A technique for rapid isolation of macrophages from guinea-pig peritoneal exudates

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Preparations of pure macrophage suspensions may be required in the investigation of cell-mediated immune reactions or for the preparation of antimacrophagic serum. Methods for the preparation of macrophage suspensions of high purity from peritoneal exudates are relatively tedious and result in small yields of cells. Bloom and Bennett (1966) and Turk and Polak (1967) describe a technique in which guinea-pig peritoneal exudates are incubated in Petri dishes at 37°C in an atmosphere containing 10% CO_2 for four to 24 hours. Those cells which adhere to the glass surface and cannot be removed by vigorous shaking are released by treatment with a 1/5000 solution of ethylenediamine tetraacetic acid. They reported their cell preparations to contain 99·4% to 99·8% macrophages but yields were not given. This technique depends on the property of macrophages to stick firmly to a glass surface, which distinguishes them from lymphocytes.

An albumin gradient sedimentation method described by Vallee, Hughes, and Gibson (1947) has been applied to the problem of isolating tumour cells from human peripheral blood (Roberts, Wayne, McGarth, McGrew, and Cole, 1958) and a modification was used for a similar purpose by Spriggs and Alexander (1960). We report here the use of a simple column of two layers of bovine serum albumin to effect differential sedimentation of guinea-pig peritoneal exudate cells.

Method

Exudates were induced in Hartley guinea pigs of 450 g to 800 g weight by intraperitoneal injection of 20 ml sterile liquid paraffin. Exudates were harvested after six to 14 days by intraperitoneal injection of 50 ml heparinized Hanks balanced salt solution (5 units heparin/ml). The exudate suspensions were withdrawn and centrifuged in conical tubes at 250 g for 10 minutes. The cell pellets were taken up in 1 ml volumes of Hanks solution and transferred to a single 10 ml centrifuge tube and washed twice in Hanks solution. Cell yields ranged from 80×10^6 to 250×10^6 leucocytes. Maximum yields were obtained nine to 13 days after injection of liquid paraffin, and showed no close relation to the

size of the animal. Cell populations contained 5% to 15% lymphocytes. Considerable difficulty was encountered in the identification of some cells. A lymphocyte was considered to be a small round cell with a deeply staining nucleus, a thin rim of pale blue cytoplasm when stained with buffered Giemsa, and at most minute vacuoles.

For differential sedimentation two layers of bovine serum albumin (BSA, fraction V, Armour) were used. A column was made in a siliconed 10 ml centrifuge tube of 2 ml '20%' BSA in Hanks solution (2 g BSA + 10 ml Hanks) onto which a similar volume of '5%' BSA in Hanks solution was carefully placed so that the interphase was not disturbed. Exudate cells, taken up in 2 to 3 ml of Hanks solution, were then carefully layered onto the BSA gradient and centrifuged at 450 g for 10 minutes. The exudate cells separated out into three distinct layers. Paraffin globules floated on the surface of the Hanks solution, whilst cell debris remained on the surface of the '5%' BSA layer. Lymphocytes, erythrocytes, and some of the macrophages formed a pellet at the bottom of the tube, whilst between the two layers of BSA was a thick layer of macrophages. This layer gave yields of 20% to 50% of the total available macrophages and consisted of 99% to 100% of these cells. The majority of these macrophages contained more than one large paraffin-filled vacuole, so it seems probable that the mechanism of separation depends on a decrease in density caused by ingestion of paraffin.

This method is less satisfactory for the isolation of pure lymphocyte suspensions from the same exudate since, although lymphocyte-enriched populations may be obtained, macrophagic contamination is always present. Lymphocytes may be rapidly obtained from the same animal as peritoneal exudates using heparinized or defibrinated blood with the carbonyl iron ingestion-methyl cellulose sedimentation method of Coulson and Chalmers (1966) as modified by Hughes and Caspary (1970).

Using the technique of Bloom and Bennett (1966) we experienced a recovery of 10 to 20% of total available macrophages, sometimes contaminated with 5% lymphocytes. The BSA column offers the advantage of being a rapid (less than one hour) and consistent method of obtaining large numbers of macrophages.

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Letter to the Editor

The Disposable Glass Culture Tube as a Cuvette

Since October 1967 we have had extensive experience using disposable test tubes as disposable cuvettes in haematology for doing haemoglobin determinations and in clinical chemistry generally. The 13 by 100 mm test tubes are most convenient, but we have had experience with 12 by 75 mm and 10 by 75 mm tubes as well. We have extensively used the Coleman Jr, Coleman Jr II, and the Spectronic 20 spectrophotometers; some personal experience with instruments passing a broad beam of light through the cuvette lead to erratic results. Larger tubes, 16 mm and 19 mm diameters, are not uniform. The thin-walled disposable flint glass and borosilicate tubes made by Kimble have been mainly used. We have also had experience with Pyrex tubes by Corning.

The uniformity of the disposable test tubes seems equal to commercially available cuvettes and superior to re-used cuvettes that are scratched and routinely used as demonstrated in the Table below.

The disposable cuvette is faster and more trouble-free than flow-through cuvettes and has increased the output in our clinical chemistry section. The disposable test tube costs approximately 1·3 cents (US) each. Besides the convenience of being disposable, they are packaged clean and free of scratches.

We have recently changed over to the routine use of borosilicate as we noted the presence of residual alkali in the flint glass tubes. However, we did not encounter any interference due to residual alkali which was probably buffered by patients' specimens and/or reagents. Recently we have seen a thick-walled grade of disposable tubes from a new supplier. The thick-walled tubes are not optically uniform for use as disposable cuvettes.

We have not noticed any significant increase in day-to-day precision on aliquots of frozen pooled serum for the tests used in clinical chemistry. For haemoglobinometry, using a cyanmethaemoglobin method over a two-year period and aliquots of frozen citrated whole blood, our day-to-day precision, as 2SD, varied from 0·4 to 0·5 mg per 100 ml using specimens with mean values of 12 to 13 g per 100 ml.

We conclude that the use of 10 to 13 mm diameter thin-walled disposable glass tubes as disposable cuvettes is justified. Verification of our experience should be sought if one wishes to use disposable test tubes or instruments other than those found satisfactory in this communication.

Type of Tube	No. of Tubes	OD			2CV (%)
		Mean	Range	2SD	
Spectronic 20 cuvette, from daily reused supply[1]	42	0·468	0·455-0·490	0·020	4·2
Spectronic 20 cuvette, selected for adequate condition[2]	32	0·464	0·455-0·480	0·014	3·0
Disposable, flint glass culture tube	275	0·455	0·442-0·465	0·010	2·2

Table   *Optical densities of cuvettes and disposable tubes with rotation*

[1]On inspection, some of these were noticeably scratched.
[2]These were inspected, so that they were all unscratched.

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