JCI The Journal of Clinical Investigation

A Study in Dogs of Methods Suitable for Estimating the Rate of Myocardial Uptake of Rb^{86} in Man, and the Effect of l-Norepinephrine and Pitressin® on Rb^{86} Uptake

W. D. Love, G. E. Burch

J Clin Invest. 1957;36(3):468-478. https://doi.org/10.1172/JCI103444.

Research Article

A STUDY IN DOGS OF METHODS SUITABLE FOR ESTIMATING THE RATE OF MYOCARDIAL UPTAKE OF RB^{86} IN MAN, AND THE EFFECT OF L-NOREPINEPHRINE AND PITRESSIN® ON RB^{86} UPTAKE [1]

By W. D. LOVE and G. E. BURCH

(From the Department of Medicine, Tulane University School of Medicine and the Charity Hospital of Louisiana at New Orleans, La.)

(Submitted for publication April 20, 1956; accepted November 29, 1956)

These experiments were performed to test the accuracy of techniques which are suitable for estimating the rate of uptake of Rb^{86} by the myocardium in man. In the experimental animal it was possible to compare estimates that were based on external measurements of the type possible in man with the actual myocardial uptake of Rb^{86} determined by direct analysis of the heart after sacrifice. The rate of myocardial uptake of Rb^{86} was of interest because factors which influence it, such as the rate of coronary blood flow and the permeability of capillaries and muscle fibers, probably likewise affect the uptake of important metabolites by the heart. Since rubidium resembles potassium in its biological behavior, it might be possible to extrapolate to any gross changes in the rate of potassium uptake or concentration occurring with heart disease. As a partial test of the relationship between coronary blood flow and Rb^{86} uptake rate in the heart, l-norepinephrine or Pitressin® were administered intravenously to several dogs because of the known effects of these drugs on the coronary blood flow (1, 2). If an estimate of coronary blood flow could be obtained in intact man without catheterization of the coronary sinus, the variations in coronary blood flow in large numbers of normal subjects and patients with various types of cardiac disease could be studied. Rubidium^{86}, which has a 1.1 mev. gamma emission and a T½ of 19.5 days, has been used as if it were a tracer of potassium since the 12.4-hour T½ of K^{42} makes its use difficult.

Rubidium^{86} is not actually a tracer of potassium (3). However, rubidium resembles potassium chemically, and has biologic effects on the heart similar to those produced by potassium (4–7). These two elements are partitioned between the myocardium and plasma in almost identical ratios, and the rates of uptake of each by the various organs of the dog are qualitatively similar (8). The rates of uptake of K^{42} and Rb^{86} by the human erythrocyte *in vitro* have been found to be very nearly the same (9). The processes involved in bringing about uptake of the two elements seem to be similar in this type of cell at least, since factors reducing K^{42} uptake, such as cooling, increase in plasma potassium concentration, and the addition of iodoacetate to the plasma, produce a proportional reduction in Rb^{86} uptake (10).

The general procedure in these experiments was to maintain a nearly constant concentration of Rb^{86} in arterial blood by the continuous injection of isotope, usually for 30 minutes, while an indication of the rise of myocardial Rb^{86} concentration was obtained from a collimated, recording scintillation ratemeter placed over the precordium. From these data the turnover rate of myocardial potassium, which is defined as the fraction of myocardial potassium exchanging with the plasma per minute, and the amount of plasma cleared of Rb^{86} by 100 Gm. of myocardium in one minute were calculated using certain simplifying assumptions. The reliability of these estimates was evaluated by comparing them with the results of direct analysis of the myocardium after sacrifice.

MATERIALS AND METHODS

Mongrel dogs weighing 6.7 to 14.3 Kg. (mean 10.7 Kg.) were anesthetized with 30 mg. sodium pentobarbital per Kg. intravenously and taped to a frame so constructed that they could be held securely in a prone position over the precordial monitor. Supplementary anesthesia of 30 to 60 mg. sodium pentobarbital was occasionally necessary. Under fluoroscopic control, the frame was adjusted so that the approximate center of

[1] Supported by the R. A. Billups Fund for Research in Heart Disease and aided by a U. S. Public Health Service Grant, H-143.

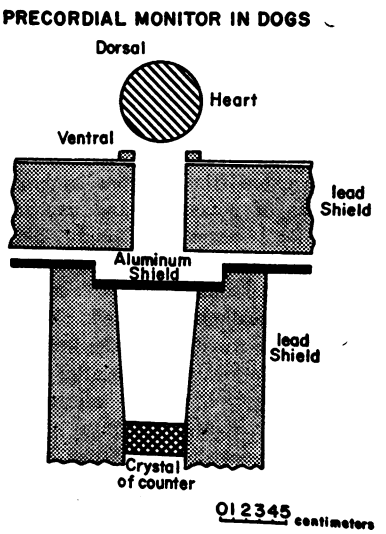

FIG. 1. SCHEMATIC REPRESENTATION OF THE ARRANGEMENT OF THE PRECORDIAL MONITOR IN PRONE, ANESTHETIZED DOGS

the ventricular mass coincided with the vertical axis of the counter crystal. After intravenous injection of 100 to 150 mg. heparin, frequent recordings of pulse and mean femoral arterial blood pressure were begun using a mercury manometer. The mean arterial blood pressure averaged 140 mm. Hg in dogs not receiving drugs, and the mean pulse rate was 160 per minute. Blood from the opposite femoral artery was allowed to flow through 300 cm. of 1.7 mm. internal diameter polyethylene tubing and returned to the femoral vein. A drop bottle was interposed, and 60 cm. of this tubing was wrapped around a probe-type Geiger-Muller tube attached to a recording ratemeter according to the principle described by Sear (11). Blood flow through the tubing was approximately 15 ml. per min., measurements being made at five-minute intervals by timed collection of 6 ml. blood.

The drugs were administered in 0.85 per cent NaCl solution at an average rate of 4 ml. per min. from a pressurized flask. Flow was regulated with a needle valve and drip bottle. The average dosage of l-norepinephrine was 2.5 μgm. per Kg. per min., and of Pitressin® [2] 0.065 pressor units per Kg. per min. Administration of the drugs was started 15 minutes before injection of Rb^{86} in order to allow any transient changes in plasma potassium to subside. When Pitressin® was used the initial rise in blood pressure caused by the drug had disappeared before measurements of Rb^{86} uptake were started. The mean pulse rate was 175 per min. and the average mean blood pressure 165 mm. Hg in four dogs receiving l-norepinephrine, while in the six dogs given Pitressin® the average pulse rate was 125 per min. and the average mean blood pressure 145 mm. Hg. The hearts of three of four dogs that received l-norepinephrine showed varying degrees of intramyocardial hemorrhage, mainly subendocardial, and a decrease in the myocardial potassium concentration. The mean potassium concentration in these dogs was 66.4 mEq. per Kg. myocardium, or 20 per cent less than in the control dogs.

Precordial monitoring was performed by means of a scintillation ratemeter [3] employing a NaI crystal and a recording galvanometer with a half-time of 5 seconds. The geometry, shielding, and general arrangement of the apparatus are indicated schematically in Figure 1. The precordial radioactivity at the end of 30 minutes of Rb^{86} infusion was approximately 20 times background. Three dogs were sacrificed *in situ* by intravenous injection of 300 mg. sodium pentobarbital, and those tissues which were within the field of the counter were removed to determine what portion of the radioactivity recorded over the precordium was actually derived from the heart. This varied from 62 to 72 per cent. Almost one-half of the precordial radioactivity arising outside of the heart was derived from the anterior chest wall, while the remaining 15 to 20 per cent originated in the posterior chest wall, lungs, and the portions of the body shielded from the counter. In six dogs in which the heart was monitored separately after sacrifice, the amount of radioactivity averaged 70 per cent of the total precordial count.

The injection of Rb^{86} in 0.85 per cent NaCl solution was made into a femoral vein by means of a 50-ml. syringe driven by a variable speed motor. The usual Rb^{86} concentration of the injectant was 12 μc. per ml.,

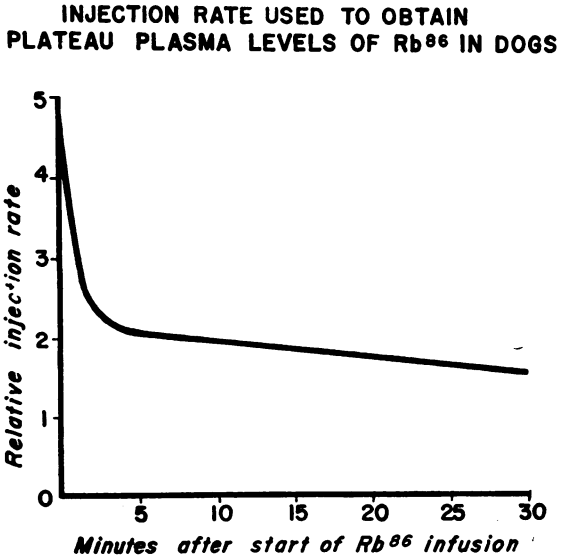

FIG. 2. THE CONTINUOUSLY DECREASING INTRAVENOUS INJECTION RATE USED TO OBTAIN NEAR CONSTANT ARTERIAL PLASMA Rb^{86} CONCENTRATION IN DOGS

[2] Supplied by Parke, Davis & Co.

[3] W. S. MacDonald Co. Type 155.

35 ml. being injected in 30 minutes. Rubidium[86] obtained as Rb_2CO_3 was neutralized with HCl and used no longer than two months after receipt in order to avoid significant contamination with long lived radio elements, such as Cs^{136}. A standard injection rate was derived by calculation from the plasma decay curve of Rb^{86} in dogs (8), with empirical modifications, Figure 2. In some cases the injection rate was altered during the procedure as required by a change in whole blood radioactivity.

Tissue specimens weighing approximately one gram were collected from several parts of the heart, from the lung, liver, and muscle of the pectoral region and spine. These samples were digested in HNO_3 for determination of radioactivity and potassium content by methods previously described (8). Specimens of arterial plasma were obtained at five-minute intervals for determination of radioactivity (12) and potassium concentration. In the initial studies plasma potassium concentration was measured using a twenty-fold dilution of plasma. However, all other plasma samples were digested with HNO_3 before dilution, and the earlier determinations were corrected to the probable values which would have been obtained with digested plasma. This correction, which amounted to a 15 per cent increase, was based upon 200 samples measured by both methods.

METHODS AND ANALYSIS OF DATA

Several assumptions were necessary to order to make calculations of myocardial potassium turnover rate and Rb^{86} clearance from the data obtained. As mentioned previously, Rb^{86} was used as if it traced potassium within the myocardium. Therefore, the mass of nontracer material was represented by the potassium content, which was assumed not to change during the procedure. The rationale of this assumption has been given above. To extend the previous study of the relative concentrations of potassium and exchangeable rubidium in the dog's heart (8), eight animals were sacrificed three to five days after intravenous injection of Rb^{86}, and the potassium and Rb^{86} concentrations of the plasma and myocardium compared. The specific activity of exchangeable rubidium in the heart and plasma have been shown to be nearly equal after 24 hours (8), so that Rb^{86} concentration would indicate relative exchangeable rubidium content. The ratio of the Rb^{86} concentration in the myocardium to that in the plasma divided by the similar ratio for potassium averaged 1.05 ± 0.08, compared with 1.14 in four dogs reported previously. In the calculations this ratio was treated as if it were unity.

The second assumption upon which calculations were based was that the ventricular myocardium constituted a mixed homogeneous compartment exchanging at a single rate with the blood. Although this is clearly not the case, any rapidly exchanging portion containing little potassium, such as the interstitial fluid, would be undetected by the methods used. Furthermore, the results did not indicate the presence of any large slowly ex-

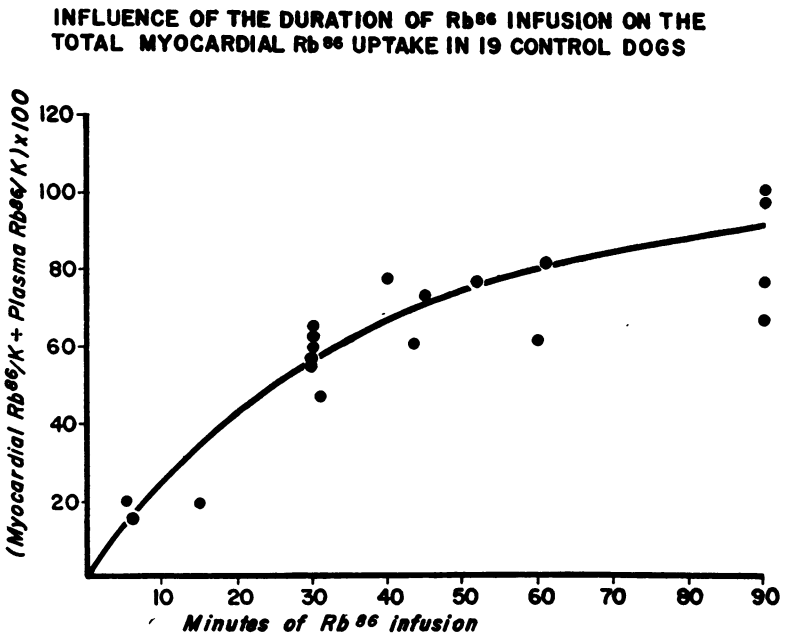

FIG. 3. THE TOTAL Rb^{86} UPTAKE IN 19 CONTROL DOGS DETERMINED AT SACRIFICE 5 TO 90 MINUTES AFTER THE START OF Rb^{86} INFUSION

The curved line represents the time course of Rb^{86} uptake in the hypothetical average dog, if the myocardium was homogeneous and exchanged Rb^{86} with the plasma at a single constant rate.

changing components. This is indicated in Figure 3 which shows the time course of the variation in individual dogs about the mean Rb^{86} uptake rate.

In making calculations from data obtained from the intact dog, it was further assumed that the precordial monitor reflected the behavior of Rb^{86} concentration in the myocardium. The actual comparison of estimates of myocardial Rb^{86} uptake made on this basis with those obtained from direct analysis of the myocardium served as the test of the usefulness of this assumption. If radioactivity over the heart reflects myocardial radioactivity, then the recording of precordial radioactivity has three attributes from which the rate of Rb^{86} uptake by the heart can be estimated. The initial rate of increase of radioactivity over the precordium is related to the initial rate at which plasma is cleared of Rb^{86} by the myocardium, while the level of precordial radioactivity at the end of the infusion of Rb^{86} is related to the maximal myocardial Rb^{86} concentration reached during the procedure and, therefore, to the average myocardial clearance of plasma Rb^{86} during the infusion. The amount of curvature of the trace of precordial radioactivity depends in part on the turnover rate of myocardial potassium. These three characteristics have been used to obtain "estimated" rates of plasma clearance and rates of turnover from the type of data obtainable in man, and these rates have been compared with their counterparts obtained by direct analysis of the heart at sacrifice. The latter have been referred to as "observed" rates of clearance and rates of turnover.

Estimated myocardial potassium turnover rate was obtained by differentiation of the time course of precordial Rb^{86} radioactivity. The continuous recording of precordial radioactivity was transcribed and the time course of its slope determined graphically. The resulting values were plotted semi-logarithmically and a single straight line drawn by inspection which appeared most nearly to represent the data. Usually there was evidence of a rapidly exchanging component during the first five minutes of the Rb^{86} infusion. This portion was ignored in choosing the predominant exchange rate, since it was considered to rise from outside the myocardium. Assuming stable potassium concentrations, the precordial radioactivity curve can be taken to represent the rise of Rb^{86}/K ratio in two compartments exchanging with plasma of constant Rb^{86}/K ratio. This may be expressed as:

$$Y_t = A(1 - e^{-at}) + B(1 - e^{-bt}) \qquad (1)$$

where

Y_t is the precordial radioactivity at any time t,

A and B are the radioactivities which would be recorded from the two compartments at complete equilibrium, *i.e.*, $t = \infty$,

b is the fraction of total non-tracer potassium entering or leaving the slowly exchanging portion per minute, and a is the similar fractional exchange rate in the fast exchanging component.

From equation (*1*)

$$\frac{dY_t}{dt} = aAe^{-at} + bBe^{-bt} \qquad (2)$$

The second term of equation (*2*), obtained graphically as previously described, was considered to represent the variation of Rb^{86}/K ratio in the myocardium.

$$b = \frac{0.69}{T\frac{1}{2}},$$

where $T\frac{1}{2}$ is the time required for the value of the second term to decrease by one-half. 100 b is equal to the per cent of the myocardial non-tracer entering or leaving the heart per minute.

Estimated initial myocardial Rb^{86} clearance. The initial rate of increase of the predominant component of the precordial radioactivity curve was obtained by evaluating the second term of equation (*2*) at $t = 0$. For each dog the resulting value was plotted against the observed initial myocardial Rb^{86} clearance rate, defined below, expressed in units of Rb^{86} cpm. per 100 Gm. myocardium per minute. The resulting empirical relationship (correlation coefficient, $r = +0.95$) was used to convert the observed initial rate of rise of precordial radioactivity in individual dogs to the units of Rb^{86} cpm. per 100 Gm. myocardium per minute. The resulting value divided by the average plasma concentration of Rb^{86} was the estimated myocardial Rb^{86} clearance, in units of ml. plasma cleared of Rb^{86} per 100 Gm. myocardium per minute.

Estimated average myocardial Rb^{86} clearance was obtained by taking advantage of the high correlation between final precordial radioactivity and the Rb^{86} concentration of the myocardium at the time of sacrifice ($r = +0.93$). The correlation coefficients between precordial radioactivity and the Rb^{86} concentration of the other organs measured were: lung + 0.85; liver + 0.59; muscle from anterior chest wall + 0.35; and back muscle + 0.54. Despite the variation in ventricular weight from 38 to 89 Gm., there was no consistent increase in precordial radioactivity with increase in heart size. Estimated average myocardial Rb^{86} clearance was defined as:

$$\frac{\text{Estimated myocardial } Rb^{86} \div \text{Mean plasma } Rb^{86}}{\text{Duration of } Rb^{86} \text{ infusion}} \qquad (3)$$

Because increasing amounts of Rb^{86} return to the plasma from the heart with increasing duration of Rb^{86} infusion, average clearances of different dogs are not comparable unless measurements are made over the same length of time. As the myocardium approaches equilibrium with the plasma, average clearance becomes an increasingly poor index of initial clearance. Therefore, mean rate of clearance reflects the true rate of myocardial Rb^{86} uptake only when the heart has attained less than approximately 40 per cent of the equilibrium Rb^{86} concentration.

Observed myocardial potassium turnover rate was calculated from the Rb^{86} and potassium concentrations of the myocardium measured at the end of the period of

Rb^{86} infusion, using the assumptions listed above. Since the myocardium was assumed to be a single compartment exchanging Rb^{86} and potassium with the plasma at a constant rate, then

$$H = C(1\text{-}e^{-bt}), \qquad (4)$$

where H is the Rb^{86}/K ratio in the myocardium at the time of sacrifice, and C is the Rb^{86}/K ratio in the heart at $t = \infty$ (C is assumed to be equal to the average Rb^{86}/K ratio observed in the plasma) and b and t are as previously defined. The values of H, C, and t were measured directly, and the turnover rate b was determined graphically.

Observed initial myocardial Rb^{86} clearance was defined as the amount of plasma at the average concentration of arterial Rb^{86} which would be required to supply the amount of Rb^{86} taken up per minute during a hypothetical moment before any Rb^{86} had begun to return from the myocardium to the plasma. This was obtained from the turnover rate and potassium concentrations as follows:

$$\text{Initial myocardial } Rb^{86} \text{ clearance} = \frac{(b)\ (\text{Mean myocardial K conc.})}{\text{Mean plasma K conc.}} \qquad (5)$$

Observed mean myocardial Rb^{86} clearance was calculated in the same manner as estimated mean myocardial Rb^{86} clearance except that the myocardial Rb^{86} concentration was obtained by direct analysis of the heart muscle.

Changes in plasma Rb^{86}/K ratio occurred during Rb^{86} infusions and caused errors in the indices of Rb^{86} uptake. The influence of such variations was tested by calculating the theoretic changes produced in the time course of myocardial Rb^{86} concentration and the resulting errors in calculations of Rb^{86} uptake. Changes in plasma Rb^{86}/K ratio were assumed to be linear to simplify calculation. It is evident from Figure 4 that, in the range of exchange rates and rates of change of plasma Rb^{86}/K ratio actually encountered and investigated, a very significant error may occur in estimations of turnover rate and initial plasma Rb^{86} clearance based on differentiation of the curve of precordial radioactivity. However, little error results in the calculations of observed initial myocardial clearance of plasma Rb^{86} and turnover of potassium which depend on the relationship of final Rb^{86}/K ratio in the myocardium to the mean plasma Rb^{86}/K ratio. The error in the estimated initial clearance would be greatly reduced if the initial rather than the mean plasma Rb^{86} concentration were used in calculating the initial clearance when a definite progressive change in plasma Rb^{86}/K ratio occurred.

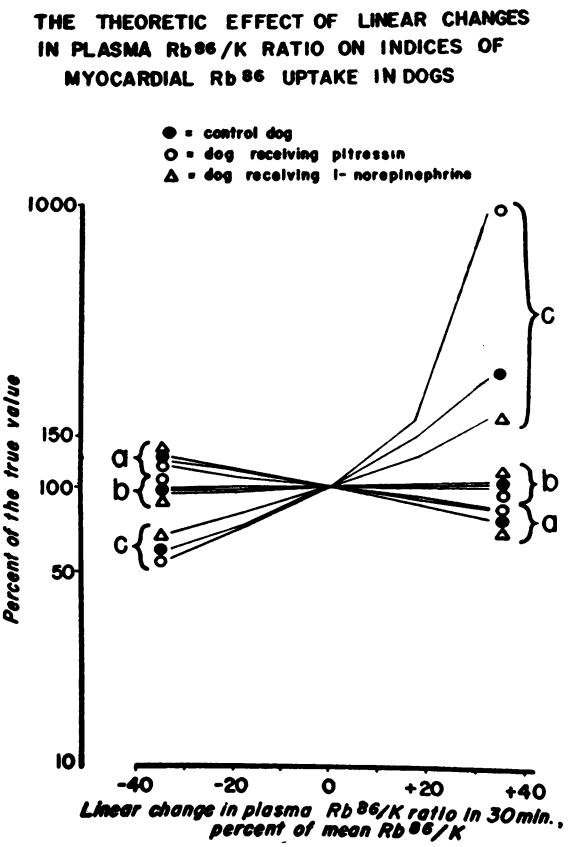

FIG. 4. THE THEORETIC EFFECTS OF LINEAR CHANGES IN PLASMA Rb^{86}/K RATIO ON THE CALCULATED INDICES OF MYOCARDIAL Rb^{86} UPTAKE IN DOGS

Since the effect depends on the actual rate of potassium turnover present, calculations were made in the range of turnover rates of myocardial potassium present in the dogs receiving Pitressin® and l-norepinephrine, as well as the control dogs. Group *a* gives the effects on the calculations of estimated initial myocardial clearance of plasma Rb^{86}, group *b* the effects on calculations of observed initial myocardial clearance of plasma Rb^{86} and turnover of potassium, and *c* the effects on calculations of estimated myocardial turnover rate of potassium.

RESULTS

An example of the type of data obtained is given in Table I. Figure 5 illustrates the time course of precordial radioactivity in a dog receiving no drug infusion, in one receiving l-norepinephrine, and in one receiving Pitressin®. The mean plasma Rb^{86} concentration and the relationship between final precordial radioactivity and Rb^{86} concentration of the myocardium were approximately equal in these three dogs.

The accuracy of estimations of turnover rate is indicated by Figure 6. Two dogs that received Rb^{86} infusions for less than 7 minutes were omitted since no record of precordial radioactivity was made, and two that were infused with Rb^{86} for 90 minutes were omitted since the myocardial

TABLE I

Data and calculations from Dog 944, wt. 14.3 Kg.

Data

	Minutes of Rb^{86} infusion					
	5	10	15	20	25	30
Rb^{86}, *cpm. per ml. plasma*	10,900	10,000	11,300	11,000	10,700	10,400
K, *mEq. per L. plasma*	3.27	3.27	3.24	3.20	3.17	3.17
Mean blood pressure, *mm. Hg*	113	112	115	118	118	119
Heart rate *per min.*	164	164	160	168	168	168

Rb^{86}, *cpm. per Gm. ventricle* (av.)	157,000
K, *mEq. per Kg. ventricle* (av.)	77.5
$T\frac{1}{2}$ from differentiation of the plot of precordial radioactivity, *min.*	54
Initial slope of principal component of curve of precordial radioactivity, converted to cpm. per Gm. ventricle per min.	9,980
Final net precordial radioactivity, converted to cpm. per Gm. ventricle	205,000

Calculations

	Observed	Estimated
K turnover of myocardium, *per cent per min.*	3.1	1.3
Initial myocardial clearance of plasma Rb^{86}, *ml. per 100 Gm. myocardium per min.*	75	93
Mean myocardial clearance of plasma Rb^{86}, *ml. per 100 Gm. myocardium per min.*	49	64

Rb^{86}/K ratio at the time of sacrifice exceeded the plasma Rb^{86}/K ratio, making it impossible to calculate the rate of turnover. Estimated turnover was consistently less than observed turnover in the dogs which did not receive drugs, averaging only one-half of the latter value. Figure 6 shows that it was not possible to predict rapid or slow rates of turnover in the control group of dogs. Abnormally low rates of turnover were apparent in two of six dogs receiving Pitressin®, since

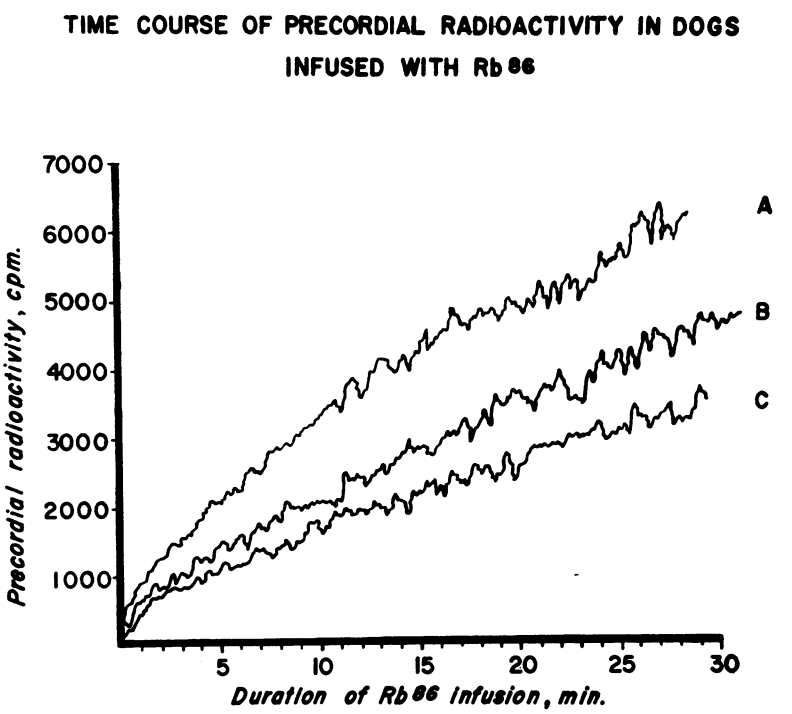

FIG. 5. THE TIME COURSE OF PRECORDIAL RADIOACTIVITY IN A CONTROL DOG (B) AND DOGS INFUSED WITH L-NOREPINEPHRINE (A) AND PITRESSIN® (C) DURING A 30-MINUTE PERIOD OF CONTINUOUS RB^{86} ADMINISTRATION

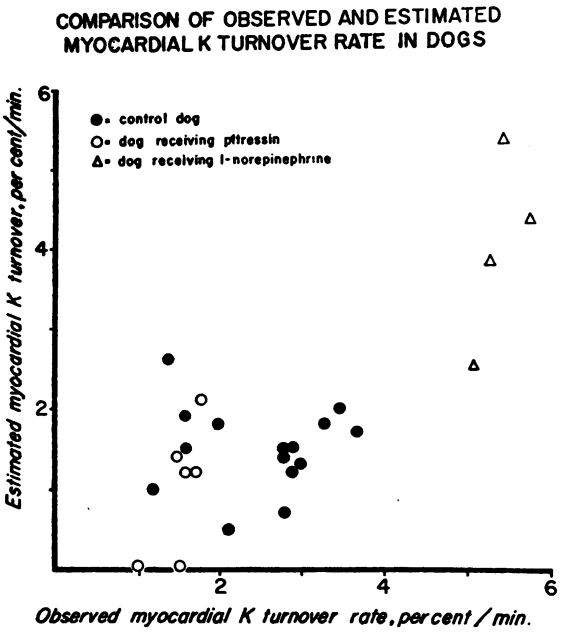

FIG. 6. COMPARISON OF OBSERVED AND ESTIMATED TURNOVER RATE OF MYOCARDIAL POTASSIUM IN DOGS

there was no detectable curvature in the tracing of precordial radioactivity. Abnormally rapid rates of turnover were predicted in all four dogs given l-norepinephrine, although the observed rates of turnover were again somewhat greater than the estimated rates.

The accuracy of estimations of the rates of initial myocardial clearances of plasma Rb^{86} is apparent from Figure 7. The standard error of estimate was 14 ml. plasma, the mean normal clearance being 70 ml. Predicted and observed clearances were necessarily equal overall because of the method of calculation.

The accuracy of estimations of mean rate of clearance in those dogs receiving Rb^{86} for 30 minutes is shown in Figure 8. The standard error of estimate was 10 ml. plasma, the mean control value being 50 ml. There was no consistent difference between the values for estimated and observed mean clearances because an experimentally derived factor was used to obtain the estimated myocardial Rb^{86} concentration from the final level of precordial radiation. In accordance with considerations discussed previously, decreased uptake caused by Pitressin® was detected, whereas the small increase in mean clearance occurring in dogs receiving l-norepinephrine was not.

Factors affecting the accuracy of the three types of estimations included the failure to maintain a constant plasma Rb^{86}/K ratio. In 18 dogs this ratio was not observed to vary by as much as 10

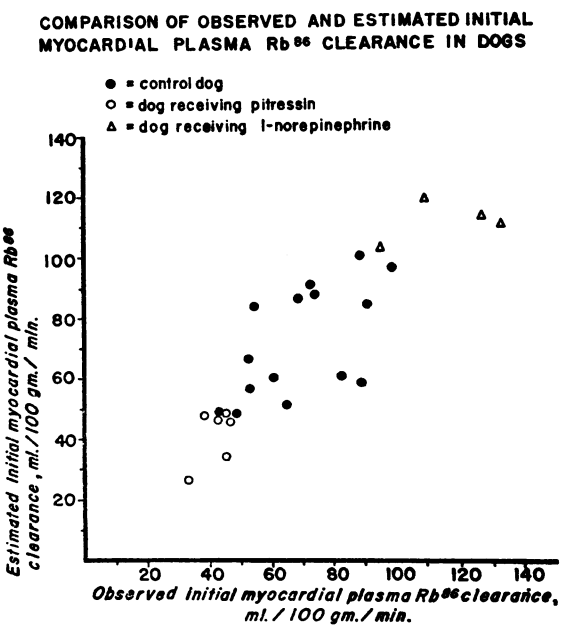

FIG. 7. COMPARISON OF THE VALUES OF OBSERVED AND ESTIMATED INITIAL MYOCARDIAL PLASMA Rb^{86} CLEARANCE IN DOGS

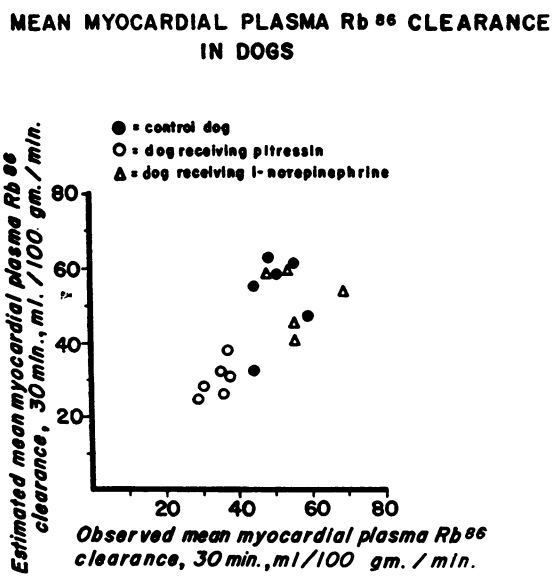

FIG. 8. COMPARISON OF THE VALUES OF OBSERVED AND ESTIMATED MEAN MYOCARDIAL PLASMA Rb^{86} CLEARANCE FOR A 30-MINUTE PERIOD IN THE DOG

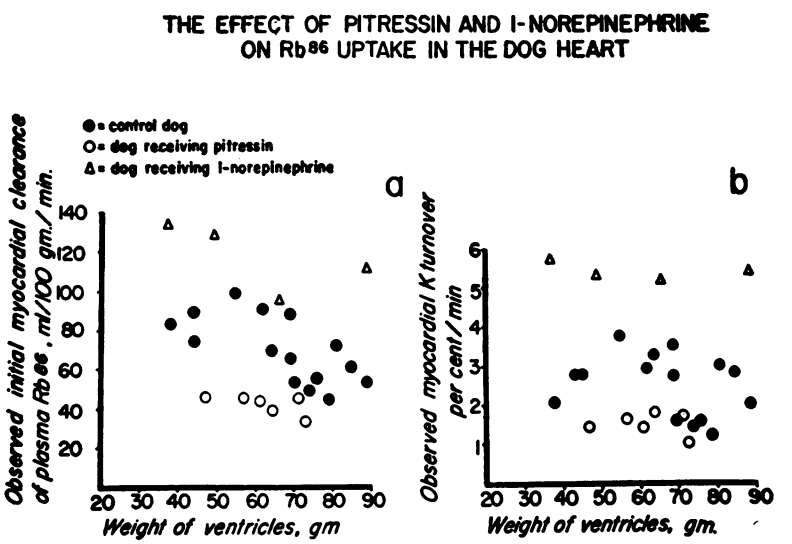

FIG. 9. THE EFFECT OF PITRESSIN® AND L-NOREPINEPHRINE ON THE OBSERVED INITIAL MYOCARDIAL CLEARANCE OF PLASMA RB^{86} (a) AND THE OBSERVED MYOCARDIAL POTASSIUM TURNOVER RATE (b) IN DOGS

per cent of the mean during the Rb^{86} infusion, while in the remaining 9 dogs the Rb^{86}/K ratio declined as much as 22 per cent or increased as much as 33 per cent of the mean value. In general these changes in plasma Rb^{86}/K ratio were associated with errors in estimated rates of clearance and turnover that were in the expected directions as outlined above, but the quantitative relationships were not consistent enough to justify the application of corrections to the estimated values. Some variations in Rb^{86}/K ratio were caused by changes in plasma Rb^{86} concentration, but frequently the variations were due to changes in plasma potassium concentration which could not be detected during the experimental procedure.

Variations in the quantitative relationship between the radioactivity observed by monitoring over the precordium and the actual myocardial concentration of Rb^{86} was the major source of error in estimations of the rates of clearance. It is apparent that such variations must be related to variations in geometry and heart size, and also to differences in the Rb^{86} uptake of the other tissues under the monitor in relation to that of the heart. However, attempts to correlate the radioactivity of the lung, liver, and muscles of the chest wall with variations in the relationship of myocardial Rb^{86} concentration and precordial radioactivity were unsuccessful. The presence of important amounts of slowly exchanging tissue under the precordial monitor would result in estimated rates of turnover which would be consistently lower than observed rates, as was the case. Dissection of the animals after sacrifice showed that 10 to 20 per cent of the precordial radioactivity was derived from skeletal muscle, which is known to exchange slowly (8). In two dogs in which the Rb^{86}/K ratio in the myocardium had nearly reached equilibrium with that of the plasma after 90 minutes of Rb^{86} infusion, there was still a continuing rise in precordial radioactivity. These slowly exchanging components could not be separated from the myocardial component in the analysis of the time course curve of the first derivative of precordial radioactivity, although in theory this might be possible after 90 minutes of infusion of Rb^{86}.

The duration of Rb^{86} infusion appeared to influence estimates of the rates of Rb^{86} uptake since infusions of 15 minutes or less provided insufficient data for the separation of rapidly equilibrating components, and infusions of long durations, *i.e.*, 90 minutes, accentuated errors caused by slowly exchanging components and variations in the comparative distribution of non-tracer rubidium and potassium.

The assumption that the right and left ventricles had the same geometrical relationship to the moni-

tor represented a variable source of error depending upon the exact location of the monitor, since uptake of Rb^{86} by the right ventricle was less than by the left. Variations in the ratio of myocardial Rb^{86}/K to plasma Rb^{86}/K that would have been present at equilibrium in the dogs studied contributed an error of unknown size to the calculation of observed rates of turnover and rates of initial clearance. The coefficient of variation of this ratio was 8 per cent in 8 similar dogs sacrificed after equilibrium was considered to have been achieved.

The effects of Pitressin® and l-norepinephrine on the rate of Rb^{86} uptake are indicated in Figure 9. Since Spencer, Merrill, Powers, and Bing (13) have shown that coronary blood flow per unit weight of myocardium varies inversely with heart size, the indices of Rb^{86} uptake have been plotted with the values for ventricular weight. The differences in mean heart rate among the three groups of dogs were relatively small, as noted previously, and there was no definite relationship between heart rate and rate of uptake of Rb^{86} within the individual groups.

DISCUSSION

It is evident that differentiation of the time course of precordial radioactivity did not result in even a gross indication of the rate of uptake of Rb^{86} by the myocardium. This method probably is more suited for conditions in which the rate of turnover is greater than that encountered in these dogs, since with greater curvature of the plot of radioactivity the graphic methods used for analysis would be expected to be more reliable and interference by slowly exchanging components less important. On the other hand, estimations of initial rate of clearance of plasma Rb^{86} by the myocardium were reasonably reliable for the detection of both increased and decreased rates of uptake. Estimations of the mean rate of Rb^{86} clearance appeared to be reliable only for the detection of diminished Rb^{86} uptake. It should be noted that if reproducible and reliable estimates of the rate of turnover of potassium by the myocardium could be combined with determinations of the rate of initial myocardial clearance of plasma potassium it would be possible to detect lowered concentration of potassium in the myocardium *in vivo,* since the rate of turnover would be relatively large compared to the rate of uptake of potassium.

It has been repeatedly observed that the rate of blood flow appears to be a major factor limiting the rate of uptake by the tissues of a variety of substances including electrolytes, water, and inert gases (14–16). If this is true in the case of the exchange of potassium between myocardium and plasma, then the rates of passage of potassium through the capillary wall, the mixing with interstitial fluid, and the entry into myocardial fibers are all very high compared to the rate of delivery of new potassium by the circulation. Therefore, the actual rate of accumulation of a tracer substance in the myocardium would be dependent for the most part on the rate at which the tracer was brought to the heart by the coronary blood. H. L. Conn and J. S. Robertson (17) have stated that in a series of dogs the amount of K^{42} entering the interstitial fluid of heart muscle from the plasma was approximately equal to the total amount delivered by the coronary blood, assuming that the data of others (18) for the coronary blood flow in dogs were applicable to the animals used in their experiments. In the studies reported here, it was found that drugs which are known to affect coronary blood flow affect the rate of uptake of Rb^{86} by the myocardium as a whole in the manner which would be expected if Rb^{86} uptake were dependent on the rate of coronary blood flow. The observed myocardial potassium turnover rate estimated with Rb^{86} in these dogs was correlated with the concentration of potassium in the plasma ($r = +0.67$), whereas the observed initial myocardial clearance of plasma Rb^{86} showed no significant correlation with the concentration of potassium in the plasma ($r = +0.24$, σ of $r = 0.25$). This is consistent with the uptake by the myocardium of all, or a constant fraction, of the tracer in coronary arterial blood. These data do not exclude the possibility that these drugs produced their effects through alteration in cellular metabolic or chemical factors. Conn and Robertson (17) have found the $T\frac{1}{2}$ value of the slowly exchanging component of myocardial potassium in the dog to average 45 minutes; whereas, in the normal dogs reported here this value was 25 minutes. The smaller size, and subsequently relatively higher rates, of

coronary blood flow of the dogs studied with Rb^{86} is one of the factors which may account for this difference.

Although blood flow may be the most important factor limiting the rate of uptake of Rb^{86} by the heart of the normal dog, it cannot be inferred that capillary or extravascular factors may not be of significance in heart disease. In addition, it is necessary to distinguish between the absolute rate of blood flow to a tissue and the effective rate of blood flow, which is judged by the clearance of a substance from the blood stream. The heart is known to be capable of increasing the degree of extraction of oxygen from arterial blood from a normal value of 70 per cent to one as great as 91 per cent (19). Although this is believed to be caused by a change in the oxygen diffusion gradients, it is possible that local circulatory readjustments such as the closing of arteriovenous shunts (20) or the re-arrangement of capillary circulation might play a role in determining the efficiency of extraction of oxygen or other substances such as Rb^{86} from flowing blood. Johnson, Cavert, and Lifson (15) have shown that in the isolated perfused heart of the dog the initial degree of extraction of D_2O from coronary blood was 100 per cent, and Conn and Robertson (17) considered K^{42} extraction by the dog heart *in situ* to be 100 per cent. However, these workers found that after less than one minute the extraction of arterial K^{42} had fallen to approximately 65 per cent. This latter figure is essentially in agreement with the experience with Rb^{86} in six dogs submitted to thoracotomy and cannulation of the coronary sinus in this laboratory. The apparent initial extraction of Rb^{86} from arterial plasma averaged 70 per cent in these dogs, although the existence of an early rapidly exchanging component of myocardial potassium would not have been detected by the methods used.

SUMMARY

1. Methods suitable for estimating the rate of myocardial uptake of Rb^{86} in man were tested in 27 dogs because of the probable relationship of Rb^{86} uptake to coronary blood flow and to possible extravascular factors affecting the exchange of metabolites.

2. Estimates of initial myocardial clearance of plasma Rb^{86} had a standard error of estimate of 14 ml. per 100 Gm. ventricle per min., the mean normal value being 70 ml.

3. For estimates of mean clearance for 30 minutes, the standard error of estimate was 10 ml. per 100 Gm. ventricle per min. and the mean 50 ml. Estimates of mean clearance were unsuited for detection of rapid uptake, although slow uptake was reliably reflected.

4. Estimates of turnover rate were much less reliable than those of clearance.

5. Infusion of 0.065 pressor unit of Pitressin® per Kg. per min. decreased the average initial plasma Rb^{86} clearance from the normal value of 70 ml. per 100 Gm. ventricle per min. to 42 ml.

6. Infusion of 2.5 μgm. l-norepinephrine per Kg. per min. increased initial clearance to 116 ml. per 100 Gm.

7. These changes in plasma Rb^{86} clearance were in the direction expected from the known effects of these drugs on coronary blood flow. The ability to detect such changes with a reasonable degree of accuracy in dogs by external monitoring over the heart supports a trial of these procedures in man.

ACKNOWLEDGMENT

We wish to acknowledge the assistance of Professor J. A. Cronvich, of the Departments of Medicine and Electrical Engineering of Tulane University, in the formulation of calculations.

REFERENCES

1. Green, H. D., Wégria, R., and Boyer, N. H., Effects of epinephrine and pitressin on the coronary artery inflow in anesthetized dogs. J. Pharmacol. & Exper. Therap., 1942, 76, 378.
2. Dörner, J., Vergleichende Untersuchungen über die Kreislaufwirkungen des Adrenalins und Arterenols im Tierexperiment. Arch. f. Kreislaufforsch., 1954, 21, 88.
3. Burch, G. E., Threefoot, S. A., and Ray, C. T., The rate of disappearance of Rb^{86} from the plasma, the biologic decay rates of Rb^{86}, and the applicability of Rb^{86} as a tracer of potassium in man with and without chronic congestive heart failure. J. Lab. & Clin. Med., 1955, 45, 371.
4. Clark, A. J., The mode of action of potassium upon isolated organs. J. Pharmacol. & Exper. Therap., 1921, 18, 423.
5. Ringer, S., An investigation regarding the action of rubidium and caesium salts compared with the action of potassium salts on the ventricle of the frog's heart. J. Physiol., 1883, 4, 370.

6. Mitchell, P. H., Wilson, J. W., and Stanton, R. E., The selective absorption of potassium by animal cells. II. The cause of potassium selection as indicated by the absorption of rubidium and cesium. J. Gen. Physiol., 1921, **4**, 141.
7. Follis, R. H., Histological effects in rats resulting from adding rubidium or cesium to a diet deficient in potassium. Am. J. Physiol., 1943, **138**, 246.
8. Love, W. D., Romney, R. B., and Burch, G. E., A comparison of the distribution of potassium and exchangeable rubidium in the organs of the dog, using rubidium^{86}. Circ. Research, 1954, **2**, 112.
9. Love, W. D., and Burch, G. E., A comparison of potassium^{42}, rubidium^{86}, and cesium^{134} as tracers of potassium in the study of cation metabolism of human erythrocytes in vitro. J. Lab. & Clin. Med., 1953, **41**, 351.
10. Love, W. D., Cronvich, J. A., and Burch, G. E., Mechanisms controlling cation concentrations in the human cell: Evidence from the effect of iodoacetate on Na and K exchange rates of the erythrocyte. J. Clin. Invest., 1955, **34**, 61.
11. Sear, H., A method for presenting liquid samples to the flat surface of a scintillation crystal. Nucleonics, 1953, **11**, No. 4, 52.
12. Burch, G., Reaser, P., Ray, T., and Threefoot, S., A method of preparing biologic fluids for counting of radioelements. J. Lab. & Clin. Med., 1950, **35**, 626.
13. Spencer, F. C., Merrill, D. L., Powers, S. R., and Bing, R. J., Coronary blood flow and cardiac oxygen consumption in unanesthetized dogs. Am. J. Physiol., 1950, **160**, 149.
14. Pappenheimer, J. R., Passage of molecules through capillary walls. Physiol. Rev., 1953, **33**, 387.
15. Johnson, J. A., Cavert, H. M., and Lifson, N., Kinetics concerned with distribution of isotopic water in isolated perfused dog heart and skeletal muscle. Am. J. Physiol., 1952, **171**, 687.
16. Jones, H. B., Respiratory system: Nitrogen elimination *in* Medical Physics, O. Glasser, Ed., Chicago, Year Book Publishers, 1950, Vol. 2, p. 855.
17. Conn, H. L., Jr., and Robertson, J. S., Kinetics of potassium transfer in the left ventricle of the intact dog. Am. J. Physiol., 1955, **181**, 319.
18. Eckenhoff, J. E., Hafkenschiel, J. H., Harmel, M. H., Goodale, W. T., Lubin, M., Bing, R. J., and Kety, S. S., Measurement of coronary blood flow by the nitrous oxide method. Am. J. Physiol., 1948, **152**, 356.
19. Case, R. B., Berglund, E., and Sarnoff, S. J., Ventricular function. VII. Changes in coronary resistance and ventricular function resulting from acutely induced anemia and the effect thereon of coronary stenosis. Am. J. Med., 1955, **18**, 397.
20. Prinzmetal, M., Simpkin, B., Bergman, H. C., and Kruger, H. E., Studies on the coronary circulation. II. The collateral circulation of the normal human heart by coronary perfusion with radioactive erythrocytes and glass spheres. Am. Heart J., 1947, **33**, 420.