THE PREPARATION OF MORPHOLOGICALLY INTACT LEUKOCYTES FROM PERIPHERAL BLOOD BY MEANS OF GRAMICIDIN AND LYSOLECITHIN

By Thomas P. Singer, M.D., Inge Lore Silberbach, and Samuel Schwartz

AN INVESTIGATION of the metabolism of intact leukocytes offers a unique opportunity for studying the normal functioning of individual cells isolated from the animal body, besides being of potential importance in the study of certain diseases, such as leukemia.

There are at present three principal methods used for the preparation of relatively pure leucocytes for such biochemical studies.

1. A preparation rich in polymorphonuclear cells may be obtained after the injection of salt or sugar solutions,1 of irritants such as aleuronat,2 or of organic solvents (mineral oil and turpentine) subcutaneously or into the pleural or peritoneal cavities. An exudate rich in leukocytes may be collected four to twenty-four hours after the initial injection. With proper technic, contamination with blood is minimal. Ninety to 95 per cent of the cells recovered after the intra-peritoneal injection of saline are polymorphonuclear cells. The injection of liquid paraffin, on the other hand, is reported to result in a predominance of lymphocytes in the peritoneal exudate.1 Though a large yield of white cells is thus obtained, there are at least three disadvantages inherent in the method. First, it is not feasible for human leukocyte studies. Second, the cells obtained may not be entirely normal either because of the presence of Menkin's exudate factor,3 a toxic substance which alters capillary permeability, or because the acidosis which develops in inflammatory loci may produce cells whose metabolic behavior is altered.4,5 Third, the method is laborious and time-consuming. In addition, it should be pointed out that the cells obtained are to a large extent freshly released from the bone marrow,6 and, therefore, may not truly represent the cells normally circulating in the peripheral vascular system.

2. Preparations rich in lymphocytes are conventionally obtained by cannulation of the thoracic duct of dogs.7 While large numbers of lymphocytes, contaminated by relatively few granulocytes, may be thus collected for several days after the operation, the surgical procedure is a painstaking and difficult one and is not feasible in smaller animals or in humans.

3. Leukocytes from human blood have been prepared by fractional centrifugation of the supernatant plasma where the sedimentation rate is rapid,8 or more commonly, by isolation of the "buffy coat" following centrifugation of the citrated or heparinized whole blood. By the use of suitable technics, such as the construction of special centrifuge tubes, it thus is possible to obtain a highly concentrated preparation of leukocytes.9 The yield of white cells, however, is not

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quantitative, and the cells obtained are still contaminated with some red cells and blood platelets.

Because of the known deficiencies of the above methods, several alternative procedures have been investigated in an attempt to quantitatively isolate morphologically intact white blood cells from venous blood. The successful use for this purpose of saponin in hypotonic saline will be reported elsewhere.10 Best results have been obtained by the combined use of gramicidin and lysolecithin, as described below.

Certain preliminary experiments by Dubos indicate that gramicidin does not morphologically or biochemically damage granulocytes obtained from the peritoneal exudates of rabbits and mice. The lytic action of this chemotherapeutic agent on erythrocytes, on the other hand, has been known for some years.12,13 Our program, therefore, included a thorough study of the action of gramicidin on leukocytes and erythrocytes.

Encouraged by the success of Laskowski in preparing pure nuclei from avian red cells by the use of lysolecithin, we decided to investigate the effect of this compound also on the differential lysis of the formed elements of mammalian blood.

Rather early in our studies it became apparent that neither gramicidin nor lysolecithin alone fulfill the criteria for a satisfactory differential hemolytic agent which could be made the basis of a method for the isolation of leukocytes. However, when these two agents were used together, morphologically intact white cells could be consistently isolated. In the following section, the details of the procedure employed will be described.

EXPERIMENTAL

1. Materials and methods. (a) Preparation of lysolecithin. Lysolecithin was prepared by the action of cobra venom* on lecithin. The substrate was isolated from egg yolk by the method of Bloor and purified by fractionation of a petroleum ether solution with acetone and alcoholic MgCl₂. The enzymatic conversion of lecithin to the hemolytic compound lysolecithin was carried out as follows. With each batch of cobra venom the minimum amount of enzyme needed to convert 0.5 grams of lecithin to lysolecithin in 24 hours at 38°C was determined, and this amount of venom (usually about 25 mg.) was dissolved in 5 ml. of 0.158 M phosphate buffer, pH 7.1. The solution was then mixed with 0.5 Gm. of pure egg-yolk phospholipids, dissolved in 15 ml. of the same phosphate buffer. The sample was incubated for 24 hours at 38° and then centrifuged for 30 min. at 3,000 rpm. The supernatant liquid was assayed for hemolytic activity as described below. The lysolecithin thus prepared is stable for at least several weeks when refrigerated, while the enzyme itself (lysolecithinase) deteriorates rapidly.

Purified lysolecithin free from cobra venom was also prepared by mixing 7.5 grams of phospholipids dissolved in 250 ml. of a 0.158 M phosphate buffer, pH 7.0, with 75 mg. of venom dissolved in 10 ml. of the same buffer. The mixture

* Crude dried cobra venom containing an active lysolecithinase was obtained from Hynson, Westcott and Dunning, Inc., Baltimore, Md.
was incubated at 38°. Aliquots of the solution were assayed for hemolytic activity every 12 hours. After 45 hours of incubation the sample was refluxed with 8 volumes of 3:1 alcohol-ether over a water bath, concentrated in vacuo, and extracted with petroleum ether. The lysolecithin was then precipitated from a cold concentrated solution by the addition of 10 volumes of acetone and \( \frac{1}{2} \) volume of saturated MgCl\(_2\) in ethanol; this procedure thus followed the essential steps of phospholipid purification. The sample was washed with acetone and dried in vacuo. Solutions of this lysolecithin powder had the same activity as crude solutions of an equivalent amount of lecithin incubated with cobra venom and behaved in the same manner with respect to the differential lysis of white and red cells in the presence of gramicidin. For this reason it was not necessary to isolate the lysolecithin for most purposes.

(b) Determination of lysolecithin activity. The activity of each batch of lysolecithin was carefully assayed as follows: Cells from heparinized human blood were washed twice with isotonic saline and made up to the original blood volume in isotonic saline. To a 2 ml. portion of such washed blood, 17.5 ml. of 0.6 per cent NaCl were added in a series of test tubes. The tubes were brought to room temperature and varying amounts of lysolecithin were added to each tube. The contents of each test tube were mixed by pouring the solution back and forth a few times between it and another tube. After three to five minutes the tube was centrifuged for two minutes at 2,000 to 2,500 rpm. An aliquot of the supernatant solution was pipetted off, and the hemoglobin measured colorimetrically. A sample of washed blood diluted to the same volume with distilled water and centrifuged served as a control for 100 per cent lysis. Results were also periodically checked by standard red and white cell counts. By varying the amount of lysolecithin used between two limits, e.g., 0.25 and 1.0 ml. per 2 ml. of blood, it was usually possible to determine in this way the minimum amount of lysolecithin needed to effect complete lysis of 2 ml. of washed blood. This minimum amount has been considered one unit of lysolecithin.

For different preparations of lysolecithin this figure varied between fairly wide limits. During the early part of our work as much as 1.5 ml. was required to lyse 2 ml. of washed blood under these conditions. More recently one unit has varied rather constantly from 0.5 to 0.8 ml. for human blood. In a few experiments on dog blood the amount of lysolecithin needed seemed to be somewhat lower.

If whole blood rather than washed cells were used, essentially the same procedure was repeated as follows. One ml. of whole blood was diluted ten times with 0.6 per cent NaCl and varying amounts of lysolecithin solution were added. The minimum amount of lysolecithin required to cause complete lysis of this blood was then noted and used from then on with this batch of material. This quantity was usually about the same as the amount required to lyse washed cells from 2 ml. of whole blood, that is, approximately twice as much lysolecithin was required to lyse the same number of red cells in whole blood as in washed blood. As noted above, it was necessary to assay the purified, dried lysolecithin only once, since the preparation was stable indefinitely. Since there appeared to be a strict proportionality between the volume of blood used and the amount of lysin required, it was necessary to increase proportionately the amount of lysolecithin if more than 2 ml. portions of blood were used.
(c) Microscopic examination of isolated leukocytes. The centrifugate of the hemolyzed blood was washed once with saline and then suspended in several drops either of homologous plasma or of a 7 per cent solution of dried bovine plasma. A smear of this suspension was made and stained with Wright's stain in the usual way.

Suspension of the sediment in plasma before smearing and drying was essential if morphologically intact cells were to be obtained. Considerable shrinkage and distortion of the cells occurred if they were dried directly in the saline suspension. The plasma protein film around the cells prevented this deleterious effect.

(d) Gramicidin preparation. In the experiments to be described, crystalline gramicidin* dissolved in 100 per cent propylene glycol (5 mg./ml. and 1 mg./ml.) was used. Such solutions, when stored in the ice chest, retained their activity over a period of several weeks. The small amount of propylene glycol used as a solvent was found to lyse the erythrocytes only to a negligible extent and did not seem to affect the leukocytes at all.

### Table 1. The Effect of Gramicidin on Human Blood

<table>
<thead>
<tr>
<th>Concentration of gramicidin, (micrograms/ml. of solution)</th>
<th>Washed or whole blood</th>
<th>Total volume (ml.)</th>
<th>Time after addition of gramicidin</th>
<th>Extent of lysis of erythrocytes (%)</th>
<th>Microscopic appearance of white blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Whole</td>
<td>10</td>
<td>1 hour</td>
<td>8</td>
<td>Most WBC injured</td>
</tr>
<tr>
<td>25</td>
<td>Whole</td>
<td>10</td>
<td>3 hours</td>
<td>76</td>
<td>All WBC injured</td>
</tr>
<tr>
<td>100</td>
<td>Whole</td>
<td>10</td>
<td>1 hour</td>
<td>69</td>
<td>Many WBC swollen or fragmented</td>
</tr>
<tr>
<td>100</td>
<td>Washed</td>
<td>10</td>
<td>3 hours</td>
<td>96</td>
<td>All WBC lysed</td>
</tr>
<tr>
<td>100</td>
<td>Washed</td>
<td>10</td>
<td>5 minutes</td>
<td>86</td>
<td>WBC normal</td>
</tr>
<tr>
<td>100</td>
<td>Washed</td>
<td>10</td>
<td>11 minutes</td>
<td>83</td>
<td>Little injury to WBC</td>
</tr>
<tr>
<td>100</td>
<td>Washed</td>
<td>10</td>
<td>45 minutes</td>
<td>83</td>
<td>15% of WBC injured</td>
</tr>
</tbody>
</table>

Conditions: To 2 ml. of heparinized human blood gramicidin dissolved in propylene glycol at a concentration of 5 mg./ml. and 1 mg./ml. was added. Lysis was estimated colorimetrically by Hb. determination. Cells were suspended in 0.6 per cent NaCl in each instance.

2. The effect of gramicidin. In general, lysis of human and dog blood cells by gramicidin was very slow. Two to three hours were required for complete or nearly complete lysis of the red cells in 2 ml. of blood, even at a concentration of 100 y of gramicidin per milliliter of solution, i.e., 2 mg. gramicidin per 2 ml. of whole blood, diluted to 20 ml. with 0.6 per cent NaCl. This was much too slow to be suitable for metabolic experiments, which should be started as soon as possible after withdrawal of the blood.

It is known12 that certain plasma constituents, notably sugars, inhibit the hemolytic effect of gramicidin. For this reason cells washed free from plasma by two to three centrifugations in 0.9 per cent NaCl were also studied. The lysis of washed cells was indeed more rapid than that of whole blood, but it was not rapid enough for our purposes.

In addition, with either whole blood or washed cells, the leukocytes showed

* Purchased from Wallerstein Laboratories, New York, N. Y.
evidence of definite damage before lysis was complete and by the time all the
erthrocytes had been destroyed many or most of the white cells were fragmented.
Table 1 is typical of numerous experiments which illustrated some of these phenom-
ena.
3. The effect of lyssolecithin. Both the purified and the crude lyssolecithin prepara-
tions described above rapidly and completely hemolyzed red cells in washed or
whole human blood. The action of lyssolecithin on blood cells has been studied
under a variety of conditions. The general conclusions derived are listed below:
1. Lysis of washed cells is faster than that of cells in whole blood.
2. Lysis is faster in hypotonic saline (0.6 per cent) than in normal saline.
3. There seems to be a definite stoichiometric relation between the amount of
lyssolecithin needed for complete hemolysis and the volume of blood to be lysed.
This is rather constant with various blood samples.

Table 2. The Effect of Lyssolecithin (Cobra Venom) on Human Blood Cells

<table>
<thead>
<tr>
<th>Amount of lyssolecithin used (ml.)</th>
<th>Time of lysis (min.)</th>
<th>Extent of lysis of erythrocytes (%)</th>
<th>Microscopic picture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>2.5</td>
<td>100</td>
<td>50% of leukocytes badly damaged</td>
</tr>
<tr>
<td>0.20</td>
<td>3</td>
<td>95</td>
<td>25% of leukocytes damaged</td>
</tr>
<tr>
<td>0.1</td>
<td>3</td>
<td>75</td>
<td>Very little damage to leukocytes; many RBC left</td>
</tr>
<tr>
<td>0.25 lecithin (control)</td>
<td>3</td>
<td>0</td>
<td>Perfect WBC</td>
</tr>
</tbody>
</table>

Conditions: In each case once-washed cells from 2 ml. of whole blood were suspended in their
original volume of 0.9 per cent NaCl and then diluted to 20 volumes with 0.6 per cent NaCl and the
amount of lyssolecithin indicated (0.25 ml. = 1 unit), giving a total volume of 20 ml. Experiments
using whole blood diluted with 0.9 per cent NaCl gave essentially similar results with more pro-
longed hemolysis time.

Though the erythrocytes are attacked preferentially, coincidental damage
to white cells is seen before the red cells are completely lysed, and when the latter
disappear, a considerable portion of the leukocytes are badly damaged.
Table 2 illustrates a typical experiment on the effect of lyssolecithin (crude
preparation) on erythrocytes and leukocytes.

4. The combined effect of lyssolecithin and gramicidin. The method which gave in
all respects the most satisfactory results involved the use of a mixture of gramicidin
and lyssolecithin in a definite ratio. Although lysis with the mixture of the two
substances seemed no more rapid than with the fastest component alone, the
deleterious effects of the two agents on leukocytes appeared to cancel each other
inasmuch as the use of a definite proportion of lyssolecithin to gramicidin resulted
in leukocytes which were perfectly normal in appearance, with no erythrocytes
or ghost cells remaining. This was true even though the concentration of either
component used alone was sufficiently high to produce visible damage to the
leukocytes. The method is applicable to either whole blood or washed cells, and
it has been used with a large number of samples of human and dog blood.
As in the case of gramicidin or lysolecithin alone, plasma inhibits the lysis somewhat, but this can be easily overcome by keeping the ratio of the two lysing agents constant and increasing the absolute concentration of each component.

In general, the method finally adopted may be described as follows. Washed heparinized blood was placed in a centrifuge tube and enough 0.6 per cent NaCl was added to make a final tenfold dilution. A sufficient amount of solution of gramicidin in propylene glycol (mg./ml.) was added to make the concentration of gramicidin 100 y per ml. Simultaneously or immediately afterward the predetermined number of units of lysolecithin required to lyse this amount of blood was added.* The blood in the tube was quickly mixed by pouring it back and forth between two centrifuge tubes. After 3 to 5 minutes at room temperature lysis was visibly complete. The tube was centrifuged for two to three minutes at 2,100 rpm and the supernatant was discarded. The residue was rapidly washed by mixing and centrifuging it with 5 to 10 ml. of isotonic saline (three minutes at 2,100 rpm) and the supernatant was again discarded. The white residue of leukocytes was now ready for use.

Smears of this residue were made for microscopic examination as described in 1 (c). Figures 1 and 2 are microphotographs of typical preparations from whole and washed human blood, respectively.

It might be noted that the cell membranes are visibly more intact than they

* To illustrate this: If it was desired to lyse 2 ml. of blood and if the lysolecithin solution was found to contain 1 unit of activity in 0.6 ml. of solution, then 2 ml. of washed blood plus 17 ml. of 0.6 NaCl were mixed with 0.4 ml. of gramicidin and 0.6 ml. of lysolecithin.
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Fig. 2. Leukocytes isolated from washed cells of human venous blood by the gramicidin-lysolecithin method (X 975)

Table 3. The Combined Action of Lysolecithin and Gramicidin on Human Blood

<table>
<thead>
<tr>
<th>Lysing Agent Added</th>
<th>Lysolecithin (ml.)</th>
<th>Gramicidin (ml.)</th>
<th>Medium (5%, NaCl)</th>
<th>Time of lysis (min.)</th>
<th>Extent of lysis (%)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 1.5 (1 unit)</td>
<td>0.4</td>
<td>0.6</td>
<td>5</td>
<td>99</td>
<td>Undamaged leukocytes; few ghosts</td>
<td></td>
</tr>
<tr>
<td>2. 0</td>
<td>0.4</td>
<td>0.6</td>
<td>5</td>
<td>15</td>
<td>Undamaged white cells; many red cells left</td>
<td></td>
</tr>
<tr>
<td>3. 0</td>
<td>0.4</td>
<td>0.6</td>
<td>45</td>
<td>83</td>
<td>25% of WBC injured; most WBC in poor condition</td>
<td></td>
</tr>
<tr>
<td>4. 1.5</td>
<td>0</td>
<td>0.6</td>
<td>5</td>
<td>92</td>
<td>Few RBC left; most WBC in poor condition</td>
<td></td>
</tr>
<tr>
<td>5. 1.5</td>
<td>0</td>
<td>0.6</td>
<td>10</td>
<td>100</td>
<td>All WBC lysed</td>
<td></td>
</tr>
<tr>
<td>6. 1.5</td>
<td>0.4</td>
<td>0.9</td>
<td>10</td>
<td>100</td>
<td>All white cells in good condition, but slightly shrunken. No RBC or ghosts</td>
<td></td>
</tr>
</tbody>
</table>

Conditions: 2 ml. of washed human blood made up to tenfold final dilution by addition of saline followed by gramicidin and/or lysolecithin at 0 time. 1.5 ml. of this lysolecithin preparation contained one unit of lysis activity. The gramicidin-solution used contained 5 mg./ml. of propylene glycol.
are in cells prepared by the saponin method. The differential counts in these and in other sediments agreed well with the differential counts in the untreated whole blood.

Table 3 compares the effect of a combination of lysolecithin and gramicidin with that of either component alone. As seen in experiments 1 to 5 in this table,

**Table 4. Lytic Effects of Various Mixtures of Gramicidin and Lysolecithin**

<table>
<thead>
<tr>
<th>Lysing Agent Added</th>
<th>Blood preparation</th>
<th>Time of lysis (min.)</th>
<th>Extent of red cells lysis (%)</th>
<th>Microscopic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysolecithin (ml.)</td>
<td>Gramicidin (ml.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. o.8</td>
<td>0</td>
<td>Washed cells</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>2. o.8</td>
<td>0.4</td>
<td>Washed cells</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>3. o.8</td>
<td>0.2</td>
<td>Washed cells</td>
<td>6</td>
<td>99</td>
</tr>
<tr>
<td>4. o.8</td>
<td>0.2</td>
<td>Washed cells</td>
<td>6.5</td>
<td>100</td>
</tr>
<tr>
<td>5. o.8</td>
<td>0.8</td>
<td>Washed cells</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>6. o.8</td>
<td>0.4</td>
<td>Washed cells</td>
<td>9.5</td>
<td>100</td>
</tr>
<tr>
<td>7. o.6</td>
<td>0.4</td>
<td>Washed cells</td>
<td>9.5</td>
<td>99</td>
</tr>
<tr>
<td>8. 1.2</td>
<td>0.4</td>
<td>Washed cells</td>
<td>9.5</td>
<td>100</td>
</tr>
<tr>
<td>9. 1.6</td>
<td>0.8</td>
<td>Washed cells</td>
<td>9.5</td>
<td>100</td>
</tr>
<tr>
<td>10. o.8</td>
<td>0.4</td>
<td>Whole blood</td>
<td>8.0</td>
<td>95-98</td>
</tr>
<tr>
<td>11. 1.6</td>
<td>0.8</td>
<td>Whole blood</td>
<td>10.0</td>
<td>100</td>
</tr>
</tbody>
</table>

Conditions: As in table 3, except that 0.8 ml. of this lysolecithin preparation was equal to one unit of lysis activity. In all cases the initial sample was diluted with 0.6 percent NaCl.

A mixture of gramicidin (100 γ/ml.) and lysolecithin (1 unit) gave complete lysis within a few minutes, yielding uninjured white blood cells, while either component alone gave incomplete lysis in the same time interval, and when allowed to act for a longer period of time severely injured the leukocytes. Comparison of experiment 1 with experiment 6 shows that 0.9 per cent NaCl as a medium worked just as well as hypotonic saline, but the cells appeared somewhat smaller.

5. *The effect of variation in the gramicidin:lysolecithin ratio.* The ratio and not the
absolute amounts of gramicidin and lysolecithin determines whether the white cells will be morphologically intact or badly damaged. A 25 to 50 per cent deviation from the correct ratio will result in a poor preparation. There is a relatively wide margin of safety, however, in the absolute amounts of the two lysing agents used, provided the ratio is kept constant. Doubling the concentration of both seems to make no difference in the final result. This situation is illustrated by the data in table 4. Thus in experiments 2 to 5 the lysolecithin (1 unit) was kept constant while the gramicidin concentration varied from the optimum of 100 γ/ml to 50 γ/ml and 200 γ/ml. The effect was a gradually increasing injury to the leukocytes. In experiments 6 to 8, on the other hand, the gramicidin concentration was constant (100 γ/ml.) and the lysolecithin varied. The effect, again, was damage to the leukocytes when the optimum ratio was departed from. In experiment 9 of table 4, the ratio was kept constant, but the amount of each component was doubled. The result was complete lysis of the red cells and morphologically altered, excellent white cells. In experiments 10 and 11 similar findings on whole blood are recorded.

6. The effect of experimental conditions on the lysis. There is a wide margin of safety in the time allowed for hemolysis; centrifugation at either four minutes or twelve minutes after addition of the lysing agents gave equally satisfactory preparations. This behavior is unlike that of saponin, where the factor of time is quite important.

The actual process of lysis, regardless of the lysing agents, is faster in hypotonic than in isotonic saline solution. Dilution of the blood, even with saline, also speeds up the hemolytic reaction considerably. A tenfold final dilution of whole blood with 0.6 per cent NaCl, as described, seemed to yield optimum results. While white cells became somewhat swollen in this solution, they resumed their normal shapes when placed in plasma or isotonic salt solution.

Heparinized blood (1 drop of 10 per cent heparin per 10 ml whole blood) has been used routinely in this study. Both citrate and oxalate appeared to injure the white cells somewhat.

The method has been found to be applicable to samples of whole dog blood as well as to that of humans.

DISCUSSION

Nothing definite can be stated at present regarding the mechanism of action of this combination of lytic agents. It is possible that gramicidin and lysolecithin form a compound which is hemolytic for red cells but not for white cells, or it may be that they overcome each other's harmful effects on white cells in some unknown way. Direct evidence is not available on these points since no attempt has been made to crystallize material from the mixed solution. No evidence for compound formation, however, was obtained from the study of the ultraviolet absorption spectra of these substances alone and when mixed in the proper proportions; the absorption spectra of the components were additive in the mixture. This, of course, does not prove that compound formation does not occur. In this regard it is of interest to note that Wilbur and Collier have found that lysolecithin and saponin have different and possibly antagonistic hemolytic effects.
Attempts have been made to evaluate the quantitative nature of the white cell recovery. The evidence must, in a sense, be indirect, since it is difficult to get a perfectly homogeneous suspension of the prepared sediment for white cell counts. However, counts on the hemolyzed blood before centrifugation agree well with those of the same volume of blood diluted only with saline. Furthermore, no white cells are found in the hemolyzed supernatant solution after centrifugation. Finally, as noted earlier, differential counts of the recovered white cell sediment agree well with those of the original whole blood. It might be pointed out that if any white cells were being destroyed and lost, one would expect to find partially destroyed white cells in the recovered sediment. This is not the case. One may safely conclude, therefore, that the white blood cells are quantitatively recovered by the procedure described.

While it is our hope to test the validity of the method eventually by investigating the metabolic and biochemical properties of the leukocytes before and after their isolation, this has not yet been possible. At the present time, therefore, no statement can be made as to whether or not the leukocytes isolated are biochemically intact.

SUMMARY

A method involving the combined use of gramicidin and lysolecithin is described for the rapid and apparently quantitative isolation of morphologically intact leukocytes from circulating blood. The mixture of these two compounds gives rapid and complete lysis of erythrocytes. The use of either gramicidin or of lysolecithin alone in sufficient amount to completely lyse the red cells results in morphological damage to the leukocytes. For some as yet unknown reason, however, the combined use of these two agents in a definite ratio results in cancellation of this leukocyte damaging effect. The cells thus prepared appear to be consistently more intact morphologically than any yet prepared in this laboratory.

REFERENCES

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THOMAS P. SINGER, INGELORE SILBERBACH and SAMUEL SCHWARTZ