

Dimethyl Sulfoxide: Failure to Penetrate Encysted *Artemia* Embryos. (32457)

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Dimethyl sulfoxide (DMSO) is an aprotic polar solvent, the biological properties of which have become the subject of considerable interest. It has been shown to be useful in preventing damage to living cells preserved at low temperatures(1-5), although it is somewhat toxic to actively growing cells(5,6). DMSO apparently penetrates most biological membranes very freely, permeating frog skin, for example, ten times more rapidly than does urea(7). It also causes marked increases in permeability, leading some workers to suggest that it would be a useful vehicle for topical application of pharmaceuticals(8-10). In this report evidence is presented showing that encysted embryos of the brine shrimp, *Artemia salina*, are impermeable to DMSO.

Methods. The encysted embryos used in these experiments were obtained from General Biological Supply House, Chicago, Ill. The test solutions (25 ml) were placed in Petri dishes of 90 mm diameter, and 15 mg of dry eggs were added. They were kept at room temperature (approximately 22°) and observed periodically at 36× magnification.

Results and discussion. The desiccated encysted embryo of *Artemia salina* is in a dormant state, developed to the blastula or gastrula stage. The external appearance is that of a hollow ball which has been pushed in on one side. When placed in aqueous salt solutions, water is taken up rapidly and the cyst swells to a fully rounded appearance within 30 minutes. Within one day the outer shell breaks and a membrane-enclosed larva emerges. This hatches into a free-swimming nauplius shortly thereafter. The salinity of the medium can be varied widely with little effect on the viability(11).

In 0.15 M NaCl, the sequence of events was as described above. In 2.1% DMSO by volume,* which is approximately isosmotic to 0.15 M NaCl, the results were similar except that motile nauplii were never observed.

Eggs placed into solutions containing 2.1% DMSO plus 0.05 to 0.15 M NaCl hatched into motile nauplii within 2 days. The observation that motile nauplii were not obtained in salt-free dilute solutions of DMSO is not surprising, as the organism's requirement for sodium ions, low concentrations of DMSO had no noticeable effects.

With DMSO concentrations of 20 to 100% the eggs never became rounded, even after immersion for as long as 9 days. This suggests that DMSO is unable to penetrate the cysts. Alternatively, one might imagine that it did in fact penetrate, but was toxic in these high concentrations. The failure of the eggs to swell might then be attributed to leakage of material out of the cysts, resulting in little or no gain in volume. To test for this possibility, eggs which had been immersed in 20 to 100% DMSO for 9 days were washed and reimmersed, this time in 0.15 M NaCl. Their behavior was indistinguishable from that of untreated eggs immersed in 0.15 M NaCl, and motile larvae of normal appearance were obtained within 2 days. This result supports the conclusion that DMSO failed to penetrate during the 9-day exposure. Finally, eggs were allowed to swell by immersion in 0.15 M NaCl for one hour, then placed into 20 to 100% solutions of DMSO. Such eggs reverted to the appearance of desiccated eggs within 30 minutes, as would be expected if the cysts were impermeable to DMSO.

Summary. These results demonstrate the impermeability of *Artemia* cysts to DMSO. There was no evidence of DMSO penetration even after 9 days, and hydrated cysts shrunk rapidly when placed into hyperosmotic DMSO solutions. DMSO, at 2.1%, appears to have

* DMSO at 2.1% by volume is 0.3 M. However, DMSO is quite hygroscopic, and no correction has been made for the water content of the solvent stock.

no particular toxicity to the eggs or to the free-swimming nauplii.

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Breaking Strength of Chick Bones as an Indication of Dietary Calcium and Phosphorus Adequacy.*† (32458)

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It is an accepted fact that the levels of dietary calcium and phosphorus regulate bone ash content. Harms *et al*(1) reported that maximum growth could be attained with much lower levels of calcium or phosphorus than those indicated to be required(2), provided the calcium-phosphorus ratio was optimum. However, in many instances bone ash was increased by higher dietary levels of the two minerals than were required for normal growth. The value of the increased bone ash has been questioned.

In most calcium or phosphorus assays, tibia ash has been used as the main criterion for evaluation of dietary adequacy(3). The purpose of this study was to determine whether the increased bone ash was beneficial to the chick for providing greater bone strength, and to develop a method for measuring bone strength.

Procedure. The basal diet used in this

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TABLE I. Composition of Basal Diet.

Ingredient	%
Degerminated corn meal	51.70
Cerelose	5.40
Soybean meal (50% protein)	34.00
Alfalfa meal (20% protein)	3.00
Iodized salt	.40
Micro-ingredients*	.50
Variable†	5.00
% Phosphorus	.30
% Calcium	.26

* Supplied per kg of diet: 7,000 I.U. vit A, 2200 I.C.U. vit D₃, 770 mg choline chloride, 40 mg niacin, 4.4 mg riboflavin, 20 mg pantothenic acid, 22 µg vit B₁₂, 125 mg ethoxyquin, 20 mg iron, 2 mg copper, 200 µg cobalt, 1.1 mg iodine, 100 µg zinc, 175 mg manganese and 125 µg zoalene.

† Composed of monosodium phosphate, calcium carbonate and builder's sand.

study contained .26% calcium and .30% phosphorus (Table I). Nine experimental diets were fed which involved 3 levels of calcium at each of 3 levels of phosphorus. Calcium and phosphorus levels were chosen which had been found to produce a wide variation in tibia bone ash(1). Reagent grade monosodium phosphate (NaH₂PO₄ · H₂O) and reagent grade calcium carbonate (CaCO₃) were used to supplement the basal diet.