

both PVP and Dx is intriguing in view of Jay's finding that albumin had the reverse effect<sup>2</sup>. Jay also reported a significant decrease in surface area.

Several methods are in use for assessing cell deformability: cell filtration through materials of known porosity (Teitel<sup>16</sup>) the rate of centrifugal packing (Sirs<sup>17</sup>) and the micropipette technique<sup>15,18</sup>. These various methods have not been correlated; ours reflects the cell's ability to change rapidly its geometry as it must to traverse fine channels in the microcirculation and spleen. By our method, deformability decreased uniformly and significantly as polymer concentration increased. The mean pressure required to draw cells into a micropipette increased from a control value of  $21.4 \pm 0.04$  kPa to  $22.6 \pm 0.28$  kPa (5.6%) as the concentration of PVP was raised to  $5 \text{ g} \cdot \text{l}^{-1}$  and increased further to  $23.8 \pm 0.29$  kPa (11.2%) at a concentration of  $7 \text{ g} \cdot \text{l}^{-1}$ . The increase in pressure implies a decrease in deformability as we defined it above. In Dx the mean pressure increased from a control value (using a different pipette) of  $23.6 \pm 0.04$  kPa to  $24.8 \pm 0.37$  kPa (5.1%) at a concentration of  $5 \text{ g} \cdot \text{l}^{-1}$  and continued to increase uniformly up to  $27.9 \pm 0.41$  kPa (18.2%) as concentration rose to  $50 \text{ g} \cdot \text{l}^{-1}$ . An increase in volume alone decreases deformability<sup>19</sup>. The observed increase in volume plateaued at a polymer concentration of about  $5 \text{ g} \cdot \text{l}^{-1}$  but deformability continued to decline as concentration increased. This was particularly marked for Dx at concentrations up to  $50 \text{ g} \cdot \text{l}^{-1}$  and it implies a stiffening of the membrane in addition to the change in volume. The change in volume is not an artefact. The osmolarity of the suspending medium changed by less than 1% with concentration change and the refractive index of the medium increased by less than 0.05% relative to that of water at concentrations beyond which changes in volume were not observed. In any case an increase in refractive index is expected to decrease the apparent volume as measured optically.

From these preliminary studies we conclude that the interaction of neutral polymers with the RCM is complex and different from that of other macromolecules<sup>2-4</sup>. Both PVP and Dx cause an increase in volume, the former by a

swelling in the region of the 'dimple' and the latter by swelling of the rim. Both polymers decrease the deformability of the cell but more than can be accounted for by the change in volume. Some molecular rearrangement must be involved in a stiffening of the membrane. Such a molecular change may be in the peripheral membrane protein. Spectrin appears to influence red cell shape<sup>20-22</sup> and there is evidence that spectrin interacts with integral membrane glycoproteins which are exposed to the suspending medium<sup>22-24</sup>.

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## Immunoglobulin synthesis by cord blood lymphocytes

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**Summary.** The IgG, IgA and IgM synthesis by adult peripheral blood and cord blood lymphocytes incubated alone and with pokeweed mitogen was quantitated. The cord blood lymphocytes produced no immunoglobulin even with mitogen stimulation while the adult peripheral blood lymphocytes responded to the mitogen with a significant ( $p < 0.04$ ) increase in immunoglobulin production.

Pokeweed mitogen (PWM) induces polyclonal immunoglobulin synthesis *in vitro* by adult peripheral blood lymphocytes (PBL)<sup>1</sup>. In contrast, this mitogen has been shown qualitatively to have minimal effect on immunoglobulin synthesis by lymphocytes derived from cord blood<sup>2</sup>. Quantitation of immunoglobulin synthesis by cord blood lymphocytes (CBL) has not been reported. Therefore, we prepared PWM response curves with CBL's and simultaneously cultured adult peripheral blood and cord blood lymphocyte preparations with PWM. Immunoglobulin synthesis was then quantitated with radioimmunoassays. PBL's and CBL's were isolated using the ficoll-hypaque method<sup>3</sup> from 20-30 ml of heparinized blood drawn from 6

separate placentas (cord blood) and 6 separate adult donors. A differential was performed on dried smears using nonspecific esterase staining<sup>4</sup> to facilitate cellular identification and counting was performed with a hemocytometer. 2 CBL response curves to PWM (Gibco) were prepared using mitogen concentrations starting with 0.1 ml of undiluted mitogen followed by serial dilutions in media to 1:80. In addition to the 2 CBL response curves, 6 PBL and 6 CBL culture sets were prepared. The sets included 1. lymphocytes alone, and 2. lymphocytes plus PWM. PWM was added at a concentration previously determined to give maximal stimulation of PBL's (0.1 ml of 1:5 mitogen dilution) in preliminary studies. Each culture contained

$2 \times 10^6$  lymphocytes suspended in 2 ml of media (RPMI 1640 containing glutamine, penicillin, streptomycin and 10% fetal calf serum). The total volume of media and cells/ml media remained constant for each experiment. Viability was monitored post incubation on the basis of dye exclusion and found to be greater than 70% in each culture. The amount of IgA, IgG and IgM in the culture media at zero time and after 7 days incubation was measured using a double antibody (Ab) competitive radioimmunoassay<sup>5</sup>. The difference between the immunoglobulin concentration measured after 7 days incubation and at zero time represented the amount released by the cells in culture.

The data collected were not normal in distribution. Therefore, the 2-tailed probabilities of Wilcoxon's signed rank statistic were calculated for their analysis.

There was no detectable immunoglobulin synthesis by CBL's in either the mitogen dose response study or the 6 CBL culture sets. The median IgA, IgG, and IgM synthesis by unstimulated PBL's was 210, 220, and 100 ng respectively. When PWM was added the median IgA, IgG and IgM synthesis increased significantly ( $p < 0.04$ ) above the control values reaching 1320, 1320 and 2420 ng respectively.

Previous in vitro studies have indicated that cord blood lymphocytes differentiate primarily into plasma cells containing intracytoplasmic IgM with minimal IgA or IgG differentiation detectable<sup>2</sup>. Perhaps the intracytoplasmic IgM was present in these cultures, but no release was measured in the media.

It is pertinent that fetal lymphocytes in laboratory animals have been induced to synthesize all 3 classes of immunoglobulin using variations in the culture conditions<sup>6,7</sup>. These studies suggest that fetal cells are capable of differentiating but may require a specific stimulus.

In this regard, the B lymphocytes of human milk appear

similar to those of cord blood<sup>8,9</sup>. Milk lymphocytes have been shown to synthesize only IgA in vitro even though IgM, and IgG bearing B cells are present<sup>10,11</sup>. Interestingly, a human milk cellular factor has recently been described which selectively stimulates IgA synthesis and may explain in part this unusual immunologic observation<sup>12</sup>. Milk lymphocytes also respond with minimal immunoglobulin synthesis to PWM and in this regard too are similar to the lymphocytes of cord blood<sup>9</sup>.

In summary, we have quantitated the immunoglobulin synthesis by cord blood lymphocytes in 7 day cultures. These cells alone produced no immunoglobulin and there was no response detected to PWM at any concentration studied. There was, however, a significant ( $p < 0.04$ ) increase in the synthesis of all classes of immunoglobulin by PBL's when PWM was added to the media.

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## Penicillin-induced formation of ribonuclease in rice (*Oryza sativa* L.) endosperm and its inhibition by abscisic acid<sup>1</sup>

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**Summary.** Penicillin stimulates the formation of ribonuclease in embryoless rice (*Oryza sativa* L.) endosperm and enhances gibberellin-induced response. Penicillin-induced RNase production is completely inhibited by abscisic acid.

The antibiotic effect of penicillin is mediated by blocking some stage in the biosynthesis of bacterial cell wall mucopeptide<sup>3-5</sup>. While elucidating its role in higher plant metabolism, it has been demonstrated that penicillin enhances chloroplast pigment formation in intact rice (*Oryza sativa* L.) seedlings<sup>6</sup> and in isolated mungbean (*Phaseolus aureus* L.) cotyledons<sup>7</sup> greening in presence of light; the penicillin effect is mediated through its influence on nucleic acid and protein synthesis. In embryoless rice endosperm, penicillin induces gibberellin biosynthesis which in turn stimulates the synthesis of  $\alpha$ -amylase<sup>8</sup>. Gibberellic acid greatly enhances the synthesis and release of  $\alpha$ -amylase<sup>9</sup> by cereal aleurone layers. In addition to  $\alpha$ -amylase, endosperm tissue (aleurone layers plus the starchy endosperm) produces a variety of hydrolases<sup>10,11</sup> following GA treatment. For the purpose of further defining the action of penicillin in higher plants, and to test its possible implication in gibberellin controlled processes, we have studied whether ribonuclease is also formed as a result of penicillin action. We present here evidence that penicillin is

able to stimulate the synthesis of RNase in deembryonated rice endosperm which is accompanied by increased protein synthesis.

**Material and methods.** Rice (*Oryza sativa* L.) seeds were made huskless and rinsed several times with sterile distilled water. The embryo portion of the seed was discarded from the grain. Batches of 10 such endosperm halves (embryoless half seeds) were placed in the incubation medium in autoclaved Erlenmeyer flasks and incubated in a revolving rotator at 28°C for the stipulated period. The medium contained 10  $\mu$ moles of acetate buffer (pH 4.8), 80  $\mu$ moles of  $\text{CaCl}_2$ , penicillin in different concentrations, and other test chemicals in a total volume of 2 ml<sup>10</sup>. Sterile solutions were used throughout the experiments. Following incubation, the half seeds were washed thoroughly to remove the chemicals adhering on the surface, and homogenized with cold 0.05 M sucrose-citrate buffer (pH 6.0), then centrifuged at  $10,000 \times g$  for 15 min in Sorvall RC-2B refrigerated centrifuge. Ribonuclease (RNase) activity was measured according to Cherry<sup>12</sup>. Reaction mixture included 0.1 ml of