

## External Assessment of Myocardial Metabolism with C-11 Palmitate In Vivo

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**The externally detected rate of clearance of C-11 palmitate ( $[^{11}\text{C}]$ palmitic acid) from isolated hearts varies directly with  $\text{CO}_2$  production from neutral lipids and with physiological indexes of myocardial oxygen consumption. The present study was performed to determine whether myocardial metabolism could be quantified noninvasively in vivo in a fashion analogous to that in the isolated heart. Opened chest, male rabbits were injected with C-11 palmitate (100–200  $\mu\text{Ci}$ ) and coincidence counts were detected externally with two NaI(Tl) crystals so placed that their collinear field of view encompassed the heart. The monoexponential rate of clearance of tracer—obtained from the portion of the residue-detection curve reflecting metabolism of fatty acid incorporated into neutral lipids—correlated directly with induced changes in tension-time index after injections into the left atrium ( $r = 0.96$ ,  $n = 12$ ), right atrium ( $r = 0.86$ ,  $n = 14$ ), and ear vein ( $r = 0.93$ ,  $n = 14$ ). Clearance of labeled palmitate from the vascular pool within the field of detection (determined with both C-14 palmitate and red blood cells labeled with  $\text{C}^{15}\text{O}$ -hemoglobin) was rapid and did not significantly affect measurements of palmitate clearance from the heart itself.**

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The use of positron-emitting nuclides (e.g., C-11), rather than the conventional single-photon emitters used clinically, offers several advantages, including: (a) convenient quantification of radioactivity in tissue, since coincidence detection of the two annihilation photons permits electronic collimation; (b) study of C-11 substrate metabolism that is identical to that occurring physiologically (1); and (c) elimination of distortions resulting from superimposition of normal and abnormal zones of myocardium, through the use of reconstructive tomography facilitated by the ease of compensating for the attenuation of annihilation radiation (2).

In previous studies we have demonstrated that residue detection of C-11 palmitate, a tracer for fatty acid, provides an index of myocardial metabolism based on the rate of clearance of C-11 radioactivity from isolated, perfused rabbit hearts (3). Both (a) carbon dioxide

production from prelabeled neutral-lipid stores, and (b) the rate of decline of C-11 radioactivity during a monoexponential portion of the residue-detection curve, correlated with induced changes in tension-time index and peak  $dP/dt$  (3). Under control conditions in isolated perfused hearts, changes in the rate of clearance of labeled palmitate were dependent on metabolism rather than on flow, since flow was maintained constant. Under such conditions, however, in which recirculation is excluded, clearance of labeled fatty acids in isolated hearts may differ from that found in vivo, which may be influenced by recirculation, by variation in blood flow as well as metabolism, and by fluctuation in levels of circulating fatty acids, catecholamines, and regional myocardial neutral-lipid pools. Accordingly, the present study was undertaken to determine whether clearance of C-11 palmitate, measured from residue-detection curves, could provide an index of global ventricular metabolism in vivo, and to define time intervals that should be suitable for metabolic studies in patients in whom radioactivity would be detected serially in selected regions of myocardium by positron-emission transaxial tomography.

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METHODS

**Animal preparations.** Thirty-eight fed, male rabbits weighing 2–3 kg were anesthetized with sodium pentobarbital intravenously (30 mg/kg), intubated, and placed on a variable-phase respirator. The heart was exposed through a median sternotomy and catheters were inserted into the left and/or right atrium and left ventricle. The left-ventricular catheter was connected to a pressure transducer\* for continuous monitoring of left-ventricular pressure and heart rate. Each catheter was filled with heparinized saline and subsequently used for administration of tracer. Two lead-shielded NaI(Tl) crystals were positioned on either side of the chest with a colinear field of view encompassing the heart and chest wall. The crystals were adjusted before each study after insertion of a gallium-68 positron source within the detection field to obtain a ratio of 10:1 for singles to coincidence counts. Lead collimators with apertures of 3 cm were used to minimize distortion resulting from detection of random counts from noncardiac structures. Carbon-11 palmitate was prepared as previously described, and complexed to 0.4 mM defatted bovine serum albumin (3). After baseline recordings of left-ventricular pressure and heart rate, 100–200  $\mu$ Ci of C-11 palmitate (in 0.5 to 1.0 cc) were injected as a bolus into the right- or left-atrial catheter, or intravenously into a marginal ear vein. Coincidence detection was performed as previously described (3).

**Relationship of clearance of C-11 palmitate to determinants of myocardial oxygen consumption and substrate utilization.** Changes were induced in myocardial energetics, reflected by tension-time index and peak dP/dt, to determine their effect on the rate of cardiac clearance of C-11 palmitate as measured by residue detection. Although coronary flow undoubtedly changes under these circumstances, the changes in metabolic rate—as reflected by the fractional decline of radioactivity in fatty acid initially incorporated in the intracellular lipid pools—should be independent of flow per se, since they correlate with CO<sub>2</sub> production in other systems (3, 4). In some animals, C-11 palmitate was injected into the left atrium, before and after administration of epinephrine (50  $\mu$ g) intra-atrially to increase contractility, tension development, and heart rate (5). However, since epinephrine enhances lipolysis (in addition to its hemodynamic effects) results were compared with those obtained with C-11 palmitate injected intravenously or via the right atrium, before and after infusion of methoxamine to increase afterload without directly affecting lipolysis (6). In selected experiments, systolic blood pressure was decreased by phlebotomy in order to diminish tension-time index. Coincidence counts were collected for 10 min, and hemodynamics were restored to baseline before the next injection in the sequence. Tension-time index and peak dP/dt were obtained as described previously (7).

**Measurement of the rate of clearance of radioactivity from the vascular compartment.** To evaluate the contribution of intravascular C-11 palmitate to the observed curves, vascular time-activity curves were obtained after atrial injections of autologous red blood cells labeled with C<sup>15</sup>O (200  $\mu$ Ci) as a reference tracer. Since palmitate is extracted by the heart, liver, and other organs, and is therefore largely removed from the vascular space after the first pass, the number of counts remaining in blood, based on curves obtained with C<sup>15</sup>O-red blood cells, is much greater than the counts that would be expected for curves obtained with palmitate itself. Therefore, the systemic-blood time-activity curve for palmitate was obtained from rabbits injected via the left atrium with 5  $\mu$ Ci of [<sup>14</sup>C]-1-palmitate. 0.2-ml blood samples being collected from the left-atrial catheter following a saline flush on each occasion at 30-sec intervals for 10 min (8). To calculate the time course of washout of labeled palmitate from the vascular space within the field of detection, after left-atrial administration of palmitate, the time-activity curve for C<sup>15</sup>O carboxyhemoglobin was corrected by multiplying each data point by the percentage of counts of C-14 palmitate remaining compared with peak C-14 palmitate counts at the corresponding times. An accurate estimate of blood-pool contamination of the washout curve cannot be made from only the C-11 counts in blood samples, since these take no account of the blood volume within the field of view of the detectors, or of tissue attenuation of the radiation.

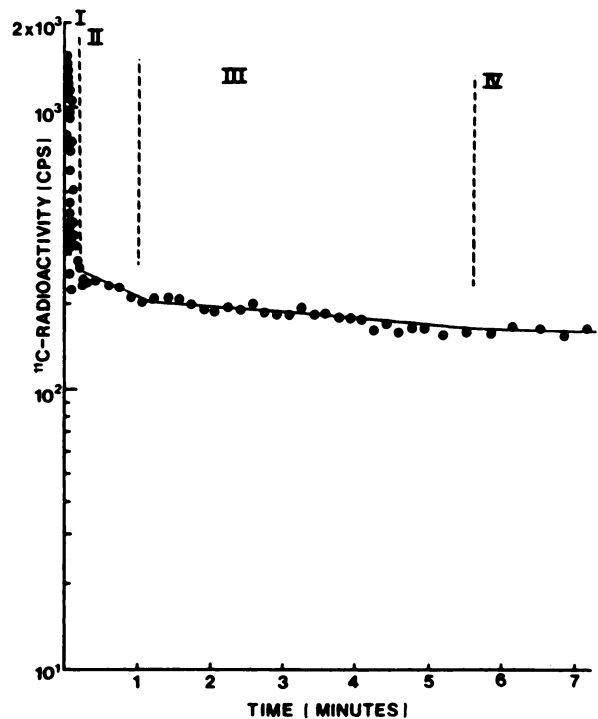


FIG. 1. Residue-detection, cardiac time-activity curve of C-11 coincidence count rate after bolus injection of C-11 palmitate into left atrium.

**Time-activity curves of C-11 palmitate in skeletal muscle and liver.** The curves recorded for the detection of cardiac residues might be distorted by the extraction of C-11 palmitate by skeletal muscle in the chest wall within the field of detection. Accordingly, a second pair of Na(Tl) crystals was used to provide simultaneous detection of radioactivity in the right hip region after left-atrial injection of C-11 palmitate. In separate experiments, time-activity curves were also obtained from detectors positioned on either side of the liver, since the liver extracts and releases fatty acids actively.

**Statistical analysis.** Phase 3 of residue-detection, time-activity curves (Fig. 1), was assessed by monoexponential regression analysis, with slopes expressed as the natural log of coincidence counts per minute. The relationship between cardiac clearance of C-11 palmitate and determinants of myocardial oxygen demand was analyzed by linear regression using the method of least-squares approximation.

## RESULTS

**Analysis of C-11 palmitate cardiac-residue detection curves.** A characteristic time-activity residue detection curve of C-11 palmitate, obtained after left-atrial injection, is shown in Fig. 1. Although each of the designated phases of the curve reflects influences of several processes, and thus represents a summation of several component curves, it is convenient to characterize the curve in terms of four relatively distinct phases, each of which is dominated by one process. These include:

Phase 1: appearance of tracer in the vascular space and its rapid washout.

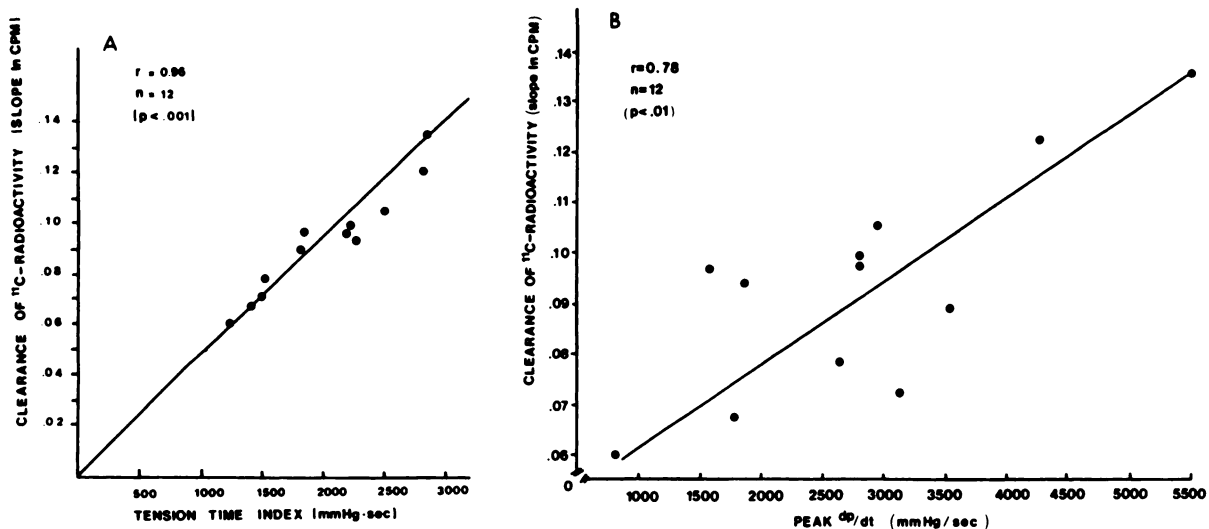
Phase 2: extraction of C-11 palmitate, with some back diffusion and washout of tracer into the vascular space.

Phase 3: monoexponential clearance of C-11 palmitate that has been incorporated into neutral lipids (3); and Phase 4: a flat portion reflecting turnover of tracer in stable neutral lipids and phospholipids.

Phase 3 was selected to define intervals that should be suitable for analysis of whole-heart aerobic metabolism. Delineation of Phase 3 was accomplished by (a) first visualizing the apparently linear portion on a plot of the logarithm (ln) of counts (corrected for physical decay) versus time; (b) obtaining best-fit regression lines conforming to inclusive sets of data from the temporal boundaries of this region and progressively more central portions; (c) calculation of the correlation coefficient ( $r$ ) for each regression; and (d) selection of the data set that includes the largest total number of points and provides an  $r$  value exceeding 0.95. The duration of Phase 3 from hearts of animals receiving tracer administered from the same site varied by less than 10%.

**Relationship between the rate of cardiac clearance of C-11 palmitate and physiological determinants of myocardial oxygen consumption and substrate utilization.**

**Left-atrial injection.** The cardiac clearance of C-11 palmitate in Phase 3 of the residue-detection time activity curve occurred during the interval from 1 to 6 min after bolus injection into the left atrium. Figure 2A shows the relationship between clearance of C-11 radioactivity from the heart and tension-time index. Increases in myocardial tension-time index are accompanied by an accelerated clearance of C-11 palmitate from the heart ( $r = 0.96$ ,  $P < 0.001$ ). Figure 2B illustrates the relationship between peak  $dP/dt$ , an index of contractility of the left ventricle, and cardiac clearance of C-11 palmitate ( $r = 0.78$ ). These data suggest that despite accompanying and variable changes in coronary flow, recirculation, and alterations in myocardial lipid pools in vivo, the rate of clearance of C-11 palmitate from the



**FIG. 2.** Correlation of C-11 palmitate clearance rates with (A) tension-time index, and (B) peak  $dP/dt$  after left-atrial injection. Regression lines were calculated by least-squares approximation.

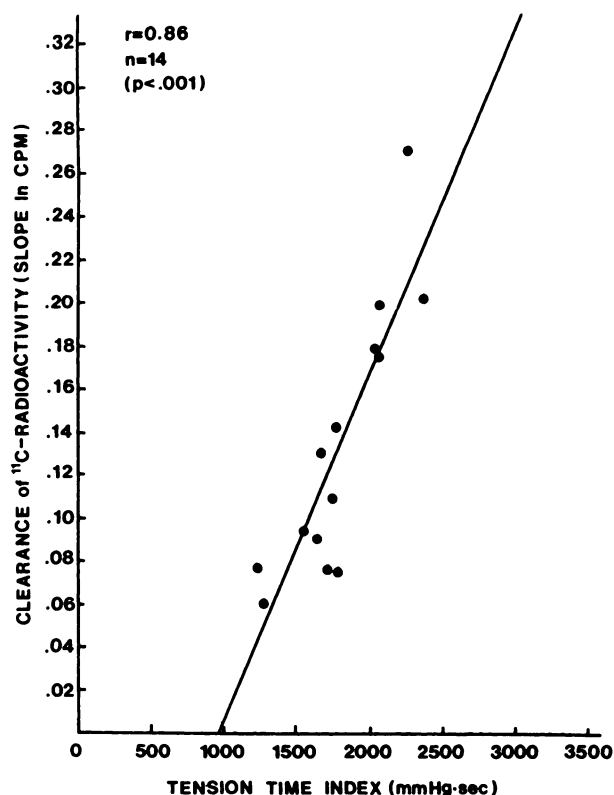


FIG. 3. Correlation between C-11 palmitate clearance rate and tension-time index after right-atrial injection of tracer. Regression lines were calculated by least-squares approximation.

heart reflects metabolic demand.

**Right-atrial injection.** In contrast to the curves obtained after left-atrial injection, all those observed after right-atrial injection of C-11 palmitate had a somewhat more brief Phase 3, with its duration persisting from 1 to 3 min after injection. After 3 min, the time-activity curve was flat when corrected for physical decay of the nuclide. The effect of alterations in tension-time index on the cardiac clearance of C-11 palmitate during Phase 3 is shown in Fig. 3. Although the duration of Phase 3 is briefer after right-atrial injection, the relationship between the rate of clearance of C-11 radioactivity and tension-time index is similar to that obtained after left-atrial injection ( $r = 0.86$ ,  $P < 0.001$ ). In this group of hearts, tension-time index was increased by administration of methoxamine, an agent known to exert pressor effects without augmenting contractility (6). Consequently,  $dP/dt$  may decrease in the face of increased cardiac work.

Increased cardiac clearance of C-11 palmitate accompanied the increased tension-time index evoked by the drug. Correlations with  $dP/dt$  would not be expected, of course, under these conditions, since the independence of cardiac work and  $dP/dt$  after administration of methoxamine has been observed by others (9).

**Intravenous injection.** Figure 4 shows the relationship between clearance of C-11 palmitate from the heart and

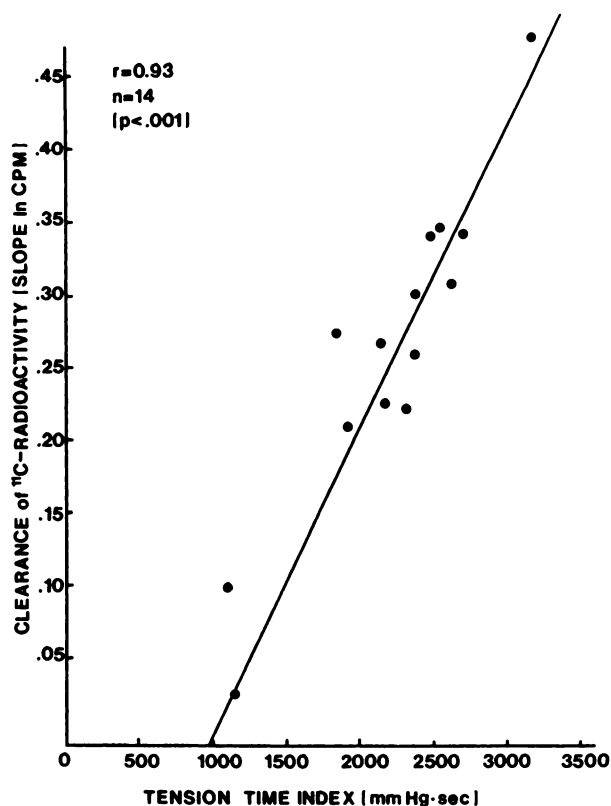


FIG. 4. Correlation between C-11 palmitate clearance and tension-time index after i.v. injection. Regression lines were calculated by least-squares approximation.

tension-time index after i.v. administration of tracer. As was the case after left- or right-atrial injections, intravenously administered C-11 palmitate was cleared at a rate proportional to tension-time index ( $r = 0.93$ ,  $P < 0.001$ ). However time-activity curves of C-11 palmitate after i.v. injection of tracer exhibit an earlier appearance of Phase 3 (30 sec after injection) than that seen after right-atrial injection.

**Vascular clearance of radiolabeled palmitate.** Figures 5, 6, and 7 show the clearance of C-14 palmitate from the vascular space, as obtained from sequential blood samples after left-atrial, right-atrial, and i.v. injections of both C-14 palmitate and  $C^{15}O$  red blood cells. Corrected blood clearance of radiopalmitate, based on the clearance of  $C^{15}O$  red blood cells and C-14 palmitate, is complete within 15 sec after intra-atrial injection, and within 6 sec after i.v. injection. In contrast, there is much slower clearance of radioactivity remaining in the myocardium within the entire field of view encompassing the heart. Thus, residual activity within the vascular space is minimal and does not significantly influence Phase 3 of the recorded cardiac time-activity curves of C-11 palmitate.

**Time-activity curves in skeletal muscle and liver.** Peak coincidence counts of C-11 palmitate in skeletal muscle after left-atrial injection were less than 1% of peak radioactivity recorded from the field of view encompassing

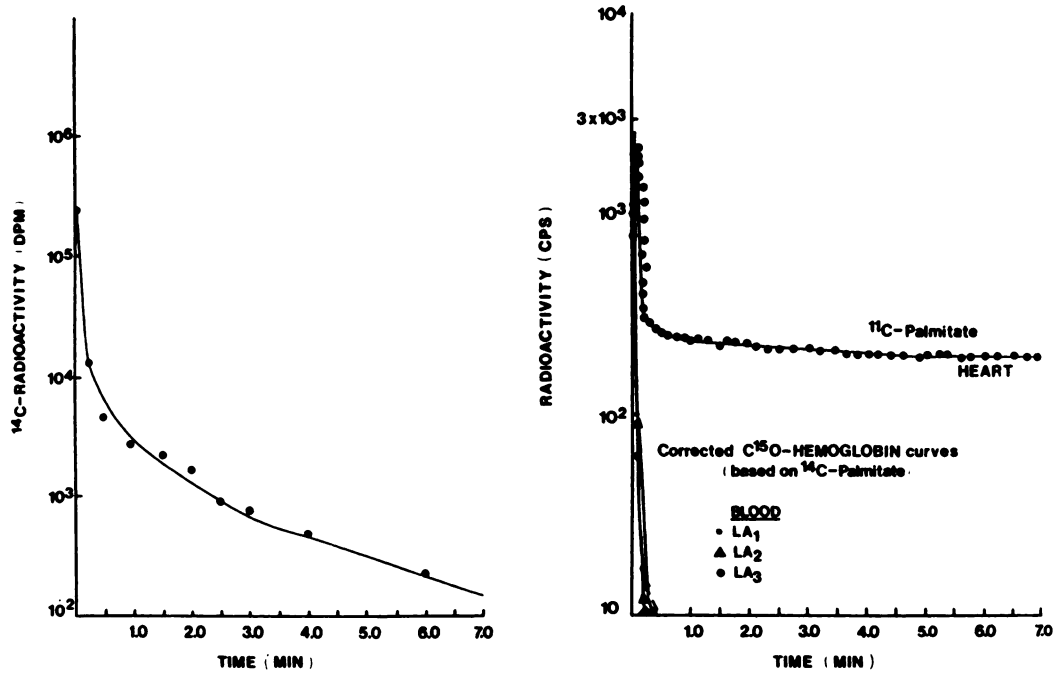


FIG. 5. Left: time-activity curve of C-14 radioactivity after left-atrial injection of C-14 palmitate. Right: time-activity curve of C<sup>15</sup>O-hemoglobin in blood, corrected for fractional rate of the observed decline of radioactivity in serial blood samples after three left-atrial injections of <sup>14</sup>C-palmitate (LA<sub>1</sub>, LA<sub>2</sub>, and LA<sub>3</sub>).

the heart, and radioactivity in skeletal muscle during Phase 3 was minimal. Because the absolute amount of radioactivity sequestered in skeletal muscle was so small, accurate determination of clearance rates for activity

within this type of tissue was not possible. In contrast to the heart, C-11 palmitate accumulated, rather than declined, in the field of view that encompassed the liver.

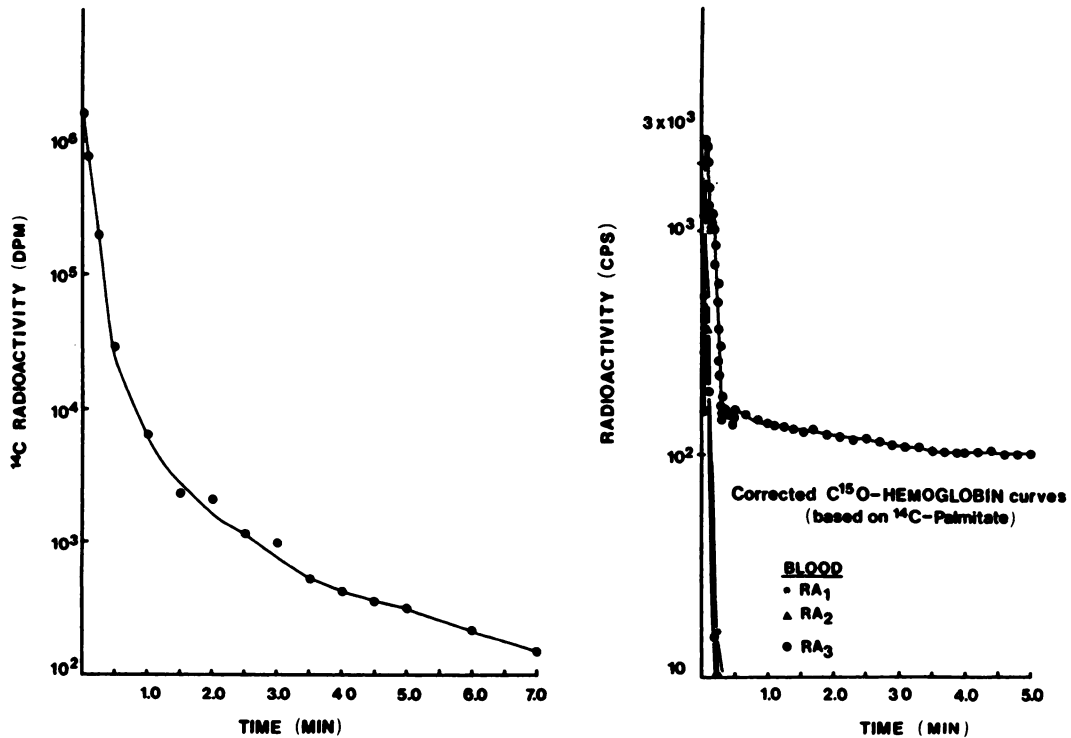


FIG. 6. Left: time-activity curves of C-14 palmitate after right-atrial injection of C-14 palmitate. Right: corrected time-activity curve of C<sup>15</sup>O-hemoglobin in blood corrected for fractional rate of decline of radioactivity in blood after three right atrial injections (RA<sub>1</sub>, RA<sub>2</sub>, and RA<sub>3</sub>) of C-14 palmitate.

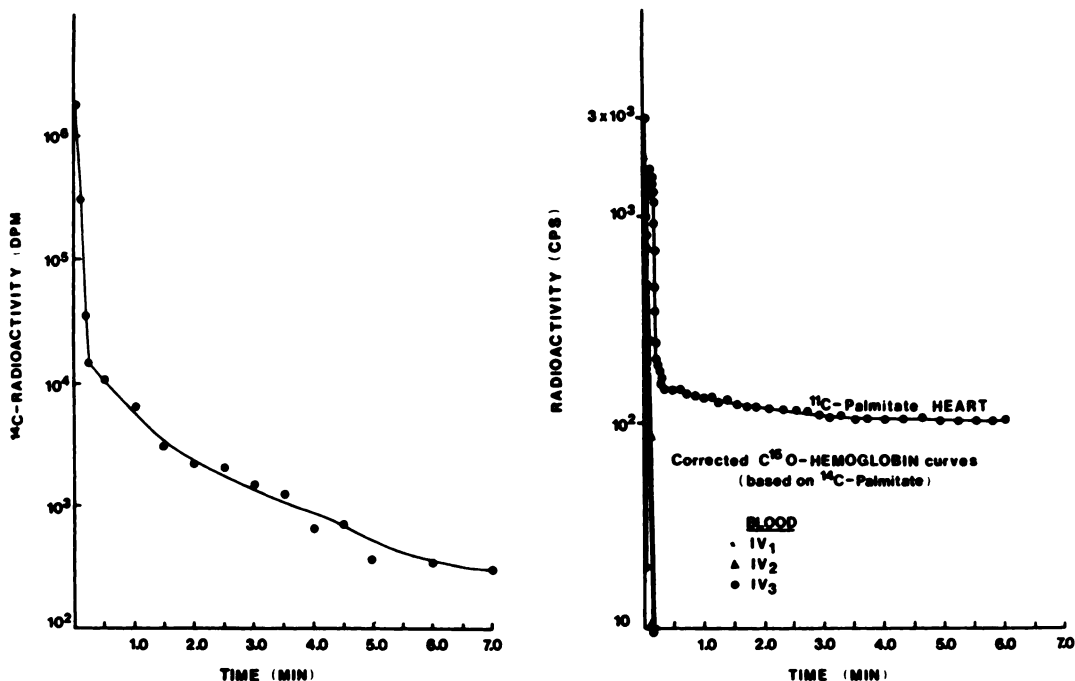


FIG. 7. Left: time-activity curves of C-14 palmitate after three i.v. injections (IV<sub>1</sub>, IV<sub>2</sub>, and IV<sub>3</sub>) of C-14 palmitate. Right: time-activity curve of C<sup>15</sup>O-hemoglobin in blood corrected for observed activity in blood samples obtained serially after i.v. injections of <sup>14</sup>C-palmitate.

DISCUSSION

Fatty-acid oxidation is the primary source of energy for the heart under aerobic, physiological conditions. Results from several laboratories have demonstrated that utilization of fatty acid is augmented in response to increased myocardial oxygen demand in vitro (4, 10). We have previously demonstrated that in isolated hearts the rate of clearance of C-11 palmitate reflects the rate of fatty-acid oxidation over a wide range (3). The present study was performed to determine whether an analogous relationship exists in vivo, despite the influences of recirculation, lactate, catecholamine levels, alterations in flow, and/or alterations in myocardial lipid constituents (4, 11). Results indicate that changes in whole-heart myocardial palmitate metabolism can be detected and quantified through the rate of clearance of C-11 palmitate detected externally after intra-atrial or i.v. administration of tracer in vivo.

In this study, we analyzed the rate of clearance of C-11 palmitate from the heart during Phase 3 of the time-activity curve rather than the fraction or absolute amount of palmitate extracted, so that results would not be distorted by the alterations in delivery and uptake of tracer that may accompany changes in myocardial work or blood flow. Recirculation of labeled substrates is not likely to distort the measurements of palmitate clearance substantially, since radioactivity in the vascular space was found to be minimal within 15 sec after administration of tracer. It is possible that clearance of C-11 radioactivity from the heart could be influenced by the rate of washout of <sup>11</sup>CO<sub>2</sub> or metabolic products of neu-

tral-lipid catabolism. Thus, changes in coronary venous flow that accompany work-dependent changes in coronary arterial flow could result in disparities between clearance and overall oxidative metabolism at very high flow rates. However, the relationship between clearance of cardiac palmitate and tension-time index was linear throughout the wide range studied (tension-time index range, 1200 to 5556 mm Hg/sec).

The duration of Phase 3 of C-11 palmitate cardiac residue-detection curves was found to depend somewhat on the site of injection. Thus, Phase 3 was found to occupy the interval from 1 to 6 min after left-atrial injection, from 1 to 3 min after right-atrial injection, and from 0.5 to 3 min after peripheral-venous injection. One possible explanation for the observed dependence of appearance time and duration on injection site is that the availability of C-11 palmitate to the left ventricle is an important determinant of the duration of Phase 3 of the time-activity curve. Left-atrial administration of C-11 palmitate provides delivery of tracer into the left ventricle and coronary arteries as a bolus that is relatively undiluted with blood. In contrast, right-atrial and, even more, peripheral i.v. injections result in delivery of tracer admixed with venous blood, with consequent diminution of blood-pool radioactivity within the field of detection. Thus, more peripheral injection sites are associated with relatively early apparently complete loss of tracer from the blood pool, in part related to a dilution phenomenon. The progressive diminution in the duration of Phase 3 with progressively peripheral injection sites may be attributable to the relative predominance of earlier in-

corporation of tracer into more slowly metabolizing selected phospholipid and neutral-lipid pools in organs other than the heart, such as the liver.

Results of this study indicate that a noninvasive index of global myocardial metabolism reflected by tension-time index can be obtained in vivo based on analysis of residue-detection curves of C-11 palmitate after left-atrial, right-atrial, or i.v. administration. Preliminary experiments in dogs given 8–12 mCi of C-11 palmitate, and tomography with a fast scan system, PETT V, showed that adequate counting statistics could be achieved for collection of time-activity curves (12). Thus, results of the present study demonstrate a duration of Phase 3 that appears sufficient to permit imaging studies in vivo for the assessment of regional and global myocardial metabolism. They also suggest that regional myocardial metabolism quantified serially by positron-emission reconstructive tomography in vivo should be feasible as soon as sufficiently fast-scanning multiple-slice instruments, currently developmental, become available for clinical use (13, 14).

## FOOTNOTE

\* Statham 23-db

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### ABSNM EXAM

The American Board of Science in Nuclear Medicine will conduct its second examination for persons practicing nuclear medicine science on Monday, June 23, 1980 in Detroit, MI. The examination will occur on the day preceding the SNM Annual Meeting.

Those desiring certification by the Board who meet the education and experience requirements are encouraged to apply. Application form and further information may be obtained from the Secretary of the Board:

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To be assured of consideration, applications must be postmarked no later than May 23, 1980.