**Brief Report: Method**

**A Quantitative Rebuck Technic**

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Increasing interest in the biology of the macrophage and its role in disease has made desirable a simple and acceptable technic for obtaining these cells in useful quantity and in a fairly pure state from patients and healthy persons. The technic here described fulfills these requirements. It was inspired by and modified from the "quantitative Rebuck technic" of Perillie and Finch but is simpler, more acceptable to the person under study, and suitable for more prolonged periods of observation. Its use for the comparison of "wandering cell" response of cancer patients and healthy subjects has been reported and current unpublished work indicates that it is useful for the collection of fairly pure populations of neutrophils or macrophages for tissue culture, and for the study of cellular responses to antigenic stimuli such as homograft sensitivity.

**METHOD**

A small area on the volar surface of one forearm is cleansed with alcohol and anesthetized by ethyl chloride spray. An area roughly 1 cm. in diameter is then abraded by scraping with a number 21 Bard Parker scalpel blade while the skin is stretched taut, until the dermal papillae are seen as tiny red spots. This is sufficient to induce an exudative reaction without bleeding. To this point the technic is identical with the method used in this laboratory for the standard qualitative type of Rebuck "skin window" technic, but instead of applying a microscope cover glass directly to the abraded area, a small flat cup-like vessel containing a physiologic salt solution is applied so that the exuded cells pass directly into the fluid.

The "cup" is the Sykes-Moore tissue culture chamber from which the glass window on one side is omitted. This chamber is available commercially from Bellco Glass Inc., Vineland, N. J. It consists of an outer stainless steel ring into which is fitted a circular microscope cover glass and a rubber gasket which occludes four holes which pass radially through the steel ring. A second steel ring screws into the first compressing the gasket so that it is tightly sealed to the glass, thus forming a shallow cup (Fig. 1). The assembled device may be sterilized by boiling or autoclaving, or the components may be sterilized separately and assembled aseptically just before use.

The sterile empty cup is placed over the abraded area of skin with the open side in contact with skin and is held in place with adhesive tape so placed as to leave access to at least two of the holes in the steel ring (Figs. 2 and 3).
Fig. 1.—Sykes-Moore chamber as used for collection of the cellular exudate. The four components—inner and outer steel rings, rubber gasket and single glass—are shown below. One of the four holes through which the chamber is filled can be seen in the assembled chamber in the Petri dish.

The chamber is then filled with an isotonic salt solution such as Gey’s tissue culture solution containing 0.15 per cent EDTA. EDTA was added because it kept the cells well dispersed, presumably by preventing fibrin formation, although no clotting was detected when the anticoagulant was omitted.

We have avoided the routine use of any protein or antibiotics in the fluid because antigenic materials might alter the cellular reaction or induce a sensitivity.

A 27G needle is pushed through one of the holes, penetrating the rubber gasket, into the empty cup to provide an air vent. The fluid is then carefully introduced by syringe and needle through another of the holes. (see Fig. 3). The chamber will hold approximately 1 ml but we usually insert 0.8 ml., leaving a small air bubble which reduces the possibility of leakage. After removing the needles the chamber is secured more firmly by an Ace bandage applied sufficiently tightly to hold the chamber in place without causing discomfort or congestion of the hand.

After any desired interval the fluid in the chamber is removed by again inserting one needle as an air vent and aspirating the fluid into a syringe and needle inserted through another of the holes. Residual cells may be flushed out by refilling and reaspirating fluid from the chamber immediately, and should be added to the original harvest and the total volume recorded. The chamber may then be refilled in the same manner for another period of observation. If the objective is to obtain macrophages, the chamber should be left in place for 24 hours after the first application and the first collection of cells discarded because polymorphonuclear neutrophils predominate. The cells collected in the subsequent few hours will include a high percentage of macrophages (large mononuclear cells with abundant cytoplasm and an open reticular nucleoplasm), usually over 50 per cent from healthy
Fig. 2.—Abraded area on forearm of a test subject, showing the manner in which the epidermis is denuded by repeated scraping with a scalpel blade. Dermal papillae can be seen as tiny spots in the shaded area.

persons. We have often left the chamber in place for a second 24-hour period without complaint or technical difficulty.

When the chamber is finally removed, the abraded area may be covered with a simple dressing (Band-aid) but needs no further attention. A scab will form but the area should be completely reepithelialized in 6 to 8 days.

The harvested cell suspensions may be counted in the same manner as blood counts. In our hands the yield is usually between 1 and 10 million nucleated cells. Cell types are determined by centrifuging the suspension and smearing and staining the pellet in the same manner as blood smears. The cells may also be utilized for tissue cultures, cinemicrography, etc.

When the objective is to study specific immune responses, antigens can be introduced into the chamber fluid. In current studies of homograft sensitivity, we are introducing the target cells either as a suspension in the chamber fluid or as tissue culture monolayers grown on the inner surface of the cover slip prior to its application to the person under study.

As compared with the qualitative Rebuck technic in which the exuded cells attach directly onto a cover glass, there is a slower and less complete transition from the initial granulocyte exudation to a preponderantly macrophage response. In studies of normal subjects, the cells collected between 24 and 26 hours usually contained 40 to 60 per cent granulocytes, whereas the cover slip technic usually gives less than 30 per cent granulocytes during that period. This suggests that the presence of the salt solution or the EDTA which it
Fig. 3.—Chamber in place and being filled. Needle on left serves as a vent. After filling the chamber, needles are removed and an elastic bandage is used to protect the chamber and hold it firmly in place.

contains, or the presence of or changing of the fluid, may act as more of a continuing irritant than the glass cover slip alone.

Summary

A simple technic is described by which the cells exuded from a skin abrasion can be collected as a cell suspension suitable for quantitation and for further study of the living cells. The technic is identical in principle to the quantitative modification of Rebuck's "skin window" technic which was devised by Perillie and Finch, but the smaller flatter chamber is more easily affixed to the arm and rarely leaks and can be left in place overnight without interfering with sleep. Hence, it is more acceptable to the subjects under study and technically more satisfactory. It provides a method of obtaining normal cells and of studying the cellular reaction to various antigenic or nonantigenic stimuli.

Summario in Interlingua

Es describite un simple technica per medio del qual le cellulas exsudate ab un abrasion cutance pote esser collectionate como un suspension cellular appropriate pro quantification e pro studios additional de cellulas vive. In principio, le technica es identic con le modification quantitativa del technica de Rebuck del "fenestra cutanea" que esseva ideate per Perillie e Finch, sed le plus micre e plus plan camera es plus facilmente affixabile al bracio, sufre escappamentos solo rarmente, e pote esser lassate in sito durante le nocte sin
interferer in le somnio del subjecto. Per consequente, illo es plus acceptabile ab le puncto de vista del subjectos studiate e es technicamente plus satisfacente. Illo provide un methodo pro obtener cellulas normal e pro studiar le reaction cellular a varie stimulos antigenic o non-antigenic.

REFERENCES


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