Ion Diffusion Delay in the Beating Mammalian Heart.* (29190)

SIDNEY S. SCHREIBER, MURRAY ORATZ, AND MARCUS A. ROTHSCHILD
(Introduced by Milton Levy)
Radioisotope Service, Veterans Administration Hospital, Department of Medicine, New York University School of Medicine, and Department of Biochemistry, New York University School of Dentistry

Inherent in any perfusion experiment measuring ion exchange is the problem of diffusion delay of the ion passing through the extracellular spaces into the surrounding medium. It has been demonstrated that the rates of movement of ions and gases in bathed tissues are dependent on the thickness of the tissue tested, and diffusion delay may influence the interpretations of cellular ionic movement(1-3).

Studies of heart and skeletal muscle potassium exchange have suggested that at least some of the intracellular K is relatively "bound" and that the bound fraction does not exchange as readily or as rapidly as the rest of the cardiac K(4). In the working guinea pig and frog hearts, in which the myocardium was perfused through the coronary circulation via the ventricles and aorta, cardiac potassium exchanged at two rates(5,6), and the "fast" compartment exchanged at a rate roughly 10 times that of the "slow" compartment. If extracellular diffusion delay were significant, evaluation of this rapidly exchanging compartment would be difficult.

The present report presents data to show that the passage of ions (measured by washout of the cation Na^{24+} and the anion I^{131-}) through the extracellular spaces in the coronary perfused working mammalian heart is very rapid and does not interfere with analyses of either slowly or rapidly exchanging K compartments.

Methods. Guinea pigs weighing 250-300 g were used in all the experiments. As described previously(6), the entire heart was prepared for perfusion *in vitro* with mammalian Ringer's solution containing a bicarbonate buffer (pH 7.45 $\pm$ 0.1 when actively aerated with 95% O_2 5% CO_2 and a K concentration of 4 meq/liter. In this preparation, the perfusion load was presented to the left ventricle which then pumped it out the aorta against a pressure of 50 mm Hg. Thus, coronary perfusion was maintained at 50 mm Hg during systole. The cardiac rate was maintained electrically at 160/min, or the hearts were allowed to beat spontaneously at a rate of 150-180/min. The temperature of the heart was maintained by passing the perfusion and bath fluid through heat exchange columns. The perfusion load presented to the left ventricle was 8 ml/min.

Washout of Na^{24+} or I^{131-} was carried out by first perfusing the heart with Ringer's solution containing the desired isotope for 25-60 minutes followed by perfusion with non-radioactive Ringer's. Radioactivity released by the heart was almost immediately removed by a rapidly changing bath solution and for all practical purposes, the counts recorded by the counter were those within the heart itself. The rate of washout was ascertained as described previously by continuous counting of radioactivity remaining in the heart(5). The counts/minute remaining in the preparation were plotted on semi-logarithmic paper as a function of the time of washout, and rates of washout determined from the resultant curve(5,7-9). Tissue slices used in these experiments were prepared from the intact guinea pig hearts with a Stadie fresh tissue microtome. The slices were 400-500 μ in thickness and approximately 1 cm diameter. The slices were equilibrated with I^{131-} for 25 min in aerated Ringer's solution, then washed with rapidly agitated and changing non-radioactive perfusate. Each experiment included 12-14 slices. At 2-3 minute intervals, a slice was removed and total radioactivity assayed. Counts/gram were plotted on semi-logarithmic paper as a function of time of washout and rate of washout

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TABLE I. Washout of Na^{24+} and I^{131-} in the Beating Heart and Tissue Slice.

Exp No.	Temp C	Fast component: Rate (min^{-1})	Fast component: % of total*	Slow components: Rate 1 (min^{-1})	Slow components: % of total	Slow components: Rate 2 (min^{-1})	Slow components: % of total
Working heart							
1 Na^{24}	37	.87	61	.14	17	.022	22
2 "	"	.69	90	—	—	.020	10
3 "	"	1.16	58	.17	20	.035	22
4 "	"	1.30	80	.35	10	.020	5
5 "	"	.90	80	.17	17	.010	3
6 "	"	1.04	75	—	—	.060	25
	Mean 1-6	.98 ± .08†	76				
7 Na^{24}	25	1.70	70	.58	20	.060	10
8 "	"	1.40	78	.46	17	.077	5
9 "	"	.58	71	—	—	.073	29
10 "	"	.85	81	—	—	.079	12‡
11 "	"	.77	61	—	—	.032	39
	Mean 7-11	1.06 ± .21†	72				
12 I^{131}	25	1.07	78	—	—	.033	22
13 "	37	.85	90	—	—	.090	10
14 "	"	.98	80	—	—	.110	20
15 "	"	1.20	90	—	—	.090	10
16 "	"	1.02	79	—	—	.010	21
	Mean 12-16	1.02 ± .06†	83				
Tissue slices							
1 I^{131}				.11			
2 "				.07			
3 "				.11			
	Mean tissue slice exp			.09 ± .01†			

* The slopes of the components are represented by the rate constants (min^{-1}), and the intercepts with the ordinate by % of the total original counts in heart or ventricle. Since zero time value for cardiac radioactivity could not be exactly determined, the first minute count was used in calculations of % of total. Thus, it is recognized that the size of the fast component given here is a minimum value and is probably larger.

† ± S.E. of mean.

‡ In this experiment, there was an even slower compartment with a rate constant of 0.025 min^{-1} containing 7% of total initial radioactivity.

determined from the resultant curve by standard curve analysis(7-9).

Results. The data for Na^{24+} and I^{131-} washout are presented in Table I, and a representative study in Fig. 1. Washout of both ions occurred at 2 or more rates similar to those observed in the frog and dog(5,7,8) with almost 80% of the total initial radioactivity exchanging at a "fast" rate. Only a few seconds were required for the radioactivity in the bath and ventricular cavity (from the isotope loading procedure) to be washed away with stable Ringer's solution. The radioactivity remaining in the heart was then recorded at minute intervals. Since the rate of washout was so rapid, the true "zero" time value of radioactivity within the heart was not known. Hence, the fast component size, calculated from the first minute assay for initial cardiac radioactivity, is a minimum value, and the extracellular ion must have represented a higher figure than the 80% described above.

The mean washout of Na^{24+} fast component was 0.98 ± 0.08 min^{-1} at 37°C and 1.06 ± 0.21 min^{-1} at 25°C. The difference is not significant. I^{131-} fast component washout at 25°C was 1.07 min^{-1} in one study compared to a mean of 1.02 ± 0.06 min^{-1} for the anion at 37°C. There was no significant difference between Na^{24+} and I^{131-} at both temperature levels.

In contrast, washout of I^{131-} at 37°C from rapidly agitated tissue slices was 0.09 ± 0.01 min^{-1}, at least 10 times slower than the mean washout of the intact, coronary perfused working heart.

Discussion. Washout of Na^{24+} in the

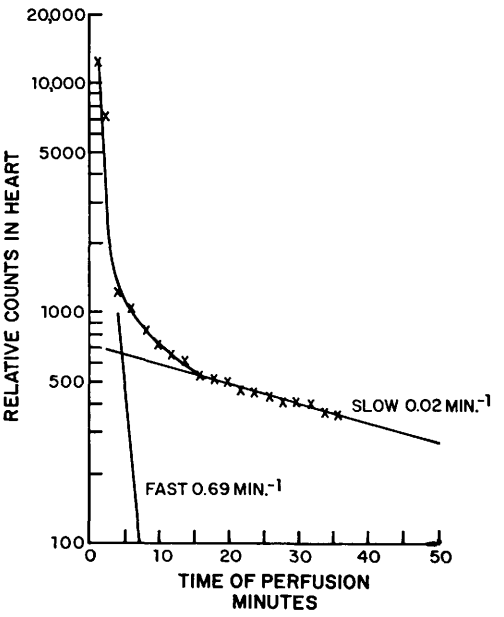

FIG. 1. Washout of Na^{24+} from beating heart previously equilibrated with the isotope for 25 min. Washout in this experiment is at 2 rates, the fast rate representing the major part of Cardiac Na^{24+} leaving the heart at 0.69 min^{-1}.

beating heart at various temperatures and frequency of beat has been reported as diphasic(5), triphasic in the frog(7) and dog (8), and with 4 components in the frog atria (9). The results of the present study are consistent with earlier reports in showing that the slowly exchanging Na^{24+} compartment may not be physiologically uniform in all cases. It is not the purpose of this study to evaluate the slowly exchanging compartments. This has been carefully done previously(7-9). The slowest rate of transfer is presumed to represent intracellular Na(5, 7-9) with the intermediate rate compartment located in the connective tissue of the heart (8) or within the cell(7). Johnson, in simultaneous studies with sucrose and Na^{24+} demonstrated that the very slow and the intermediate compartments could be explained by intracellular sodium(7). Whatever the location of the slower exchanging compartments, there seems to be no disagreement that the fastest rate, comprising the largest fraction of Na (nearly 80% in the present study) represents extracellular sodium transfer(5,7-9).

Extracellular diffusion assumes vital importance in ion exchange studies in tissues examined by the submersion technique without direct capillary perfusion. Despite rapidly flowing fluid past a small submerged muscle, Keynes demonstrated that diffusion delay increased with the thickness of the muscle and could be of significance even in tissue a few mm in thickness(1). It has also been demonstrated that the rate constants of Na^{24+} washout increase in beating frog atria compared to quiescent tissue(9). Thus, measurements in preparations such as papillary and atrial muscle may be questioned unless conditions of flow rate, rate of contraction and tissue thickness are known and corrections for diffusion delay are made. The latter point is further emphasized by the data presented above which demonstrate that the washout of I^{131-} is significantly slower in the thin tissue slice (400-500 micra) than from the full thickness ventricle, provided the ventricle is actively working and perfused through its own coronary circulation.

The finding that cardiac K exchanges at two rates in the beating heart(5,6) poses the problem that the fast rate represents extracellular K exchange. That this is not so is evident from the size of the rapidly exchanging compartment (50-60% of the total K) (5,6). Furthermore, both sodium and iodide washout rates in the guinea pig heart were 10 times faster than the washout of K^{42} previously reported (*e.g.*, a mean value of 1.03 min^{-1} for Na^{24+} and I^{131-} compared to 0.11 min^{-1} for K^{42}(6)). In addition, when cardiac perfusion loads and heart rates were maintained, the washout of Na^{24+} and I^{131-} was not significantly affected by a decrease in temperature from 37 to 25°C. In contrast, rates of transfer and washout of the fast K compartment were decreased with this reduction of temperature with a $Q_{10} > 2$ suggesting that the latter has a chemical rate limiting step(10).

Thus, the present data indicate that both positively and negatively charged ions leave the extracellular spaces in the working, coronary perfused heart at so rapid a rate, that ion diffusion delay in passing through the extracellular space does not play a significant

role in evaluation of K exchange in this preparation. Furthermore, the data support the thesis that the rapidly exchanging K fraction does not represent significant extracellular localization of this ion.

Summary. Rates of washout of Na^{24+} and I^{131-} were measured in the working perfused guinea pig heart *in vitro* at 37° and 25°C. Washout occurred at 2 or more rates with at least 80% of the ions comprising the fast component and exchanging at a mean rate of 1.03 min^{-1}. There were no significant differences between the washout rates of the Na^{24+} and I^{131-} at the 2 temperatures studied. Washout of tissue slices 400-500 μ thick occurred at a single rate one-tenth that of the fast component in the working perfused heart. The data indicate that ion diffusion delay in passing through the extracellular spaces does not play a significant role in evaluation of K transfer in this preparation, and they also support previous findings that the fast component of K exchange does not represent extracellular localization.

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Use of Tris(Hydroxymethyl)Aminomethane Buffers in Cultures of Diploid Human Fibroblasts.* (29191)

GEORGE M. MARTIN (Introduced by E. P. Beneditt)
Department of Pathology, University of Washington School of Medicine, Seattle

The control of the concentration of hydrogen ions is one of many chronic frustrations of cell and tissue culture workers. All of the commonly employed media rely upon a bicarbonate system for buffering and ordinarily employ an atmosphere of 5% CO_2 in air. Although some ingenuous feed-back devices have been invented for control of the CO_2 concentration in open systems(1), many experiments are more conveniently carried out in closed systems and it is certainly desirable to maintain stock cultures in sealed containers to minimize contamination and evaporation. We describe here our experience with tris (hydroxymethyl)aminomethane buffer systems in cell cultures with the hope that others will be encouraged to investigate tris-containing media. We have confirmed the results of Swim(2,3) indicating the relatively low toxicity of tris for cultured human skin fibroblasts. When used in conjunction with low concentrations of bicarbonate (which is essential), excellent pH control is obtained with good, but variable growth. Our preliminary experience suggests that the variable results might be attributable to the mode of preparation of stock salt solutions with resulting variations in concentrations of calcium and magnesium.

Materials and methods. All cell cultures were diploid human "fibroblasts" established for 2-10 months from newborn foreskins by either trypsinization or explant techniques and incubated at 36°-37°C. Stock cultures were maintained in 6 ounce prescription bottles and were free of pleuropneumonia-like

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