flowing blood.* This, as has been emphasized, is a prerequisite for studies that approximate to the *in vivo* condition.

TECHNIQUE

The stages in obtaining a preparation for microscopical examination are as follows:

(1) The skin is cleansed with a pad moistened with ether. This has a number of effects.

(a) it tends to remove micro-organisms from the dermal surface;
(b) it seems to act to a certain extent as a local anaesthetic agent;
(c) it removes oil from the epidermis which might contaminate the specimen and prevent the even spread of the blood droplet.

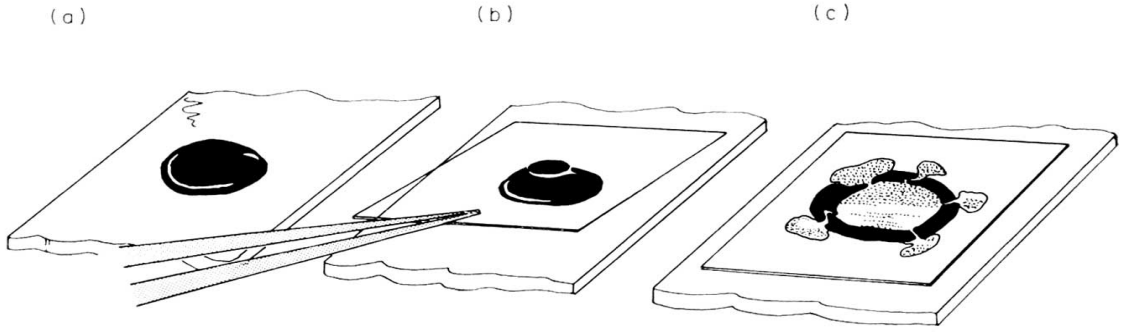

Fig. 1. Diagrammatic view of three stages in preparing the single-drop slide. (a) At commencement of preparation, with fingerprick blood specimen on standard slide. (b) Addition of a No. 1½ coverslip at 7–8 min (a series of slides is normally examined at 60-sec intervals, commencing after 5 min have elapsed). (c) Appearance after several minutes. The position of undulating pseudocapillary structures is visible in the peripheral area of the blood sample. Further flow of the sample through the channels is due to capillarity between the slide and its coverslip and will continue for as long as 20 min in favourable circumstances.

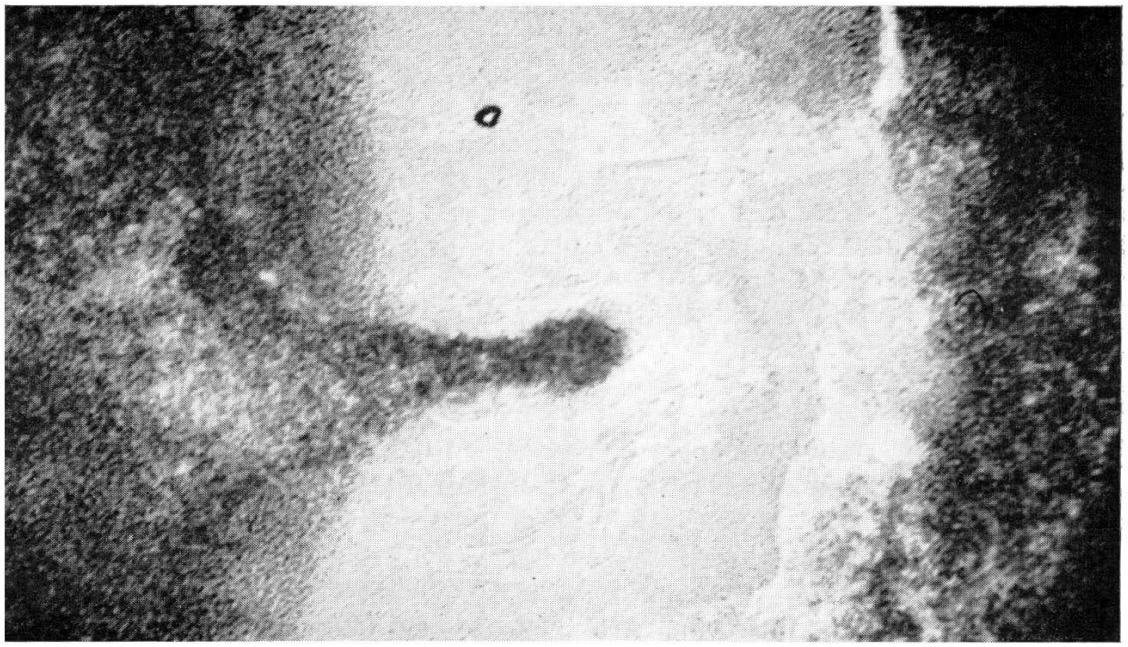

Fig. 2. Low-power appearance of peripheral area of preparation after application of coverslip. Note the 'tongue' of flowing blood moving through the coagulum gel from left to right.

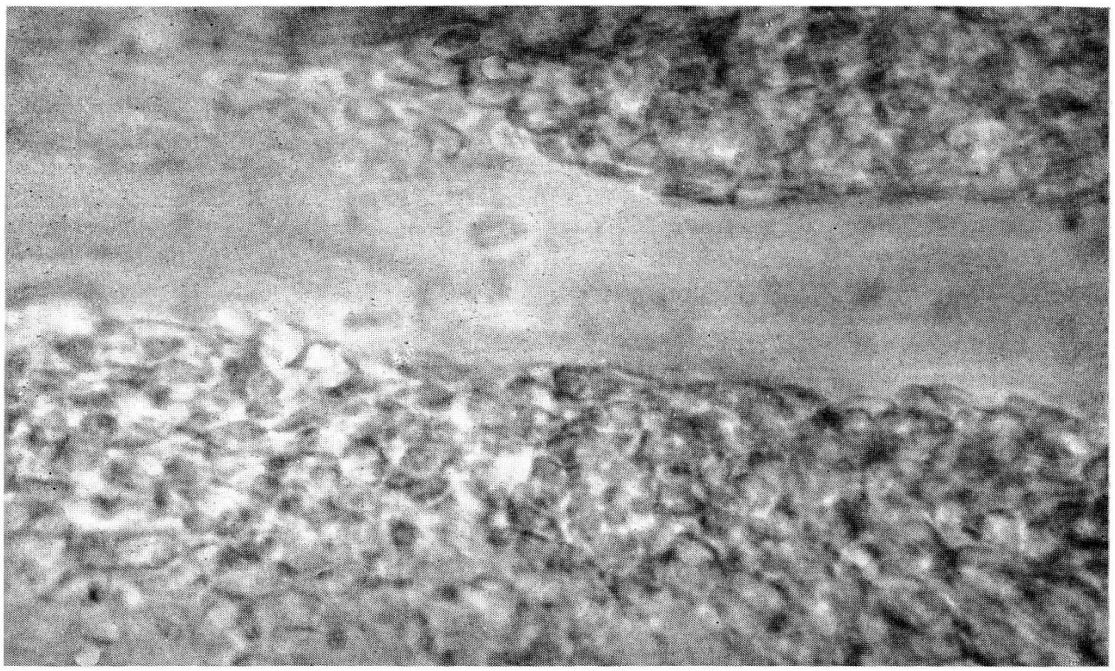

Fig. 3. High-power light ground appearance of pseudocapillary 4 min after Fig. 1. Moving cells flowing across the field show as blurred streaks. Note tissue-like appearance of stationary cells around the pseudocapillary itself.

(2) The nailbed is lanced with a Hagedorn needle, a Hemolet ® or a splinter of glass from a freshly-broken slide. A stopwatch is started at this time.

(3) Successive drops of blood are collected on 3 × 1 in. slides and are set down in series on the bench or in a slide tray.

(4) At intervals of 60 sec, commencing after—say—5 min, slides are taken for examination. If this time is found to be too long they may naturally be taken earlier, though it has been found that approximately 7 min is optimum.

(5) A coverslip is added to the slide and is lowered gently into position with a seeker or mounting needle. No. $1\frac{1}{2}$ coverslips are recommended for high-power use. The drop of blood may be seen to spread but may not yet break through the peripheral hardened area.

(6) The slide is placed on the microscope stage and examined with a low-power objective. Areas where the haemostatic pressures lead to breakdown of the peripheral 'ring' are clearly seen as erythrocytes, carried by the moving plasma, begin to migrate towards them.

(7) Examination under high-power magnifications will show the configuration of cells within the pseudocapillaries that form. In particular, the changes during coagulation, and the means by which more general hæmostasis is effected, may be clearly observed.

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