which, when the automatic apparatus is used, is valid.

A more serious difficulty is that of standardization
and is not peculiar to Pearson's method; it is solved by
the use of a commercial standard serum of the Versatol
type. Only comparison with a standard serum of good
quality is valid (Rivin et al., 1958), for artificial titrated
solutions never have exactly the same degree of humidity
as the serum and the slightest difference in the quantity of
water causes enormous differences in the intensity of
Liebemann's or Salkowski's reactions.

Since the time of the circuit is rigorously constant, it
has been possible to study the influence of continual
variations in temperature (Fig. 2). A temperature of
65°C. appears best because between 56°C. and 72°C.
variations have very little effect on the development of
the colour; thus even a small variation in the thermostat
does not produce appreciable errors (Fig. 3). Furthermore,
at this temperature the levels of the colour for free
cholesterol and for esterified cholesterol are identical
(Girard and Assous, 1962).

SUMMARY

The use of an automatic apparatus with a strictly con-
tant time for the reaction and the mixing processes
eliminates the errors observed in the manual methods.

An installation is proposed in which the temperature
is fixed in such a way that reproducibility is good such
as was never achieved manually with series including
many samples.

REFERENCES

be about 0.5 mm in diameter. It was then connected to a mouthpiece by a short glass adapter and a piece of polyethylene tubing. The fine glass pipette was guided into the narrow stem of the 'oil bottle' and then the narrow tip of the pipette was placed just within the lymphocyte-monocyte layer. Gentle mouth suction was applied to draw the lymphocytes and monocytes into the pipette.

Aliquots of the lymphocyte suspension were spread on glass slides and stained with Giemsa stain. Differential counts were performed on these preparations. Staining of the cells with trypan blue was performed according to the method of Pappenheimer (1917).

To test viability directly, lymphocytes, both of autologous or homologous derivation, were sealed in an Algire type micro-diffusion chamber constructed of Millipore filter type HA (0.45μ pore size) and lucite. Chambers, each containing about 10⁸ lymphocytes, were placed subcutaneously into 32 patients. These chambers were removed two to four weeks later and stained with Giemsa and Feulgen stains.

RESULTS

Table 1 shows the result of 10 consecutive lymphocyte separations in people with normal total white blood counts and differentials.

The degree of purity of the lymphocyte-monocyte fraction ranged from 99.5% to 91.0%. The number of red cells present per 100 white blood cells ranged from zero to 8. The morphology of the cells obtained with this technique was normal as judged by Giemsa staining (Fig. 2). Trypan blue staining showed 0% to 3% of the separated lymphocytes to be stained.

These lymphocyte-monocyte fractions placed in diffusion chambers were transformed into fibroblasts and giant cells, indicating that these cells were viable. The growth of homologous lymphocytes was no different than that of autologous lymphocytes. The persistence of the cellular homograft was proven by the presence of the female chromatin marker in the nuclei of female cells growing in diffusion chambers implanted into male patients.
**Technical methods**

**TABLE I**

<table>
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<th>Case No.</th>
<th>Total W.B.C. (per c.mm.)</th>
<th>% Lymphocytes and Monocytes in Differential Count</th>
<th>Differential after Separation per 200 Cells</th>
<th>No. R.B.C. per 200 W.B.C.</th>
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**DISCUSSION**

The oil bottle is used commercially to measure the amount of sediment in oil. The advantage of the oil bottle for collecting human lymphocyte-monocyte fractions is that in one step it concentrates the cellular elements from a large volume of plasma into a narrow tube, and thus circumvents the need for multiple centrifugations and pooling of lymphocytes from several ordinary test tubes. Also the calculation necessary for various types of ‘middle constricted’ bottles is not needed. Because only one centrifugation is required, the time between the collection of blood and the final collection of lymphocytes is under one hour. The viability of cells collected in this way is demonstrated by their ability to proliferate in subcutaneously placed micro-diffusion chambers. Since the minimal numbers of cells necessary to ‘seed’ a diffusion chamber is not known, the percentage of the viable cells present cannot be determined using this method. However, trypan blue staining indicated a high percentage of viable cells. This technique should also be suitable for finding tumour cells in peripheral blood, since tumour cells and lymphocytes are usually found in the same fraction; large numbers of pure platelets or polymorphonuclear leucocyte fractions may also be harvested using this technique.

**SUMMARY**

Attention is called to a commercially available ‘oil bottle’, well suited for the purpose of separating lymphocytes and monocytes and other cell fractions from human blood. A technique utilizing this bottle has been found to be rapid, simple, and reliable; viable lymphocyte-monocyte fractions are obtained.

**REFERENCES**