On the residual water content of dried but viable cells[1]

J.S. Clegg, A.C. Zettlemoyer and H.H. Hsing

Laboratory for Quantitative Biology, University of Miami, Coral Gables (Florida 33124, USA) and Center for Surface and Coatings Research, Lehigh University, Bethleham (Pennsylvania 18015, USA), 29 November 1977

Summary. We have examined the residual water in dried cysts of the brine shrimp, *Artemia salina,* by gas-bombardment techniques at reduced pressures and temperatures. This treatment reduced, but did not remove all the residual water, the lower limit being about 0.0069 gH_2O/g dried weight. The significance of such small amounts of water to cell hydration is assessed. The treatment did not appreciably reduce the viability of this cyst population.

Residual water is usually defined as the small amount of tightly-bound water remaining in materials that have been dried over strong desiccants and/or at low pressures for long periods of time. Its occurrence and probable importance have often been noted[2-7] but details of its nature are poorly understood. It is also well known that a variety of organisms, from diverse taxa, have the ability to remain viable even when their cellular water contents are reduced to the residual level, in which case they enter an ametabolic condition known as cryptobiosis[8-10]. Whether or not these organisms can survive the total removal of their water is extremely difficult, and likely impossible, to determine since the methods commonly used to remove residual water, such as exposure to temperatures in excess of 100 °C for relatively long periods of time, with or without vacuum, can be expected to do a great deal of thermal damage independent of considerations of water removal. Consequently, loss of viability in these cases cannot be ascribed with any confidence to the removal of residual water.

We have examined this issue using gas-bombardment and surface-area methods at low temperatures and reduced pressures. The experimental biological system chosen was the cysts (encysted embryos) of the brine shrimp, *Artemia salina.* These cysts, about 200 µm in diameter, consist of a mass of 3000–4000 cells covered by a complex shell[11], and their properties with regard to desiccation are well known[12]. A study of the tolerance of these cysts to various kinds of environmental insults has been carried out by Iwasaki[13], who also reviews the earlier literature.

Artemia cysts, purchased from San Francisco Bay Brand, Inc., Menlo Park, California, were processed and stored as previously described[14]. Viability of cyst populations was determined by counting the number of cysts giving rise to swimming larvae (nauplii) after incubation in sea water at 23–25 °C for 72 h. At least 500 cysts were used for each assay. Residual water contents were estimated by weight loss of $CaSO_4$-dried cyst samples when heated at 103–105 °C in an oven for 24 h at 1 at[14]. Populations of cysts treated this way are not viable by our assay. A large sample of $CaSO_4$-dried cysts was shipped to Lehigh University where the gas bombardment studies were performed as described next.

5 g of $CaSO_4$ dried (viable) cysts were placed in the sample tube of a Brunauer-Emmett-Teller volumetric apparatus[15] and exposed to 12 cycles of the following regime. The tube was first evacuated to 10^{-5} torr and then cooled to the temperature of liquid nitrogen. The cysts were now bombarded with pure nitrogen vapor (650 mm pressure) for 0.5–1 h. The tube was then allowed to warm up to room temperature while continuously pumping with a liquid nitrogen trap and a mercury diffusion pump, backed up with a mechanical pump. After an hour of pumping, the cycle was repeated 11 additional times. Surface area was measured by standard procedures using krypton (Kr), before and after the 12-cycles of treatment. The cyst sample was sealed off under pure nitrogen and returned to the University of Miami where viability and residual water

1 Supported in part by grant PCM-24037 from the US National Science Foundation.
2 H.T. Meryman, Ann. N.Y. Acad. Sci. *85,* 729 (1960).
3 R. Neihof, J.K. Thompson and V.R. Deitz, Nature, Lond. *216,* 1304 (1967).
4 D. Greiff and W.A. Rightsel, Appl. Microbiol. *17,* 830 (1969).
5 T.W. Rowe, in: Current Trends in Cryobiology, p. 61. Ed. A.U. Smith. Plenum Press, New York 1970.
6 J. Levitt, Responses of Plants to Environmental Stresses. Academic Press, New York 1972.
7 A.S. Sussman and H.A. Douthit, A. Rev. Pl. Physiol. *24,* 311 (1973).
8 D. Keilin, Proc. R. Soc., Lond. B *150,* 149 (1959).
9 J.H. Crowe and J.S. Clegg, (Ed.), Anhydrobiosis. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pennsylvania 1973.
10 N. Grossowitz, S. Hestrin and A. Keynan, (Ed.), Cryptobiotic Stages in Biological Systems Elsevier, New York 1961.
11 F.J. Finamore and J.S. Clegg, In: The Cell-Cycle: Gene-Enzyme Interactions, p. 249. Ed. G.M. Padilla, G.L. Whitson and I.L. Cameron Academic Press, New York 1969.
12 J.S. Clegg, Trans Am. microsc. Soc. *93,* 481 (1974).
13 T. Iwasaki, in: Inst. Internat. Froid, Commission C-1, 79-88. Sapporo, Japan 1973–1975.
14 J.S. Clegg, J. exp. Biol. *61,* 291 (1974).
15 S. Brunauer, Physical Adsorption of Gases & Vapors. Princeton University Press, New Jersey 1943.
16 A.C. Zettlemoyer, E.D. Schweitzer and W.C. Walker, J. Am. Leath. Chem. Ass. *41,* 253 (1946).

Effects of gas bombardment treatments on *Artemia* cysts*

	Kr area (m^2/g)	Viability (% nauplii)	Sample number	Residual water (g/g dried cysts)
Before treatment	0.67	86.2 ± 1.3 SD (n = 8)	1	0.0167
			2	0.0143
			3	0.0177
			4	0.0153
			average:	0.0160
After treatment	0.64	84.2 ± 2.0 SD (n = 8)	1	0.0058
			2	0.0063
			3	0.0077
			4	0.0078
			average:	0.0069

* The sample number refers to the sequential order in which the cysts were weighed after removal from $CaSO_4$ (before treatment) or upon opening the sealed tube of cysts (after treatment). The time required between samples was between 30 and 45 sec.

content determinations were performed within 2 weeks of the last gas-bombardment cycle. The results of all these studies are summarized in the table.

Although the Kr-surface area measured before the 12-cycle treatment appears to be slightly greater than that measured after treatment, the values fall within expected experimental error and little more can be said on this issue. The last column in the table indicates that the experimental treatment considerably reduced but did not remove all residual water from $CaSO_4$-dried cysts, the average amount remaining being about 0.0069 g/g. No significant difference in cyst viability resulted from the treatment.

This estimate for residual water should be considered a maximum in terms of the cellular component of the cysts for the following reasons. First, the outer surface of the shell is very hygroscopic and it is extremely difficult to prevent the rapid uptake of very small amounts of water vapor during transfer of the cysts from the sealed tube to the balance for reference-weighing before heat-treatment began. That this uptake during transfer actually occurred is indicated by the consistent increase in the values obtained for the sequential samples of treated cysts. Thus, it is clear that at least some of the residual water of the cysts must be located in the shells, rather than the cellular component. Second, it is not unlikely that at least some of the weight loss due to heating could result from volatilization of small amounts of organic compounds from the cysts. In this regard it can be pointed out that the same treatment used on *Artemia* cysts in the present work has previously been reported to remove all detectable water from intact kangaroo tendon which binds water tenaciously[16] but does not contain the organic diversity present in intact cells. In view of these considerations and the data given here, we conclude that these cells can remain viable with residual water contents significantly lower than 0.0069 g/g.

It is worth inquiring into what such low water contents actually mean. Suppose we take the upper limit for our estimates of cyst residual water as the amount actually present in the cells after the gas bombardment treatments. The following calculations suggest that this amount of water is negligible in terms of cellular hydration requirements. Thus, the protein content of *Artemia* cysts is close to 48% of the dried weight[11]. Using the value commonly found for protein hydration (0.3 gH_2O/g dried protein) it follows that the protein present in the cysts would require about 0.144 g of water. Consequently, a residual water content of 0.0069 g/g can provide only about 5% of the amount of water needed for protein hydration alone.

Perhaps the most remarkable result of this study is that the cells somehow managed to withstand exposure to repeated cycles of the gas bombardment regime without apparent damage. The issue of residual water aside, such a result further testifies to the extraordinary ability of these cells to survive environmental insults[13].

Effect of ethylene glycol on transcription of *Neurospora crassa* conidial genome[1]

R.K. Chaudhuri

Department of Botany, Univeristy of Calcutta, 35, Ballygunge Circular Road, Calcutta-700019 (India), 7 October 1977

Summary. Ethylene glycol (EG) was found not to alter DNA sequences in *Neurospora crassa* conidia, though it is believed to be mutagenic in nature. Molecular hybridization revealed 20% increase in whole RNA transcripts in EG-treated conidia, which indicates that while untreated conidia increase RNA synthesis by 2.35fold, treated conidia are inhibited and undergo only a 1.2fold increase. Thus there is an inhibition in potential RNA synthesis, though some RNA synthesis goes on in presence of ethylene glycol.

Mutagenic action of ethylene glycol (EG) was reported in rice plants[2,3] and it was noticed that germination was greatly reduced after EG treatment[3]. Polyethylene glycol (PEG) is now extensively used in protoplast isolation without any side effect[4]. After EG treatment, conidial germination was found to be arrested[5]. But treated conidia did not lose their vitality and 75% of these were found to germinate, within 4 h, after 7 days with 18% EG treatment (unpublished data). Increase in conidial weight, volume, nuclear, mitochondrial and ribosomal numbers were reported by earlier workers[5,6]. These observations suggest that EG does not inhibit synthesis of essential macromolecules. Present work reports, that with 18% EG treatment, base sequences of *Neurospora crassa* conidial genome do not alter significantly. Also it reports slight increase (20%) in its whole RNA transcription after EG treatment.

Materials and methods. Neurospora crassa (FGSC No. 74A) conidia, 4 days old, were used for the present investigation. Incubation of conidia was maintained at 30 °C with constant agitation in 18% (v/v) EG solution with Fries' minimal medium for different periods. In control experiments conidia were found to germinate within 4 h in minimal medium. Conidia and germinating mycelia were collected, washed repeatedly with TNE buffer, pH 7.8, and stored, if

Treatment (h)	True DNA-RNA hybrid*		True DNA-DNA hybrid**		Relative HAP adsorption DNA-RNA hybrid	
	Without EG treatment	With EG treatment	Without EG treatment	With EG treatment	Without EG treatment	With EG treatment
0	14.00	13.90	92.80	92.00	1.00	1.00
4	25.90	14.00	93.00	-	1.85	1.01
8	29.00	14.20	-	92.60	2.07	1.02
24	33.00	16.50	93.20	92.50	2.35	1.19
48	32.80	16.70	93.50	92.00	2.35	1.20

*^{32}P DNA concentration: 2 µg/ml^{-1}; RNA concentration: 4 mg ml^{-1}. **^{32}P DNA concentration: 2 µg/m^{-1}; unlabelled DNA concentration: 0.5 mg ml^{-1}; incubation mixture: 0.4 M phosphate buffer, pH 6.8+0.6 M NaCl+0.01 M EDTA+50% formamide; h of incubation: 220 (maximum); temperature: 37 °C.