A One-Drop Cryoscope: The Tonicity of Frog and Goldfish Sera[1]

A very promising device which provides objective register of freezing point depressions *via* recording potentiometer on relatively small samples (0.05–0.1 ml) and an automatic nucleation feature to prevent undercooling, has recently been described[2]. This instrument, however, was not used with biological materials. This paper will describe its use with such materials and present a simple modification in the design of the sample chamber for samples of 0.02 ml.

The modified sample chamber was essentially a glass tube with a thermistor sealed in its bottom. A glass tube (4 mm I.D. by 15 mm long) was one-third filled with liquid silicone rubber sealant (Dow Silastic 891) and allowed to harden. The thermistor probe (Glennite 32PB2) was prepared by grinding off most of its glass covering with a motorized fine stone. The thermistor leads were threaded through the bore of a short 18-gauge needle which was forced through the hardened sealant. The needle was removed and the probe pulled through the silicone rubber by its leads until the thermistor bead just reached the top of the sealant. A rubber grommet adapted the chamber to a larger diameter (7 mm I.D.) tube to provide a holder. Finally, leads and sample chamber were sealed in their holder with more sealant.

The design of the 20-μl chamber was shown to be valid by demonstrating that linearity obtained between concentration (in the biological range) and scale readings of the freezing points. A 100 mOsm concentration difference equalled 30 scale units on the recorder[3] chart. It was further demonstrated that published values for the tonicity of a representative organism, *Rana pipiens*, may be reproduced with ease by use of the modified chamber.

Frogs, *Rana pipiens*, in apparent good health were obtained from a local supplier and kept in a laboratory tank containing a small amount of tap water (equivalent to 20 mOsm KCl) for four days at 23°C before bleeding. Blood was drawn directly from the ventricles and centrifuged under oil to pack the clot and cells and remove other potential nucleation centers. 20-μl samples of the serum were run in the cryoscope and the results are presented in the Table. Frog serum has an average tonicity equivalent to 200 mOsm KCl or to $0.65 \pm 0.05\%$ NaCl. This value agrees with that obtained by KROGH[4] and MACALLAM[5]. It is lower than that reported by HOBER[6] and ADOLPH[7]. However, the former was derived from frogs held in a more concentrated environment than ours (equivalent to 57 mOsm KCl) whereas the latter was an average synthesized from a number of species.

The equivalent tonicity for the serum of the goldfish *Crassius auratus* (L.), standard xanthic, common comet was also determined. The value obtained (Table) was equivalent to 289 mOsm KCl or to $0.92 \pm 0.05\%$ NaCl. Although this value has not been previously reported, its correctness is substantiated by the following. The measurement falls in the general range of that reported for fresh-water teleosts[8], and luxurious *in vitro* growth of goldfish tails occurs in the media used for human skin[9], whose tonicity falls in the 0.9% NaCl range[10].

Zusammenfassung. Es wird eine einfache Modifikation eines Kryoskopes zur objektiven Messung von Gefrierpunktserniedrigungen im Bereiche von 20 μl beschrieben. Das Instrument eignet sich u.a. zur Bestimmung der Gefrierpunktserniedrigungen des Wirbeltierblutes.

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The tonicity of frog and goldfish sera, in equivalent osmolality and % NaCl

Animal	Number of determinations	Average Equivalent KCl (mOsm) ± standard error	Average Equivalent NaCl % ± confidence interval (95%)
Rana pipiens	28	200 ± 4.8	0.65 ± 0.05
Crassius auratus	11	289 ± 4.3	0.92 ± 0.05

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Rice Blast, Nyctotemperature, and Nitrate Reductase

That a particular genotype-nyctotemperature combination is an essential prerequisite for the occurrence of 'blast' disease of rice (*Oryza sativa* L.) caused by *Piricularia oryzae* Cav. has already been shown in this laboratory[1]. Subjecting susceptible rice plants (CO 13) to a nyctotemperature regime of 20°C gave the necessary predisposition for successful infection by the 'blast' fungus and in such plants there was an accelerated tempo of nitrogen metabolism with a considerable synthesis of the amide glutamine[2]. Conversely, when susceptible plants

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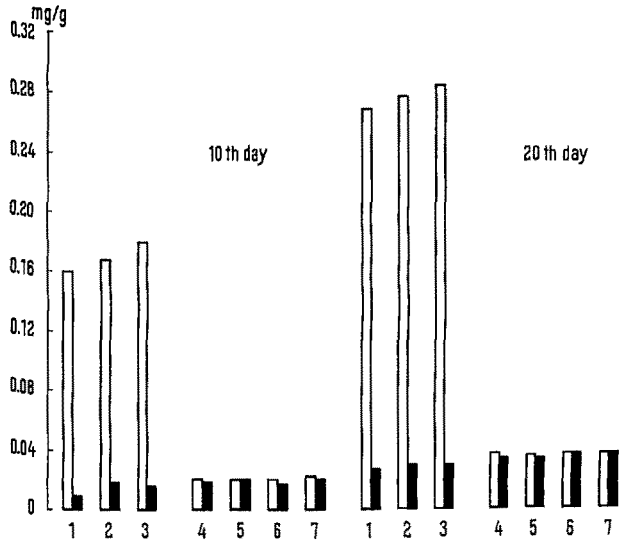
were subjected to a high nyctotemperature of 30°C nitrate accumulation resulted[3]. Although the possibility of stimulation of nitrate reductase activity in suscepts grown at low nyctotemperature was suggested[4], experimental proof for such a hypothesis is lacking. Hence a quantitative assay of nitrate reductase activity in susceptible and resistant rice seedlings grown at different nyctotemperatures was undertaken, the results of which are reported here.

Rice seedlings (*indica* varieties) susceptible (CO 13, GEB 24 and ADT 10) and resistant (CO 4, CO 25, CO 29 and CO 30) to 'blast' disease grown in Arnon and Hoagland nutrient solution were subjected to two nyctotemperatures (20° and 30°C) with a day temperature of 30°C in a thermostatically controlled miniature glasshouse with artificial light sources[5]. Leaf materials at desired age levels were collected and acetone dried powders prepared[6]. Nitrate reductase activity was measured colorimetrically (using a green filter – 540 mμ) by determining the amount of nitrite formed on incubation of the enzyme preparation with potassium nitrate (nitrite free) solution[7]. The results are presented in the Figure.

It can be seen that the variation in nyctotemperature did not affect the nitrate reductase activity in the resistant types. On the contrary, all the three susceptible types when grown at a low nyctotemperature registered a sizeable increase in nitrate reductase activity compared with those at a high nyctotemperature. This is in keeping with the postulated low reduction of nitrate at high nyctotemperature in tomato based on nitrate nitrogen accumulation recorded[8]. It has also been postulated[9] that the nitrate reducing ability of higher plants is possibly related to the genetic inheritance of nitrate reducing enzymes. The results reported here further indicate that this ability depends not only on the genotypic pattern but also on a particular genotype-nyctotemperature combination. Thus, it appears that the net effect of compatible genotype-nyctotemperature combination in susceptible rice types used by us at low nyctotemperature environment (20°C) seems to be one of stimulation of the nitrate reducing enzyme(s) leading on to incorporation of nitrogen in the amide. In the light of the recent work on the common co-factor for nitrate reductase and xanthine dehydrogenase which regulates the synthesis of nitrate reductase in *Aspergillus nidulans*[10], an extension of this idea to the problem of 'blast' would seem a worthwhile future line of investigation[11].



Nitrate reductase activity in *indica* rice varieties. 1–3: susceptible varieties; 4–7: resistant varieties. Ordinate: Nitrate reductase activity (mg nitrite nitrogen formed per g fresh weight). White columns: 20°C nyctotemperature; black columns: 30°C nyctotemperature.

Zusammenfassung. Der imperfekte Pilz *Piricularia oryzae* (Erreger von Blattflecken und anderen Schädigungen am Reis) befällt anfällige Sorten bei einer Nachttemperatur von 20°C, jedoch nicht bei einer solchen von 30°C. Die Nachttemperatur beeinflusst den Stickstoffhaushalt. Bei 20°C ist die Aktivität der Nitrat-Reduktase in den anfälligen Sorten (Figur, 1–3) wesentlich höher als bei 30°C; die resistenten Sorten (4–7) zeigen keine Unterschiede. Die Bedeutung der erhöhten Enzymaktivität für den Pilzbefall bleibt im einzelnen noch zu untersuchen.

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Some Common Features of Three Types of Insect Sensilla

Electrophysiological studies have shown that the characteristics of mechanosensory sensilla are the same as those of chemosensory sensilla. If the recording electrode is located distal of the sense cell body (as in base-, side wall-, and tip-recording of bristles or papillae), the common features are: (1) a negative receptor potential (mechanoreceptor[1,2], olfactory receptor[3], taste receptor[4]); (2) impulses with a positive and then a negative phase

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