Effects of tricaine methane sulphonate (M.S. 222) on the blood glucose levels in adult salamanders (Diemictylus viridescens)[1]

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Summary. Exposure to M. S. 222 results in hyperglycemia in adult newts (Diemictylus viridescens). A combination of higher anesthetic concentrations with reduced exposure periods reduces the magnitude of this form of stress response.

Tricaine methane sulphonate (M.S. 222) has been widely used as an anesthetic in experiments involving amphibians including studies of appendage regeneration in the adult newt, Diemictylus (Notophthalmus) viridescens [2-4]. Although, data regarding the basal blood glucose levels in the adult European Salamander, Taricha torosa[5] and both larval and adult Ambystoma tigrinum[6] are available, such values are lacking for Diemictylus viridescens. Moreover, an understanding of the metabolic response of the newt to M.S. 222-induced anesthesia is crucial in studies involving the measurement of hormones, blood glucose, liver glycogen and other metabolites. This study was undertaken to determine the concentration and duration of anesthesia eliciting minimum changes in basal glucose levels.

Methods. Adult newts, Diemictylus viridescens, from Nashville (Tennessee, USA), were kept in deionized water at 22 ± 1 °C, and fed ground beef twice weekly. Animals were allowed a minimum 2 week-period of acclimation to laboratory conditions. Medium sized animals of both sexes, weighing approximately 2.5 g were fasted for at least 48 h prior to use. Blood samples were collected from decapitated newts and from animals anesthetized in M.S. 222 (1 g/l) at 23 °C for periods of 15, 20, 30 and 45 min. An additional group was exposed for 15 min to 1.5 g/l anesthetic. M.S. 222 solution was prepared in distilled water immediately before each experiment and discarded immediately after use. Care was taken so that the control and experimental animals received the same handling prior to treatment. Each animal was isolated and anesthetized singly and the time of exposure was precisely controlled. Duplicate 10 µl blood samples were collected by cardiac puncture using heparinized lambda pipettes and blood glucose levels were immediately measured by the glucose oxidase procedure[7].

Results and discussion. A total of 64 adult salamanders were used for blood glucose analyses. A standard t-test for significance of the difference between mean blood glucose levels was performed. The results, summarized in the table show that the mean blood glucose level in decapitated animals (i.e. no anesthesia) was 25.11 ± 6.24 mg%; this was considered to be the resting level. A significant change in this value was not observed when the animals were kept in water at 23 °C for 10–30 min prior to decapitation. When the newts were anesthetized in M.S. 222 (1 g/l), mean blood glucose levels increased within 15–20 min, reached a peak (66.14 ± 35.56 mg%) after 30 min, and subsequently declined toward basal levels. However, when a higher concentration of anesthetic was used (1.5 rather than 1 g/l) 15 min of exposure effectively anesthetized the animals without elevating the glucose level. Thus, hyperglycemic response to stress can be minimized through use of a combination of higher concentration and reduced exposure period.

In addition, we observed a wide variation in the mobilization of glucose into the blood, which was presumably an expression of the individual response to stress. When newts were anesthetized in M.S. 222 (1 g/l) for 30 min, the blood glucose level of individual animals ranged from 33–145 mg%. The animals exhibited far less variation in their blood sugar levels when they were either anesthetized in 1.5 g/l of M.S. 222 for 15 min or decapitated. The latter, although probably one of the most reliable ways of obtaining basal blood glucose levels, is perhaps not the most effective way of collecting blood in a small animal because of the loss of blood consequent to decapitation. It is apparent from our study that M.S. 222 in the concentration widely used in regeneration studies (1 g/l) induces a definite stress response in adult newts, of which one manifestation is a hyperglycemic condition. The use of an efficient and quick anesthesia is extremely relevant in regeneration studies in Diemictylus viridescens, as attention is currently being focused on the control of molecular events in regeneration necessitating the measurement of various metabolites. Since several metabolic changes, involving hormones and metabolites, are induced by stress, it is essential to arrive at the shortest possible time of anesthesia to minimize such changes.

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The effects of the anesthetic, M. S. 222, on the mean blood glucose values of adult newts, Diemictylus viridescens

Treatment	Number of animals used	Duration of anesthesia (min)	Concentration of M. S. 222 (g/l)	Mean blood glucose (values ± SD)	P value (compared to decap. values)
Decapitation	10	–	–	25.11 ± 6.24	–
M. S. 222	11	15	1.5	22.40 ± 7.54	$p > 0.3$
M. S. 222	10	15	1	32.68 ± 15.69	$p > 0.1$
M. S. 222	10	20	1	31.65 ± 8.87	$p > 0.05$
M. S. 222	13	30	1	66.14 ± 35.56	$p < 0.001$
M. S. 222	10	45	1	41.17 ± 13.07	$p < 0.001$