Radiation Protection of Mice with Bone Marrow and Spleen Preserved at Low Temperature Using Polyvinylpyrrolidone

By Maxim D. Persidsky and Victor Richards

POLYVINYLPYRROLIDONE (PVP), a non-polar hydrophilic polymer, has been used by a number of investigators as a protective agent in freezing erythrocytes. Currently it is considered one of the most effective additives in the low temperature preservation of blood. An early attempt, by other investigators, to extend the scope of PVP as a preservative to nucleated cells proved unsuccessful. However, since then we have found and reported in several communications that PVP K-30 (Antara Chemicals) in a 10 per cent concentration offers effective protection in the preservation of bone marrow at low temperatures. The viability of the preserved marrow was assessed by our technique of tissue culture and phase microscopy. Although, on the average, only 29 per cent cell survival was found, the biological inertness of PVP and the long clinical experience with this compound as a plasma expander makes it a desirable additive in the preservation of bone marrow intended for clinical use. We have, therefore, further evaluated the protective effects of PVP by transplantation of preserved isologous bone marrow into lethally irradiated mice. For a comparison, bone marrow frozen with 15 per cent glycerol was also transplanted. In addition, spleen cells preserved with 10 per cent PVP were similarly evaluated.

METHODS

Bone marrow was obtained from the femurs of \((C57L \times A)F_1\) isologous mice. After homogenization, by aspiration with a syringe and straining, the cells were suspended in Hanks solution alone or in Hanks solution plus serum (3:1 ratio) containing either 10 per cent (w/v) PVP neutralized by addition of NaOH, or 15 per cent (v/v) glycerol. The spleens from an identical strain of mice were homogenized with a specially designed strain-homogenizer and suspended in the same media. The preparations were cooled at a rate of 1 C. per minute to \(-25\) C., and freezing was initiated by seeding of ice at \(-5\) C. The frozen preparations were then transferred to an alcohol bath at \(-79\) C. for 30 minutes, after which they were quickly thawed in a water bath at 37 C.

The recipient mice of the same strain, weighing 20-25 Gm., and being 12 weeks of age, were exposed to whole body x-irradiation, \(LD_{100/30}\) dose of 800 r from a 200 kvp source with a half-value layer of 1.35 mm. copper. The animals were placed in flat, round, lucite containers, perforated on both sides. Ten animals were exposed simultaneously in each container, which was positioned 50 cm. from the source. One day prior to the irradiation and 7 days thereafter, the mice received antibiotics in their drinking water.
Table 1.—Survival of Lethally Irradiated Mice after the Transplantation of Bone Marrow or Spleen

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Injection Dose*</th>
<th>Suspending Medium</th>
<th>Concentration of Preservative</th>
<th>No. Exp.</th>
<th>No. Mice</th>
<th>30-Day Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Hanks and serum</td>
<td>8</td>
<td>120</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh 1</td>
<td>Hanks</td>
<td>8</td>
<td>99</td>
<td>88.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh 1/5</td>
<td>Hanks</td>
<td>6</td>
<td>63</td>
<td>65.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen 1</td>
<td>Hanks and serum</td>
<td>8</td>
<td>81</td>
<td>67.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen 1/5</td>
<td>Hanks and serum</td>
<td>10% PVP</td>
<td>8</td>
<td>88</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>Frozen 1</td>
<td>Hanks and serum</td>
<td>10% PVP</td>
<td>5</td>
<td>56</td>
<td>76.8</td>
<td></td>
</tr>
<tr>
<td>Frozen 1/5</td>
<td>Hanks and serum</td>
<td>15% glycerol</td>
<td>5</td>
<td>57</td>
<td>62.3</td>
<td></td>
</tr>
<tr>
<td>Frozen 1</td>
<td>Hanks and serum</td>
<td>15% glycerol</td>
<td>2</td>
<td>30</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Spleen

| None        | Hanks and serum | 1                 | 10                            | 10.0     |
| Fresh 1     | Hanks           | 1                 | 10                            | 80.0     |
| Frozen 1    | Hanks and serum | 10% PVP          | 1                             | 10       | 70.0     |

*7 x 10^6 cells or one-fifth of this amount.

0.5 Gm. Polymyxin B and 12 mg. Neomycin per liter. The bone marrow and spleen cells were transplanted intravenously via the tail vein the day after irradiation. The injections consisted of 0.2 ml. of the suspensions, containing either 7 x 10^6 cells or one-fifth of this amount. Neither PVP nor glycerol were removed from the media before the infusion. The animals were divided into eight groups: the control group was injected with a blank medium, containing 10 per cent PVP; two groups received fresh bone marrow in a full or one-fifth dose; four groups received cells preserved with PVP or glycerol in a full or one-fifth dose, respectively; and the remaining group was injected with a full dose of cells frozen in Hanks solution plus serum (3:1 ratio). Each animal also received, intra-peritoneally, 3200 units of crystalline penicillin G in 0.2 ml. of normal saline. The animals were observed for a period of 30 days.

Observations

The per cent recovery of the animals receiving bone marrow transplants are summarized in table 1. The animals of the control group and of the group which received marrow frozen without a preservative were dead within 15 days. The marrow preserved with 10 per cent PVP promotes approximately 80 per cent survival when compared to the animals infused with fresh bone marrow. The one-fifth dose promotes recovery in all three groups at a proportionally lower level. The preliminary data obtained with the transplantation of spleen cells preserved with PVP indicates a high per cent recovery of lethally irradiated mice (table 1).

Discussion

The high recovery of lethally irradiated mice promoted by bone marrow preserved with PVP confirms our previous conclusions that 10 per cent PVP offers effective protection during the freezing and thawing process. Al-
though these experiments indicate a slightly lower recovery rate of the mice receiving marrow preserved with PVP than with glycerol, the traumatic effect of glycerol on cells\(^{11,14,15}\) complicates the procedure with this additive. On the other hand, PVP has no adverse effect on cells. We feel, therefore, that the simplicity of the experimental procedures with PVP and its relatively high effectiveness as a preservative make it a more desirable additive than glycerol.

**SUMMARY**

The previously reported results\(^{11-13}\) of low-temperature preservation of bone marrow with 10 per cent polyvinylpyrrolidone were re-evaluated on the radiation protection basis. The lethally irradiated mice show 80 per cent recovery after the transplantation of PVP-preserved isologous bone marrow, when compared to the recovery rate of the mice receiving fresh marrow. Slightly higher recovery resulted from the marrow preserved with glycerol. The spleen cells preserved with PVP also induce high recovery in irradiated mice.

**SUMMARIO IN INTERLINGUA**

Le previemente reportate resultatos obtenite in le preservation de medulla ossee a basse temperaturas in 10 pro cento de polyvinylpyrrolidona (PVP) eseva re-evaluata a base de experimentos de protection contra radiation. Letalmente irradiate muses recipiente isologe medulla ossee que habeva essite preservate con PVP monstrava un incidentia de restablimento equal a 80 pro cento de illo in letalmente irradiate muses recipiente medulla fresc. Levemente plus alte valores de restablimento eseva obtenite con medulla preservate con glycerol. Cellulas splenic preservate con PVP etiam produce alte procentages de restablimento de irradiate muses.

**ACKNOWLEDGMENTS**

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**REFERENCES**

9. Greaves, R. I. N., Nagington, J., and

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