Re-utilization of Lymphocytes in Lymphopoiesis. By O. A. Trowell. (*From the Radiobiological Unit, Medical Research Council, Harwell, Berkshire, England.*)*

It is generally thought, but not proved, that the life span of the lymphocyte is short, probably only a few days. But in recent experiments, in which human lymphocytes were labelled in the DNA by P^{32} (Osgood *et al.*, 1952; Ottesen, 1954; Christensen and Ottesen, 1955) or by adenine-8-C^{14} (Hamilton, 1954, 1956), it was found that labelled lymphocytes were still present in the blood after several months. In Hamilton's experiments, after 300 days the radioactivity of the lymphocyte DNA was still more than one-third of the peak activity. This means either that some lymphocytes live for more than a year, or, as Hamilton has suggested, that when lymphocytes die their nucleic acids are specifically re-utilized, without degradation, in the genesis of new lymphocytes. The purpose of this paper is to examine the re-utilization hypothesis from the histological angle and to present some evidence in its support.

In the terminology of Maximow the stages of lymphopoiesis are:—

Reticulum cell → Large lymphocyte → Medium lymphocyte → Small lymphocyte

The exact nature of the "reticulum cell" in this context is still not clear. Maximow (1928) stated that there were two separate types of reticulum cell, the one purely phagocytic belonging to the reticuloendothelial system, the other a "primitive" reticulum cell which was lymphopoietic and not phagocytic. This view was supported by Ehrich (1931) and by Cunningham, Sabin, and Doan (1925), and is still widely accepted (Maximow and Bloom, 1952; Yoffey and Courtice, 1956). The evidence, however, is unconvincing and it is equally arguable that there is only one sort of reticulum cell which is both phagocytic and lymphopoietic, a view, incidentally, which Maximow originally held in 1923.

It is well known that many effete small lymphocytes are phagocytosed by reticulum cells in the germinal centres of lymphoid tissue; their pyknotic remains, the "tingible bodies" of Flemming, are a normal histological feature. Kindred (1942) estimated that about 35 per cent of the lymphocytes produced in rat lymph nodes are eventually disposed of in this way. Ehrich (1946) went so far as to say that "the germinal centres are not only the birthplace but also the graveyard of most of the lymphocytes." Now, if these cells which have phagocytosed small lymphocytes, themselves differentiate into large lymphocytes, it is easy to see how the nucleic acids of the old lymphocytes could be specifically re-utilized in the formation of new ones. Evidence that such a process may occur was found in the following experiments.

Rat lymph nodes were cultured *in vitro* by the method of Trowell (1954) in a synthetic medium (Trowell, 1955). It was regularly found that after a few days many of the reticulum cells were in process of differentiation into large lymphocytes. Plate 100 shows cells from four-day cultures. Figs. 1 to 3 are reticulum cells, Figs. 4 to 7 are large lymphocytes, and Figs. 8 to 13 show intermediate stages between the two (for further details see Trowell, 1955). It was also observed that sometimes these "intermediate" cells still contained the pyknotic remains of phagocytosed small lymphocytes (Figs. 14 to 19). This is the crucial point. It is evidence that cells which had eaten up small lymphocytes were

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differentiating into large lymphocytes, it provides a histological basis for the re-utilization hypothesis, and it disposes of the need for "primitive" reticulum cells.

Cells like those shown in Figs. 14 to 19 have not so far been found in normal lymph nodes *in vivo*. This might be because the differentiation of reticulum cells occurs much more quickly *in vivo* than *in vitro*, so that very few intermediate cells are present. It must be conceded, however, that normal lymphopoiesis proceeds to a considerable extent by mitosis of large and medium lymphocytes; the contribution of reticulum cells is, quantitatively, uncertain. These experiments only show that re-utilization of lymphocytes can occur in rat lymph nodes *in vitro*, but they reveal a histological mechanism which could account for the rather unexpected results obtained with labelled lymphocytes in man.

De Bruyn (1948) and Ringertz and Adamson (1950) noted that the germinal centres which contained the greatest number of phagocytosed dead lymphocytes were also the most mitotically active. This suggests that dead lymphocytes may be the natural food of reticulum cells and a stimulant of lymphopoiesis. If so, it would constitute a self-regulating "feed-back" mechanism governing the total lymphocyte population of the body.

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EXPLANATION OF PLATE 100

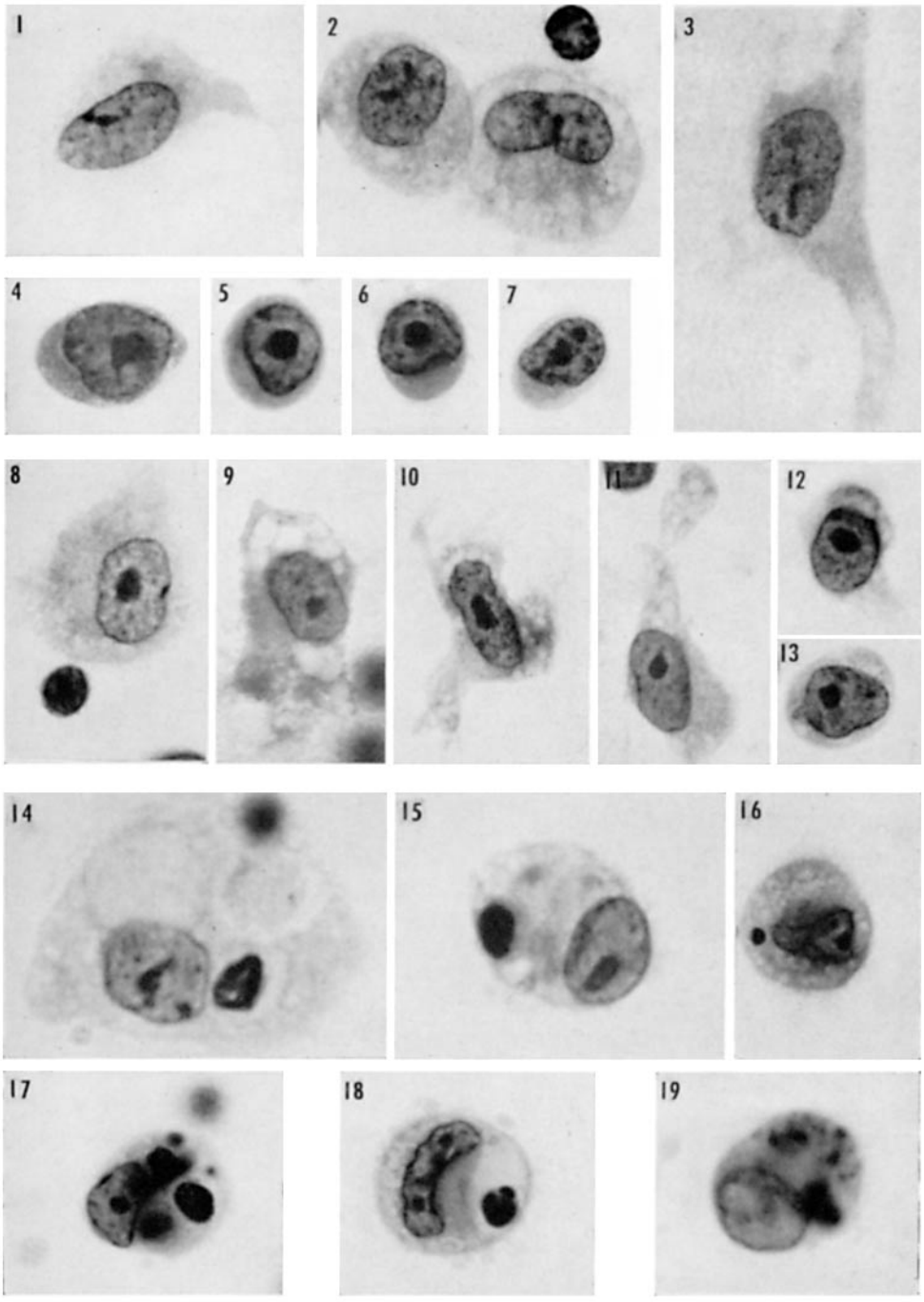
Cells from four-day cultures of rat lymph nodes. Cell suspensions, wet fixed in Heidenhain's susa, stained with haemalum. × 1400.

FIGS. 1 to 3. Reticulum cells. Note oval nucleus, thin nuclear membrane, small nucleoli, abundant faintly-basophilic frothy cytoplasm.

FIGS. 4 to 7. Large lymphocytes. Note round nucleus, thick nuclear membrane, large nucleolus, small amount of homogeneous basophilic cytoplasm.

FIGS. 8 to 13. Reticulum cells differentiating into large lymphocytes. Characters intermediate between reticulum cell and large lymphocyte.

FIGS. 14 to 19. Similar differentiating cells containing pyknotic debris of small lymphocytes in their cytoplasm, thus proving their previous or present phagocytic property.



(Trowell: Re-utilization of lymphocytes in lymphopoiesis)