

A non-phosphate-buffered physiological saline for *in vitro* electrophysiological studies on the mammalian neuromuscular junction

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There have been numerous attempts to design an 'ideal' physiological mammalian saline (see R..F. Burton, 1975). In this study, particular emphasis has been placed on designing a balanced ionic and metabolic (substrate) saline approaching that of mammalian sera (Table 1) which would be capable of maintaining 'active' metabolism in *in vitro* nerve muscle preparations. An inappropriate saline might accentuate impaired neuromuscular transmission of preparations from myasthenic patients or from animals with experimentally induced myasthenia gravis.

	Serum			
	Mouse*	Rat*	Human†	R-S saline‡
Na+	152.0	145.0	143.1	135.0
K+	6.2	6.1	4.4	5.0
Cl-	114.5	98.0	102.7	121.0
Ca ²⁺	2.1	-	2.5	2.0
Mg ²⁺	1.3	-	0.94	1.0
PO ₄ ²⁻	2.4	-	1.4	0.0
HCO ₃ ⁻	12.0	24.0	24.9	20.0-25.0
	Substrate components			
Glucose (mmol)	10.4	5.4	4.6	11.0
Pyruvate (mmol)	0.14	0.22	0.58	0.0
Lactate (mmol)	4.8	4.4	4.4	0.0
Glutamic acid (mmol)	-	-	0.47	0.30
Glutamine (mmol)	-	-	0.44	0.40
Insulin (m-i.u.)	37.0	17.3	28.0	0.0
Choline (mg/l.)	-	-	4.4	1.0-5.0
Coccarboxylase (µg/l.)	-	-	40.0	20.0

* Experimental values.

† From *Documenta Geigy*, 7th Ed.

‡ RS-saline buffered with 5.0 mmol BES. Oxygenated with 95% O₂-5% CO₂, for 40 min. pH: 7.21 (reservoir) to 7.39 (bath) at 25 °C.

The suitability of R-S (Rees-Simpson) saline for long-term (20-50 hr) *in vitro* experiments was further facilitated by replacing sodium dihydrogen phosphate with a Good's buffer, BES (Good, Winget, Winter, Connolly, Izawa & Singh, 1966), thereby avoiding precipitation of calcium phosphate and pH instability. This saline has now been used successfully with *in vitro* nerve-muscle preparations from mouse, rat, rabbit, dog, horse and human muscles. A detailed electrophysiological study on nerve-muscle preparations of the mouse diaphragm, intercostal, soleus and peroneous muscles indicated no deleterious changes in neurophysiological parameters over periods of 30 hr.

Supported by a Research Fellowship from the Muscular Dystrophy Group of G. Britain.

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