2×10^6 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[5]. 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[2]. 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[6,7]. 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[8,9]. Milk lymphocytes have been shown to synthesize only IgA in vitro even though IgM, and IgG bearing B cells are present[10,11]. Interestingly, a human milk cellular factor has recently been described which selectively stimulates IgA synthesis and may explain in part this unusual immunologic observation[12]. Milk lymphocytes also respond with minimal immunoglobulin synthesis to PWM and in this regard too are similar to the lymphocytes of cord blood[9].

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[1]

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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[3-5]. 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[6] and in isolated mungbean (*Phaseolus aureus* L.) cotyledons[7] 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 α-amylase[8].

Gibberellic acid greatly enhances the synthesis and release of α-amylase[9] by cereal aleurone layers. In addition to α-amylase, endosperm tissue (aleurone layers plus the starchy endosperm) produces a variety of hydrolases[10,11] 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.

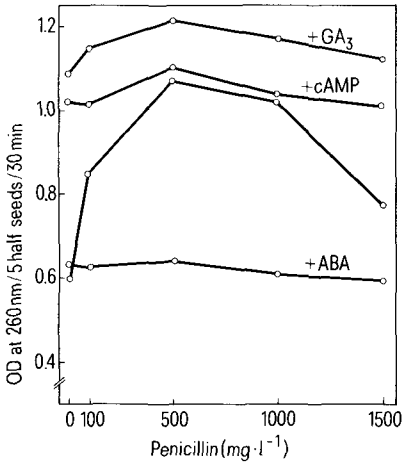
Material and methods. Rice (*Oryza sativa* L.) seeds were made huskless and rinsed several times with sterile distilled water. The embryo portion of the seed was discarded from the grain. Batches of 10 such endosperm halves (embryoless half seeds) were placed in the incubation medium in autoclaved Erlenmeyer flasks and incubated in a revolving rotator at 28 °C for the stipulated period. The medium contained 10 µmoles of acetate buffer (pH 4.8), 80 µmoles of $CaCl_2$, penicillin in different concentrations, and other test chemicals in a total volume of 2 ml[10]. 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[12]. Reaction mixture included 0.1 ml of

0.1 M KCl, 0.1 ml of 0.01 M $MgSO_4$, 1 ml yeast RNA (1 mg · ml^{-1}) in 0.05 M phosphate buffer (pH 6.0) and 1 ml enzyme in a total volume of 2.2 ml. The reaction mixture was incubated at 37 °C for 15 min and then the reaction was stopped by adding 0.2 ml of 5 N perchlorice acid and 0.2 ml of 0.1 M uranyl acetate. After centrifugation, 1 ml of the supernatant was diluted to 10 ml and the optical absorbance was measured at 260 nm in a Zeiss UV-spectrophotometer. The blank was run in a similar manner by including all the reagents, except the substrate RNA which was added after inactivating the enzyme at the end of the incubation period.

In order to study the effect of penicillin applied either alone or jointly with GA_3 on protein synthesis in rice endosperm, the rate of incorporation of labelled precursors into protein was followed in this material. Rice endosperms were incubated in medium containing 1 µCi of *Chlorella* ^{14}C protein hydrolysate in addition to other reagents and test solutions as described in the caption of the table. After 24 h incubation, the labelled endosperms were extracted with buffer and protein precipitated by 20% trichloroacetic acid. The precipitate was washed successively with TCA, ethanol, ethanol:ether, ether and finally dried. Labelled protein was dissolved in 6 N NH_4OH and transferred to scintillation vials containing 5 ml counting solution composed of 4 g of PPO (2,5-diphenyloxazole), 0.15 g of POPOP (1,4-bis2(5-phenyloxazole)-benzene) and 60 g of naphthalene per l of dioxane. These soluble samples were counted in a Beckman Liquid Scintillation Spectrometer.

Effect of penicillin with or without GA_3 on the incorporation of labelled amino acids into cellular proteins of rice half seeds

Treatment	Ct · min^{-1}/15 half seeds
Water control	515
GA_3, 10^{-5} M	1636
Penicillin (mg · l^{-1})	
100	1160
500	1485
1000	845
Penicillin (mg · l^{-1})	
100 + GA_3	1370
500 + GA_3	1693
1000 + GA_3	1648



Effect of various concentrations of penicillin applied alone or jointly with GA_3, cyclic AMP and ABA upon the formation of RNase in embryoless rice half seeds. The enzyme activity is expressed as OD at 260 nm per 5 half seeds and 30 min. Each treatment was tested in 4 replicates and the experiment was repeated several times. Incubation time 24 h. Final concentrations: GA_3, 10^{-5} M; ABA, 3.3×10^{-5} M, cyclic AMP, 10^{-5} M.

Results and discussion. The effect of penicillin on the induction of RNase in embryoless rice half seeds in the presence and absence of GA_3, cyclic AMP and ABA is presented in the figure. When applied alone, penicillin stimulated markedly the formation of RNase in rice endosperm. Of the concentrations tried here, 500 mg · l^{-1} penicillin was maximally effective in increasing RNase level by about 80% over water control. It is interesting to note that the effect of penicillin at this concentration was found to parallel closely the effect shown by GA_3 in that an almost equal amount of RNase was produced in each case. It also appears that, as regards ribonuclease formation, penicillin excelled over cyclic AMP which is thought to mimic GA action in this system[13,14]. It was further noted that when penicillin was present in the medium together with GA_3, about 40% higher activity on the average was obtained than was observed with penicillin alone. With 500 mg · l^{-1} penicillin, which produced the maximum effect when applied alone, GA effect was promoted more than 15% of that produced by GA_3 alone. With cyclic AMP, however, the effect of interaction of penicillin was almost equal to that caused by cyclic AMP alone. Similar to ABA inhibition of GA_3-induced α-amylase[15], the induction of RNase by penicillin here was totally inhibited by ABA.

The table shows that penicillin promotes the incorporation of ^{14}C-labelled amino acids into cellular proteins of the half seeds. In presence of GA_3, the effect of penicillin on the incorporation of a label became more pronounced. This suggests that the influence of penicillin and GA_3 is at least in part independent of each other.

The antibiotic penicillin produced by *Penicillia* is an important derivative of cysteine[16,17]. It is known that penicillamine, a dimethyl-substituted cysteine, is produced when penicillin is subjected to hydrolysis in vitro either by acids or with the enzyme penicillase. In the present condition, a biochemical control by maintaining the synthetic activity of the system is assumed to be exerted by the enzymes, the synthesis of which may be stimulated by S-H groups of this amino acid. In this perspective, the results obtained here may serve to indicate an indirect effect of penicillin rather than its direct antibacterial effect.

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