Differentiation of foetal and maternal erythrocytes in formol-fixed tissues

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The elution techniques of Kleihauer, Hildegard, and Betke (1957) and of Singer, Chernoff, and Singer (1951), as applied to peripheral maternal blood, have provided valuable methods for determining the severity of trans-placental bleeding. Their application to formol-fixed tissues has not proved successful in demonstrating the sites of admixture of the foetal and maternal circulations. A differential destruction technique applicable to formol-fixed placental tissue is therefore described.

MATERIALS AND METHOD

Full-term placentae were fixed whole in 10% formol saline for four days before representative blocks were cut for section. Tissues were processed in the normal way for paraffin sections and cut at 5μ . Sections were dewaxed and taken through to water. They were then exposed at 37°C to peptic digestion in an acid buffer solution. After washing in water for 10 minutes, they were then stained by Erhlich's haematoxylin and alcoholic eosin.

SOLUTIONS

- 1 Pepsin (BPC) 25 units per ml in distilled water
- 2 Glycine buffer
 - A Glycine 7.505 g
 - NaCl 5.85 g
 - Distilled water to 1 litre
 - B 0.1 N HCl

Thirty-eight ml Solution A and 62 ml Solution B provided a buffer solution at pH 1.8.

Sections were incubated at 37°C for varying periods in petri dishes containing 1.25 ml pepsin solution and 5.0 ml buffer solution, the final concentration of pepsin being 5 units/ml.

RESULTS

Incubation for periods in excess of 10 minutes resulted in complete destruction of both foetal and maternal erythrocytes. Destruction of maternal red cells was seen to start after four minutes' incubation and was complete after eight minutes. Destruction of foetal red cells was not appreciable until after eight minutes' incubation. At eight minutes a clear distinction between the two types of cell was demonstrable (Figs. 1 and 2). Little change was seen in the histological detail of the remaining tissue even after 40 minutes' incubation.

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FIG. 2. Plasma haemoglobin in samples of normal human blood.

FIG. 3. Plasma haemoglobin in 19 samples of normal blood.

haemoglobin (g/100 ml) of the control tube from the haemoglobin (g/100 ml) of the rotated tube.

Figures 2 and 3 show the effect of varying the time of rotation or concentration of Celite on the haemoglobin released from samples of normal human blood. In Table I the erythrocyte fragility of samples of normal human blood is recorded. Human subject R.K. (Table II), studied on four different occasions, revealed small variations in erythrocyte fragility.

The amount of blood or Celite and the time of rotation can be varied to improve the degree of precision and to suit the convenience of the laboratory.

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REFERENCE

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FIG. 1. Normal full-term placental tissue to show distribution of maternal and foetal erythrocytes. Haematoxylin and eosin $\times 400$.

FIG. 2. Normal full term placental tissue, after eight minutes' peptic digestion to show survival of foetal red cells within the villous capillaries. Haematoxylin and eosin $\times 400$.

REFERENCES

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Mechanical rotary device for plating out bacteria on solid medium

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Most clinical bacteriological investigations start with streaking or plating out of material containing bacteria on to the surface of solid medium so that isolated colonies appear during incubation. Further plating of single colonies may be necessary to ensure purity of the culture, or to obtain larger amounts of the bacterium under study. Plating is usually carried out by streaking inoculum on to agar in such a way that a progressive surface dilution is obtained. Conventionally, sterilized wire loops, mounted in metal handles, are used for this purpose, though some workers favour the use of glass rods with rounded ends.

Whatever method is used, plating out is tedious and a time-consuming process, and may absorb a major proportion of the work of a busy laboratory; this article describes a mechanical device designed to eliminate much of the tedium and fatigue associated with the procedure.

DESCRIPTION OF THE MACHINE

The rotary plater is essentially an electrically driven horizontal circular metal turntable with a raised lip around the circumference. This is mounted on a central spindle, grooved at the end to fit on to a shaft in the motor so that the turntable can easily be detached and replaced for cleaning and sterilizing. Turntables of different diameters can be used to accommodate various sizes of petri dish.

The rotary unit is powered by a shaded-pole motor with 90 milliamps output and 20 Watts consumption which drives a heavy-duty gear base at a constant speed of 58 revolutions per minute, and this is mounted in a stove-enamelled metal case fitted with a switch and red warning light. There are 6 feet of flex and the unit is suitable for 200/250 volt main operation.

MODE OF OPERATION AND USES

An open petri dish is placed on the rotating turntable and the wire loop loaded by touching a colony; the user sits with one or both elbows resting on the bench top beside the machine and the loop handle is held so that the wire points towards the operator. The loop is lowered to touch the circumferential surface of the agar nearest to the operator and, as the dish rotates, it is drawn radially to the centre of the plate and lifted off. In this way, a spiral track is covered by the loop, and the distance between the coils of the spiral can be varied by increasing or diminishing the rate of radial traverse. As the loop moves

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