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## THE PRESERVATION OF CELLULAR FUNCTION IN ISOLATED MAMMALIAN TISSUE/ORGAN PREPARATIONS.

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Historically, the maintenance of the physiological and pharmacological functions of isolated tissue/organ preparations from mammals has witnessed the use of a plethora of different recipes of which, few have attempted to approach the natural composition of the extracellular (serum) aqueous phase (1,4).

Conceptually, it seemed logical that a basic requirement in the design of a mammalian perfusion medium with 'universal' or 'inter-species' application should ensure (a) adherence to the physiological (serum) levels of ionic species and metabolites (1), (b) optimal utilisation of both residual (tissue) and added substrate and (c) accurate control of temperature, gaseous exchange, pH, osmolality, conductivity and 'flow' dynamics of the perfusing medium. Particular emphasis in the design of RS-I saline was placed on utilising 'free' (ionised) or 'active' levels of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ , which is reflective of the 'time-dependant' complexing of these ions with such 'active' radicals as phosphate, bicarbonate, albumen, etc.(2), as would be evident in the majority of conventional salines currently used under *in vitro* conditions (e.g. Bretag's, Krebs & Henseleit's, Turner's).

The criteria outlined were adopted in the formulation of RS-I mammalian saline, a non-phosphate buffered medium (3), and the utilisation of the controlled temperature and perfusion/perifusion characteristics afforded by a 'Res-Del Perfusion System'. Essentially, attempts have been made to maintain the myriad of continually varying electrochemical, biochemical and biophysical processes known to contribute to the overall balance of inter-dependency that exists between cell metabolism and function.

Studies to date have investigated the effects of altering glycolytic substrates, aspartate-malate/glycerol phosphate shuttles and free-fatty acid utilisation on the physiological and pharmacological responsiveness of isolated skeletal, visceral and cardiac preparations from a variety of mammalian species. Undoubtedly, the most dramatic effects were those observed using conventional phosphate buffered salines whereby, the preparations were found to function for only a matter of hours versus days in RS-I saline, e.g. rat heart (2.5 days); rat uterus (10 days); rabbit cervical ganglia (2 days); guinea pig ileum (7 days).

Results achieved to date substantiate the premise that maintaining a metabolic balance, particularly with respect to glycolysis, oxidative phosphorylation and  $\text{NAD}^+/\text{NADH}$  ratios, in the cellular components of tissue/organ systems is requisite for the validation of experiments conducted *in vitro*.

1. Burton, R.F (1975) In 'Ringer Solutions and Physiological Salines', ed. Burton, R.F. Ch. II-XI, pp 5-9 . Bristol: Wright-Scientifica .
2. Pedersen, K. O. (1973) In 'Calcium in Human Serum'; Biochemical and Clinical Aspects. PhD Thesis, University of Aarhus, Denmark.
3. Rees, D (1985) Proceedings of the Physiological Society of New Zealand, 5; 18p.
4. Robin, E.D.(1977) Clinical Science and Molecular Medicine, 52; 443- 448.