The Use of Bovine Albumin in the Examination of Bone Marrow Obtained at Autopsy

By ROBERT R. RICKERT AND ROMEO A. VIDONE

THE POSTMORTEM EXAMINATION of bone marrow smears is unfortunately still considered by many pathologists and hematologists to be of little diagnostic value. Generally, the results of such examinations are felt to yield valuable information only when the autopsy is performed within a few hours after death. It is the purpose of this report to demonstrate that this widely held belief is not entirely correct if a slight modification of the albumin method proposed by Berenbaum1 is used.

MATERIALS AND METHODS

The material used in this study was rib or vertebral body bone marrow obtained at necropsy on the pathology service of the Yale-New Haven Hospital. Direct bone marrow smears and bone marrow smears using the albumin technique described below were prepared in each case. Whenever possible. Helly (Zenker-formol) and formalin fixed bone marrow cell blocks and bone sections were also obtained and stained using hematoxylin and eosin, and Giemsa stains.

Fifty cases were studied, age one to 93 years, which included a large spectrum of disease conditions. The time between death and obtaining the marrow for study ranged from two to 23 hours. No attempt was made to evaluate the method of storage of the body (refrigeration) prior to autopsy. Most cases studied after six hours, however, were stored under refrigeration. No attempt was made to separate those cases which died with high body temperatures although many cases with high fever terminally were included in this series.

The method of preparing the bone marrow is outlined below:

1. Marrow is squeezed or scooped from the bone into a test tube containing 4-5 ml. of 5 per cent bovine albumin solution (1 part 30 per cent bovine albumin (Armour and Co.) plus 5 parts 0.85 per cent saline); 0.5-1 cubic cm. of marrow is sufficient for this purpose. The 5 per cent albumin-normal saline mixture may be made up in advance and stored under refrigeration. It has been found that it is better not to store the mixed solution for more than four weeks since a gradual deterioration in the quality of the preparation occurs following this time.

2. The marrow is broken and mixed to make a fairly uniform suspension. At this time the marrow may be set aside at room temperature for several hours or stored in the refrigerator at 4 C for as long as 24 hours with relatively little change. Although the prepared material may be stored under refrigeration for several days, it is preferable to make the smears within 24 hours, since some changes do take place which we refer to as a "refrigera-
Table 1.—Comparative Results in Fifty Cases with Both Direct and Albumin Prepared Bone Marrow Smears

<table>
<thead>
<tr>
<th>Grade</th>
<th>Quality</th>
<th>Albumin Preparations</th>
<th>Direct Smears</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of Cases</td>
<td>% of Total</td>
</tr>
<tr>
<td>4</td>
<td>Excellent</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>3</td>
<td>Good</td>
<td>21</td>
<td>42%</td>
</tr>
<tr>
<td>2</td>
<td>Fair</td>
<td>23</td>
<td>46%</td>
</tr>
<tr>
<td>1</td>
<td>Poor</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>50</td>
<td>Total</td>
</tr>
</tbody>
</table>

Mean grade for Albumin preparations: 2.42
Mean grade for Direct smears: 1.64

Albumin preparations better than direct smears — 36 cases (72%)
Albumin preparations equal to direct smears — 12 cases (24%)
Albumin preparations worse than direct smears — 2 cases (4%)

This consists of greater shrinkage and clumping of cells and loss of cellular detail. In this regard, it has been found that a short period of incubation (1–2 hours) at room temperature gives better results than proceeding immediately after suspension.

3. The tube is then centrifuged for about 10 min. The pellicle of fat and most of the supernatant fluid is drawn off. Some of the supernatant fluid is left in the tube but it is important that an excess be avoided. A 5:4 ratio of cellular deposit to supernate is ideal.

4. The deposit and supernate are then gently mixed by drawing up and down slowly in a pipet. Care must be taken since too vigorous mixing can result in excessive trauma to the cellular elements with resultant loss in the quality of the preparation. Small drops are then placed on clean slides or cover slips and smears are prepared in the usual manner. Techniques which are satisfactory for clinical material may be applied to post-mortem material as well.

5. The dried smears are then immediately stained with Wright's stain or a combination of Wright's and Giemsa or are fixed in absolute methyl alcohol for five minutes to be stained later.

6. When indicated unfixed smears may be stained for peroxidase. We have used both Goodpasture's and the Osgood and Ashworth modification of Washburn's technique with excellent results. In addition, the smears may be handled by any of the standard methods for alkaline phosphatase.

The method used is essentially that used by Berenbaum with the following differences. The mixed 5 per cent albumin in saline mixture is stored under refrigeration for a period of time not longer than four weeks. The mixed albumin and marrow is allowed to remain at room temperature for 1–2 hours before proceeding.

RESULTS

Direct bone marrow smears and albumin prepared bone marrow smears were evaluated for cytologic quality on a one to four basis and compared with bone marrow cell block sections and bone sections including marrow elements. The numbers were assigned as follows: 1-poor, 2-fair, 3-good and 4-excellent. The results of the grading are summarized in Table 1.

The albumin technique appears very useful in preserving cytologic detail particularly in the more immature cells (see Fig. 1–4) and is, therefore, most useful in the autopsy diagnosis of disease where cytologic detail is critical. As had been noted by others with direct bone marrow smears, the more mature elements, especially the segmented neutrophils appear to be the first affected by post-mortem change. This was true in the albumin prepared material as well. Stromal elements, lymphocytes, plasma cells and many eosinophils...
Fig. 1.—Direct smear of bone marrow at autopsy. 21 hours post-mortem. (AM 144). 76 year old white female with acute myelogenous leukemia. Best region of smear selected for photograph. Cell boundaries are indistinct with cytoplasm lost in many cells and poorly visualized in others. Nuclear membranes are not clear and
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resisted disintegration for longer periods of time. The erythroid elements also appeared resistant to change. Preservation of the megakaryocytes was quite variable regardless of the interval of time after death.

As might be expected, the method is most useful in the diagnosis of hematologic abnormalities at autopsy when used in conjunction with bone marrow cell blocks and sections of bone containing marrow elements. The albumin method concentrates the more loosely held cellular elements contained within the marrow cavity. It is only with tissue sections, therefore, that one can properly evaluate the relationship of the cellular elements to the other substances contained within the marrow cavity and assess the proportion of the more mature myeloid elements which tend to be lost in all of the post-mortem smearing techniques.

The method also lends itself to the application of histochemical techniques such as peroxidase (Figs. 5 and 6) and alkaline phosphatase methods (Fig. 7). Both of these techniques have been applied with success throughout the full range of time after death included in this series.

DISCUSSION

The changes occurring in the bone marrow after death are thought by some observers to be due to rapid autolysis presumably caused by “acidosis” in the agonal or early post-mortem period. As emphasized by Berenbaum and noted by others, routine fixed sections of bone and bone marrow cell blocks taken at autopsy show autolytic changes similar to those in other tissues. That death of the marrow elements is not as rapid as is commonly assumed is supported by the work of Perry et al., who demonstrated the motility of these cells many hours post-mortem. Hoffman and associates were unable to demonstrate a significant difference in the rate of cellular autolysis based on age, sex or mode of death. The environmental temperature up to several hours after death did not significantly affect the rate of change.

Berenbaum felt that the difficulty in preparing post-mortem bone marrow smears resulted largely from an inability of the cells to resist shearing in the preparation of these slides rather than especially rapid autolysis. He, there-

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nuclear detail is visualized in only a few cells. Compare with Figure 2. Wright’s stain. x 490.

Fig. 2.—Albumin prepared smear of bone marrow at autopsy in same case as Figure 1. 21 hours post-mortem. Representative field of smear selected for photograph. Cell boundaries and cytoplasm of many cells are well seen. Nuclear membranes are clear and nuclear detail is better visualized than in Figure 1. Morphology of erythrocytes is distinct in contrast to direct smear. Wright’s stain. × 490.

Fig. 3.—Direct smear of bone marrow at autopsy. Same case as Figure 1. Higher power view. This demonstrates in greater detail the poor nuclear and cytoplasmic definition of leukocytes and hazy quality of red cells. Compare with Figure 4. Wright’s stain. × 820.

Fig. 4.—Albumin prepared smear of bone marrow at autopsy. Same case as Figure 1. Same magnification as Figure 3. Numerous blasts are present with better cytoplasmic and nuclear detail, including nucleoli, than direct smear shown in Figure 3. Wright’s stain. × 820.
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fore, devised a method of suspending the cells in a viscous medium (5 per cent bovine albumin) in order to prevent their distortion during smearing. Using this method, he was able to prepare satisfactory specimens as long as several days after death.

Our experience using this method supports Berenbaum's contention that the marrow elements are not unusually susceptible to autolysis. The use of his technique has clearly expanded the period after death in which bone marrow smear examination is useful.

Summary

This report presents the findings using a minor modification of Berenbaum's albumin method of preparing bone marrow smears on post-mortem material. The method was used in 50 cases ranging in time from two to 23 hours after death and in the majority of cases (72 per cent) produced specimens of the superior cytologic quality to direct bone marrow smears obtained at the same time. This technique is especially valuable when used in conjunction with tissue sections and bone marrow cell blocks obtained at autopsy. This method has been successfully used with two histochemical techniques suggesting its usefulness in the application of other histochemical procedures in the study of autopsy marrows. The simplicity of this technique lends itself readily to routine use on a general autopsy service and should certainly be employed where hematologic disorders are suspected, especially when clinical bone marrow material is inadequate or not available.

SUMMARIO IN INTERLINGUA

Es presentate le constatationes obtenite per un minor modification del methodo de Berenbaum a albumina in le preparation de frottis de medulla ossee con material de obtention necroptic. Le methodo esseva usate in 50 casos, con intervallos post morte ab duo ad 23 horas. In le majoritate del casos (72 pro cento), le preparatos esseva de qualitate cytologic superior a illo de directe frottis de medulla ossee obtenite al mesme tempore. Le technica es specialmente utile in conjunction con sectiones histologic e blocos de cellulas de medulla ossee obtenite al necropsia. Illo eseva usate a bon successo con duo differente technicas histochimic e promitte esser de valor in conjunction con alteres de uso in le studio de specimens necroptic de medulla. Le simplicitate del methodo permette su uso routinari in le servicio necroptic general e certo debe esser empleate in casos in que il existe le sus- piscion de disordines hematologic. particularmente quando material clinic de medulla ossee es inadequate o non disponibile.

Fig. 5.—Albumin prepared smear of bone marrow at autopsy. Peroxidase preparation. 11 hours post-mortem. (AM 139). 61 year old white female with acute myelogenous leukemia. Many leukocytes contain peroxidase positive granules (stained black in photograph). Compare with Wright stained albumin prepared smear of same case, Figure 6. Osgood and Ashworth modification of Washburn’s technique for peroxidase. × 490.

Fig. 6.—Albumin prepared smear of bone marrow at autopsy. Same case as Figure 5. 11 hours post-mortem. Wright’s stain. × 490.

Fig. 7.—Albumin prepared smear of bone marrow at autopsy. Alkaline phosphatase preparation. 22 hours post-mortem. (AM 128). 19 year old white female with sepsis and "leukemoid reaction." WBC—50,000. Alkaline phosphatase positive granules stain black in photograph. × 750.
REFERENCES

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