

5th

Freiburg

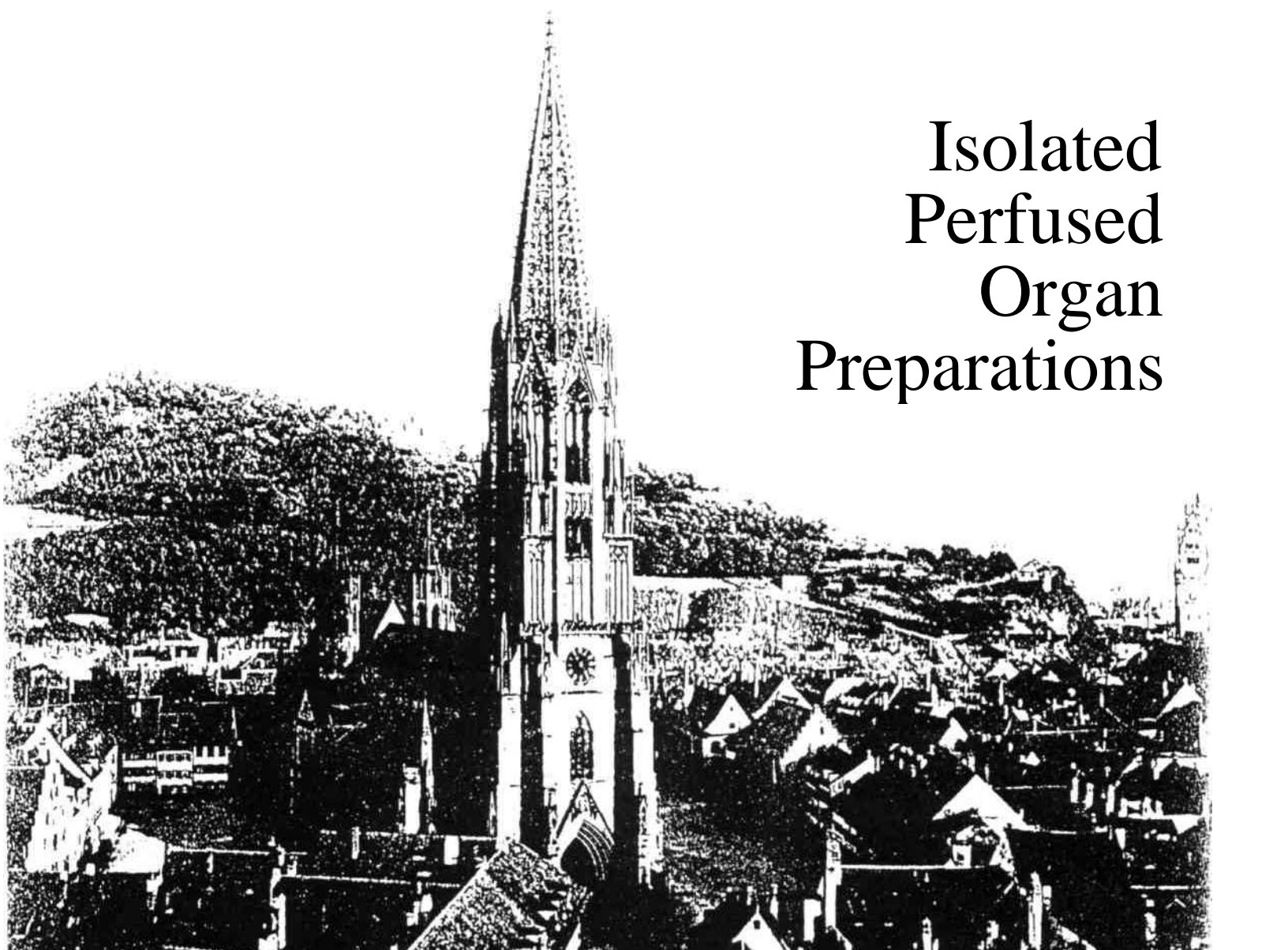
Focus on

BIOMEASUREMENT

FFB 5



February 27th and 28th, 1989

A high-contrast, black and white photograph of Freiburg, Germany. The central focus is the Freiburg Cathedral (Münster), a Gothic church with a tall, slender spire. The surrounding cityscape is visible, showing traditional European architecture with tiled roofs and narrow streets. The image is rendered in a high-contrast, almost binary style, with deep blacks and bright whites, giving it a graphic, woodcut-like appearance.

Isolated
Perfused
Organ
Preparations

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CONSIDERATION OF THE INORGANIC AND ORGANIC COMPOSITION OF MAMMALIAN PERFUSION SOLUTIONS

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Historically, the design of physiological salines dates back to the thesis proposed by the great French physiologist, Claude BERNARD (1872), who, through his clinical studies put forward his treatise on the 'milieu interieur', basically purporting that to maintain the whole (person) one should ensure that the surrounding (extra)cellular environment should be balanced in all respects.

Unfortunately, the misinterpretation or misconception of Bernard's 'milieu intérieur', as reviewed by ROBIN (1977), has led succeeding researchers to confuse the 'extracellular' with the 'intracellular' phases of cell function and the need to maintain the cell as a 'whole' entity. The initial basic 'salt' solutions for in vitro studies commenced with the simple formulation proposed by Sidney RINGER in 1883 for the isolated perfused frog heart, after which time, similarly contrived 'salt' solutions were utilised by LOCKE (1901), TYRODE (1910) and HANKS (1948) for isolated mammalian preparations. The phosphate/bicarbonate saline originally designed by KREBS and HENSELEIT (1932) was for studies on isolated homogenates of mitochondria (organelles) from pigeon liver (KREBS and EG-GLESTON, 1940) and later for his classical analysis of oxygen consumption in tissue slices of different organs from a variety of animal species (KREBS, 1950). One would add that KREBS (1950) correctly interpreted Bernard's hypothesis that the whole cell had to retain a metabolic homeostasis.

Traditionally, phosphate/bicarbonate buffers have been used in conventional solutions for perfusing mammalian tissues and organs, with questionable validity, for over 50 years and are still being currently utilised by numerous researchers. Interestingly, it has been known for 35 years that inorganic phosphate ions inhibit glycolysis (BERMAN and SAUNDERS, 1955) and, more recently, the inhibition of creatine kinase (HALL and DE LUCA, 1985) and the enzymes involved in oxygen "free radical" scavenging which have been implicated in reperfusion injury in numerous organ systems (STEWART et al., 1986).

The ensuing four decades witnessed the use of a plethora of different saline recipes for insect, Crustacea, amphibia, fish and mammals alike, of which, few have attempted to approach the natural composition of the extracellular (serum) aqueous phase for the maintenance of the physiological and pharmacological functions of isolated tissue/organ preparations.

Conceptually, it seemed logical that a basic requirement in the design of a mammalian perfusion solution with 'universal' or 'inter-species' application should, as indicated by BURTON (1975), ensure,

- (a) adherence to the physiological (serum) levels of ionic and metabolic components;
- (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 solution.

Such criteria have been adopted in the formulation of RS-I mammalian solution (REES (1985)), a non-phosphate buffered medium (Fig. 1), and the utilisation of the controlled temperature and perfusion/perifusion characteristics of the 'Res-Del Perfusion System' and the laminar flow dynamics of the Res-Del(TM) perfusion bath (Fig. 2).

In essence, a perfusion solution should attempt 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. Our results over the last ten years

(after Rees.1985)

Component	RS-I (mOsmoles/L)	
(IONIC)	KCl	
	NaCl	110.0
	CaCl ₂	1.2
	MgCl ₂	0.45
(BUFFER) -	NaHCO ₃	25.0
	BES	5.0
	D-glucose	10.0
(SUBSTRATES)	glycerol	0.11
	L-aspartate(Na ⁺)	0.02
	L-glutamate(Na ⁺)	0.30
	L-glutamine	0.40
	DL-carnitine	0.05
	Choline chloride	0.01
	cocarboxylase (TPP)	0.043
	Insulin (Porcine)	25.0 mIU/L

(carbogenate with 95% O₂/ 5% CO₂)
 [pH 7.23 - 7.41 ± 0.05 at 20-37 °C]

Fig 1 Composition of the Res-Del RS-I mammalian physiological Solution

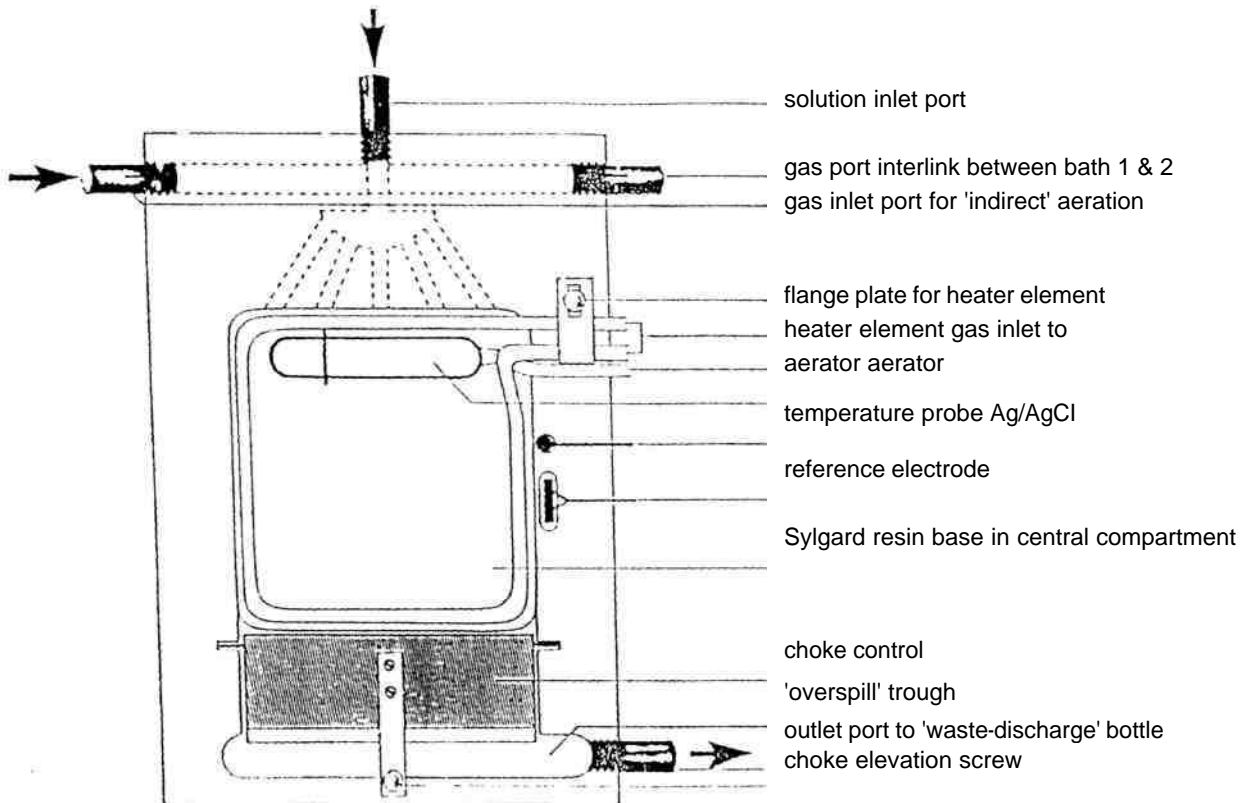


Fig 2 Res- Del perfusion hath with component modules in place

COMPONENT	SERUM	ISF	RS-I (1985)	K & H (932)
Cations (mOsmoles)				
Sodium	142	145	136	143
Potassium	4	4	5.0	5.9
Calcium (total)	2.5	2.5	1.2 *	2.5
Magnesium (total)	1 .0	1 .0	0.45 *	1 .2
TOTAL	149.5	152.5	142.7	152.6
Anions (mOsmoles)				
Chloride	101	114	118	127
Bicarbonate	27	31	25	24.8
Phosphate (Inorg.)	1 .0	1 .0	0	1.2
Sulphate	0.5	0.5	0	1.2
Organic acids	6.0	7.0	5(BES)	0
Proteins	2.0	1 .0	0.002	0
TOTAL	137.5	154.5	148.0	154.2
GLUCOSE	5	-	10	11.1
INSULIN (nmoles)	1 .4	-	1 .7	0
OSMOLALITY (mOsmoles/kg)	282	287	286	299
CONDUCTIVITY (mS cm ⁻¹)	12.4	12.8?	12.6	-

(* 'Freely Ionised')

(? Theoretical est.)

Fig. 3 Comparison of the inorganic, organic and biophysical components of human sera and ISF with RS-I and K & H physiological solutions.

have substantiated the premise that maintaining a metabolic balance, particularly with respect to glycolysis, oxidative phosphorylation and NAD/NADH ratios of the cellular components of tissue/organ systems, is requisite for the validation of experiments conducted in vitro (Fig.6a & b).

Of primary importance in the design of a 'physiological' solution is to achieve a replication of the ionic profile of the extracellular phase which, in mammalian species, is identifiable with the serum and interstitial fluid (see Fig. 3). However, a common misconception has been to adopt the 'total' ionic content of the serum which does not acknowledge the activity coefficients of each ionic species (see BURTON, 1975) and, more importantly, the serum-binding of Ca^{2+} and Mg^{2+} which will ultimately determine the 'free' or 'active' ionic profile of the physiological solution (PEDERSEN, 1973). The latter condition applies in the case of RS-I mammalian perfusion solution where a conductivity value of 12.6 mS cm⁻¹ compares with that of human serum (see Fig. 3). In addition, the state of hydration of the ionic components and extracellular pH (pH_o) will determine the state of ionisation of the viscous interstitial phase and surface membrane constituents and is therefore a determinant factor of the molecular activity of the bioelectric membrane and the very survival of the cell per se (Fig. 4).

A basic acknowledgement in the design of RS-I mammalian solution was the fact that the cell membrane lies in continuity with a 99% gel interstitial phase (ISP) so providing a 'natural' colloidal buffer phase to excess Donnan equilibrium ionic exchange across the cell membrane (Fig. 5). In this regard, it should be noted that the osmotic pressure of human plasma is 5850 mmHg (ca. 300mOsmoles), of which, only 28 mmHg is attributable to the colloidal osmotic pressure of plasma proteins (i.e., 1.4 mOsmoles).

It may be argued therefore, that since RS-I solution is of comparable osmolality to human serum, namely, 287 mOsmoles, the additional requirement of 'plasma-expanders', such as BSA, dextran,

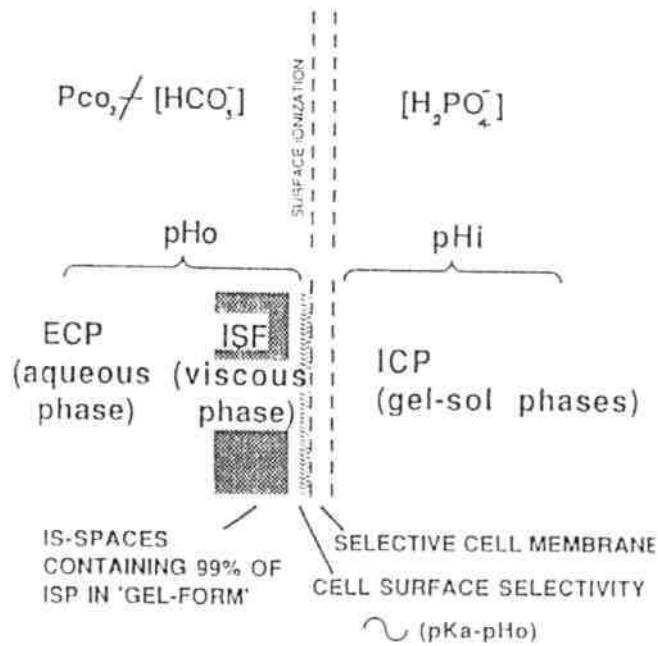
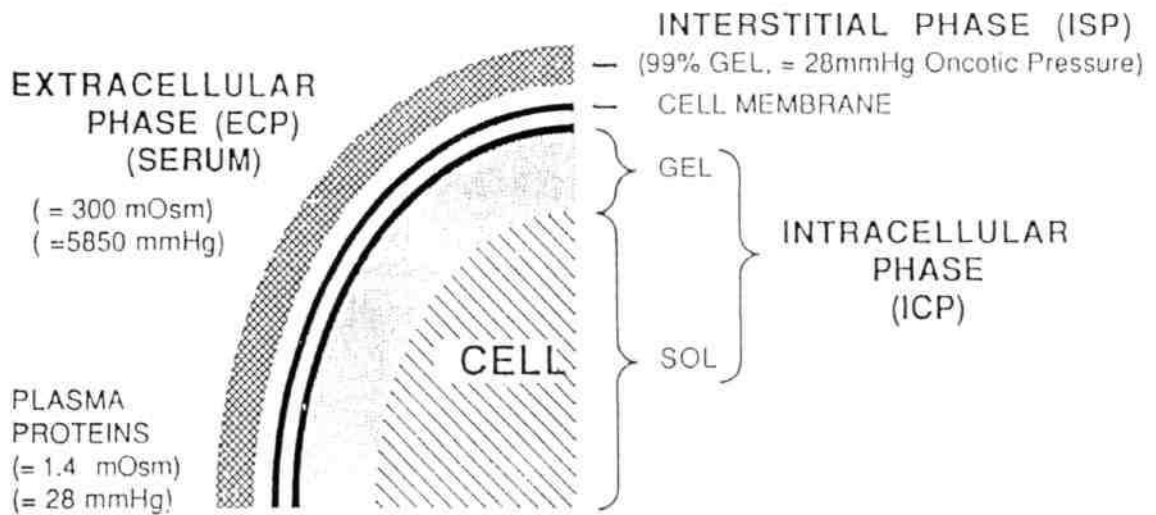


Fig 4 Biological buffer system.



GEL = POLYELECTROLYTE 'STRUCTURAL' PHASE.
 SOL = 'DYNAMIC PHASE. METABOLIC ACTIVITY'

Fig 5 Cell interface osmotic relationships.

'Haemaccel'. PVP, etc., as in other perfusion solutions, may be questioned if one considers the positive long-term results achieved utilising RS-I perfusion solution with a variety of different, isolated mammalian preparations (see Fig. 6a and b). Perhaps an exception to this theory is the mammalian kidney for our preliminary results with the perfused rat kidney have indicated that optimal glomerular filtration may necessitate the addition of crystalloid colloids to 150% of the osmotic pressure of RS-I solution for experiments lasting 4 - 24 hours to prevent oedema of the kidney tubules (BULLIVANT (1978)).

Species	Tissue/Organ	Max Days	Preparations	
		in vitro	Stored °C	Used °C
Rat	Jejunum	9.0	4	35
-	"	1.5	-	15
"	Intestine	8.0	4	35
-	-	1.3	-	35
-	Colon	5.0	-	20-35
-	Uterus	3.0	-	20-35
-	-	10.0	4	35
"	detrusor muscle	2.0	-	20-35
-	diaphragm muscle	0.6	"	35-37
-	-	2.0	-	20-35
-	Soleus muscle	1.1	-	20-35
-	Heart	0.3	-	35-37
-	-	1.2	-	20-35
-	heart-lung	0.7	-	35

Species	Tissue/Organ	Max Days	Preparations	
		In vitro	Stored °C	Used °C
rabbit	Intestine	5.0	4	37
-	-	2.0	-	20-37
-	Uterus	7.0	4	37
-	superior cervical ganglion	2.0	4	37
-	-	0.8	-	37
"	RBC's	3.0	No haemolysis at 4°C	
guinea pig	Ileum	7.0	4	37
-	Detrusor	4.0	4	37
-	"	1.0	-	20-37
mouse	Diaphragm	1.5	-	20-35
-	Intestine	0.9	-	20-35
-	n.t release diaphragm	1.5	-	20-35
"	intercostal muscle	0.9	-	20-35
-	RBC's	1.0	No haemolysis at 4°C	

Fig 6 b Functional viability of RS I maintained mammalian tissue/organ preparations.

Nevertheless, caution is advised when contemplating the addition of such oncotic agents because of various contradictory observations reported using such plasma expanders, eg., BSA - antigenicity, pH and Ca^{2+} -binding ; Dextran - loss of enzyme content; 'Haemaccel' - high (6.3mM) Ca^{2+} - ions.

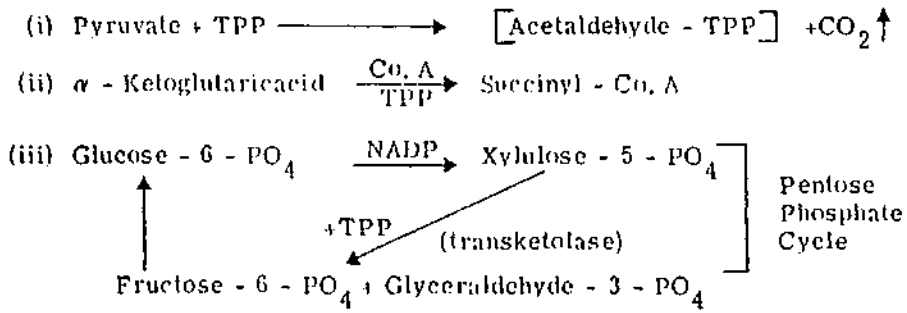


Fig 8 The involvement of Thiamine Cocarboxylase (TTP) in the Pentose Phosphate shuttle.

context, it has been found that the inclusion of thiamine pyrophosphate, glutamate, aspartate, insulin and glycerol in RS-I solution has been beneficial in sustaining,

- (a) the pentose phosphate shuttle (Fig. 0),
- (b) glycerol phosphate and aspartate-malate shuttles (Fig.9), thereby optimising mitochondrial metabolism and the generation of 'high-energy' radicles.

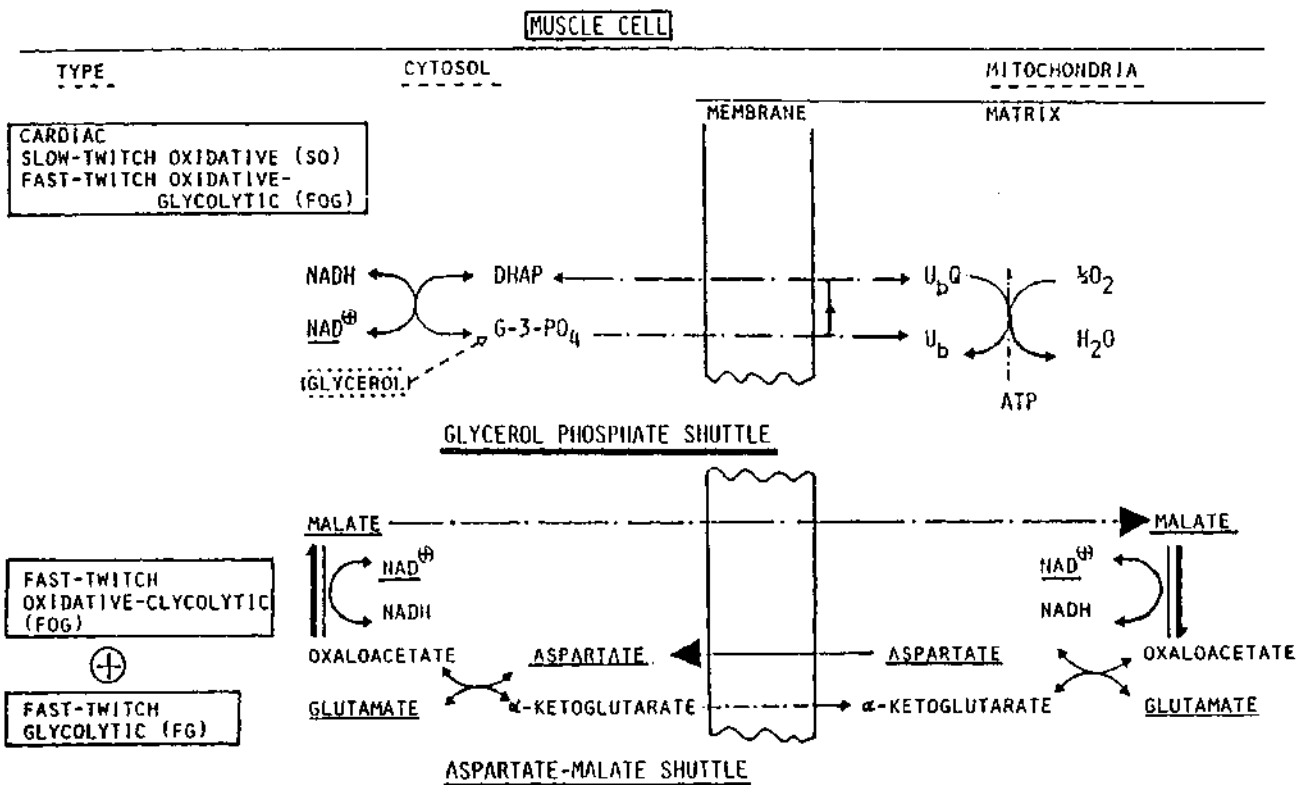


Fig 9 NAD⁺/NADH operative shuttles in SO, FOG- & FG type myofibres

One of the many long-term in vitro studies conducted (see Fig. 6a and b) has involved the classical bioassay preparation, the guinea pig ileum, which has been demonstrated to retain its physiological and pharmacological characteristics for up to 7 days (see Fig. 10a and b).

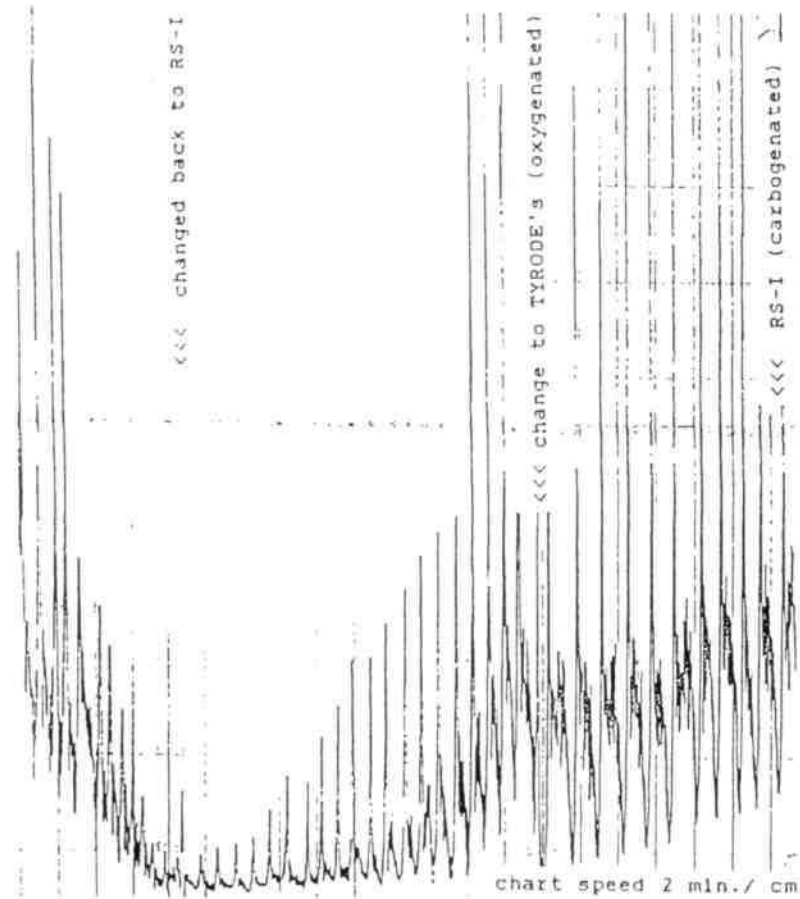


Fig. 10a Effect of alternate perfusions of TYRODE and RS-I oxygenated salines at 37°C on electrically induced contractions of isolated, guinea-pig longitudinal ileal muscle. Note the 90% decrease in activity over a 15 min. perfusion period in TYRODE's saline and the 100% recovery following 9 min perfusion with RS-I mammalian saline.

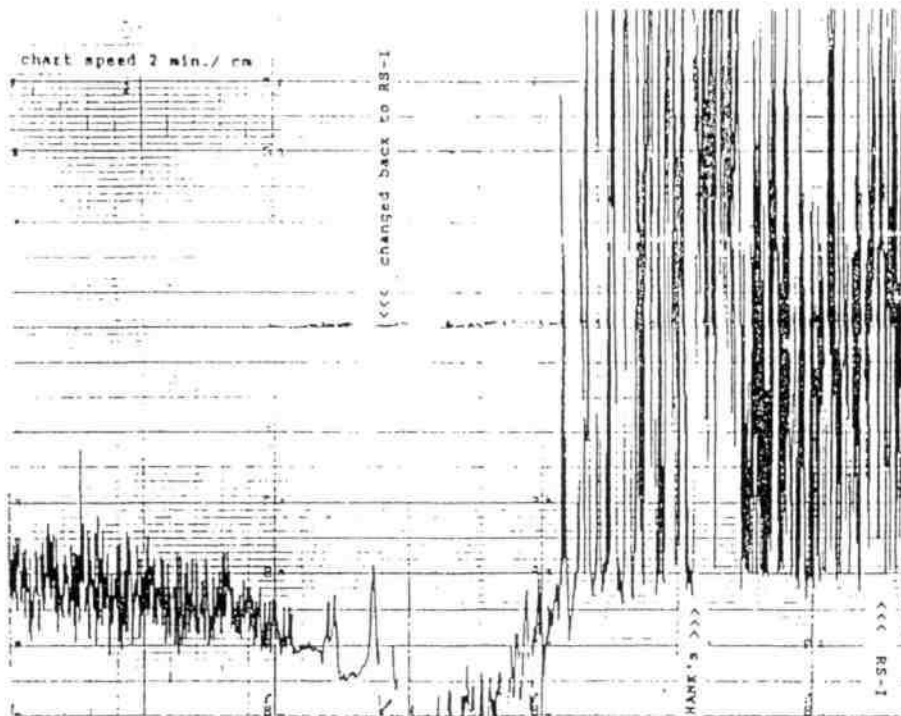


Fig. 10b Effect of alternate perfusions of HANKS and RS-I oxygenated saline at 37°C on electrically induced contractions of isolated, guinea-pig longitudinal ileal muscle. Note the immediate decrease in contractile activity following perfusion with HANK's saline and its irreversibility after 15 min perfusion with RS-I saline.

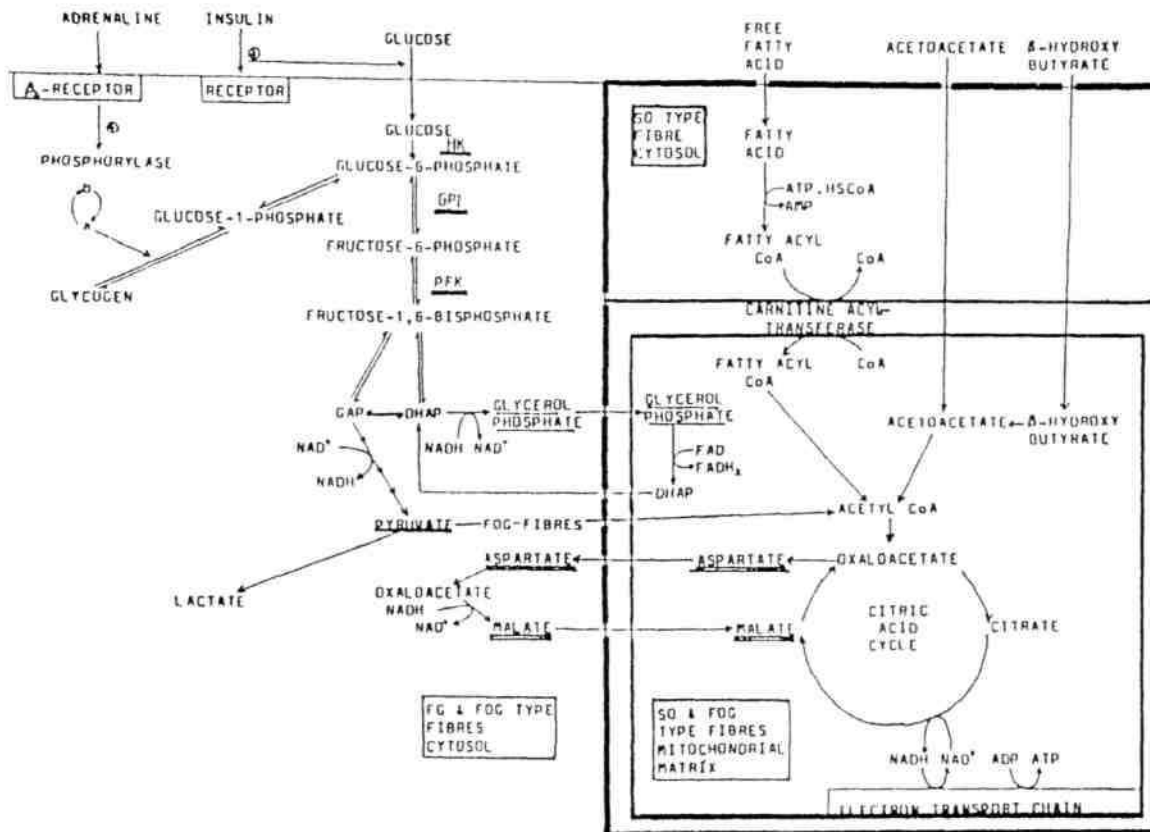


Fig. 11 Schematic diagram of the salient biochemical profiles evident in SO-, FOG- and FG-type muscle fibres.

Additionally, the inclusion of carnitine has proved essential in maintaining muscular contractility apparently by its ability to maximise residual fatty acid radical utilisation by the mitochondria in the form of acylcarnitine CoA and the generation of adenosine nucleotides.

We have found in our in vitro long-term studies that bacterial infection of isolated preparations occurs over periods in excess of 24 hours (Fig. 12) but has been adequately controlled by the inclusion of Chloromycetin (10-50 mg/l) in RS-I mammalian solution for periods of up to 48 hours. Interestingly, electron microscopic examination of hypothermically maintained isolated rat heart preparations has indicated that occlusion of the endothelial coronary micro-vasculature was caused by bacterial deposits and not, as previously reported, red blood cell sludging (M.J.TOES, unpublished data). Preliminary studies have indicated that negligible clumping of red blood cells occurs in RS-I mammalian solution for periods of up to 48 hours (REES, unpublished data).

In conclusion, it is suggested that it is the maintenance over time of the cell isovolume that must be preserved as this directly relates to metabolic homeostasis and therein the preservation of physiological and pharmacological viability of in vitro preparations.

SUBSTRATE	BACTERIA CULTURED				
	TIME (hr)	Pseudomonas aetuginosa	Flavobacterium spp.	Enterobacter clocae	Klebsiella edwardsii
De-ionised H ₂ O	0.0	+	-	-	-
RS-saline	1.0	++	+	-	-
RS-saline	8.0	+++	+	+	-
RS-saline	20.0	++	-	-	+

+ light growth
 ++ ; moderate growth
 +++ heavy growth
 - • no growth

Fig. 12 Time-Dependant bacteriological profile of physiological perfusion medium.

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