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Heterogeneity of Flow as an Explanation of the Multi-exponential Washout of Inert Gas from Skeletal Muscle[1]

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The explanation of the multi-exponential washout of inert gas tracers from skeletal muscle is a source of controversy. The two most likely explanations are a heterogeneous flow pattern and countercurrent shunting of gas. Autoradiographs of canine skeletal muscle obtained after injection of ^{85}Kr indicate that heterogeneous flow is present in this preparation which would cause multi-exponential curves. This finding does not rule out an additional contribution of countercurrent shunting to the shape of the clearance curve.

INTRODUCTION

Clearance curves of ^{133}Xe or ^{85}Kr from skeletal muscle are multi-exponential (Kjellmer *et al.*, 1967; Sejrsen and Tonneson, 1968). Kjellmer *et al.* (1967) proposed that the multi-exponential washout is due to clearance from various parallel compartments within muscle with differing flow-to-volume ratios. Sejrsen and Tonneson (1968) reached the conclusion that the multi-exponential nature of ^{133}Xe clearance from cat skeletal muscle is not likely to be caused by heterogeneous flow, but instead shunting of ^{133}Xe by diffusion between arteries and veins. We have obtained autoradiographs of canine skeletal muscle during clearance of ^{85}Kr which indicate that heterogenous flow may play the role suggested by Kjellmer *et al.*

METHODS

Male mongrel dogs weighing 20–30 kg were anesthetized with Innovar-Vet (McNeil) (0.04 ml/kg) followed by sodium pentobarbital (20 mg/kg). Additional pentobarbital was administered as necessary to maintain adequate anesthesia.

We used a calf muscle preparation which we have described in detail in an earlier publication (Mohrman *et al.*, 1973). The calf was skinned, the paw was removed, and all structures in the popliteal region except the femoral artery and vein and the femur were transected. The femoral artery was cannulated just proximal to the popliteal region and perfused by a finger pump drawing blood from the contralateral femoral artery. The femoral vein was also cannulated in the popliteal region and the blood draining the calf was returned to the animal via the contralateral femoral vein. Sodium heparin (600 units/kg + 200 units/kg/hr) was used to prevent blood clotting in the extra

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corporeal circulation and microembolization. Constant flow was set at a level [22.3 ± 3.5 (SEM) ml $(100\ g \cdot min)^{-1}$] which gave a perfusion pressure similar to the systemic arterial pressure of the dog (≃100 mm Hg).

^{85}Kr (approximately 0.1 mC) dissolved in 0.5 ml of 0.9% saline was injected into the arterial perfusion line. The disappearance of ^{85}Kr from the calf was monitored with a 2-in diameter sodium iodide crystal mounted in a collimated probe. Counting intervals of the digital rate meter ranged from 3 to 30 sec depending upon the rate of clearance of ^{85}Kr and amount of radioactivity in the tissue. A total of seven complete ^{85}Kr clearance curves were obtained from four dogs. The multi-exponential clearance curves were resolved into three mono-exponential components by a computer program which optimizes parameters according to a weighted least squares error criterion (Chandler, 1971).

Tissue autoradiographs were made to determine the anatomical representation of the resolved components of the ^{85}Kr clearance curves. For this, the circulation of the calf was arrested at a selected time after the injection of another ^{85}Kr bolus by clamping the inflow and outflow tubing. The calf muscle was immediately removed and frozen in acetone at −79°. Transverse frozen sections of the calf were prepared and placed on X-ray film for times dependent on radioactivity contained within the slices (Thorburn *et al.*, 1963).

On the basis of autoradiograph evidence (see Results) only the fastest two of the three resolved components of the ^{85}Kr clearance curves were judged to represent muscle flow. The third component appears to represent clearance from bone marrow and connective tissue. The zero time intercept and the rate constant, for each of the two fastest mono-exponential components (1 and 2) of the clearance curves was calculated by standard methods (Kety, 1951).

RESULTS

Figure 1 shows the first 30 min of a typical clearance curve of ^{85}Kr from dog calf. The bolus of saline containing the ^{85}Kr was injected intra-arterially at time zero. The clearance curve itself (dotted line) is multi-exponential as indicated by the curvature on the semilogarithmic plot. The solid lines indicate the three mono-exponential components selected by a weighted least squares error criterion which sum algebraically to give the raw data. The determination of the slope of component 3 was on the basis of the last 30 min of washout data, not shown in Fig. 1.

The zero time intercepts of components 1 and 2 for the seven curves averaged 48.9 ± 14 (SEM) and 51.1 ± 14.1% of the total radioactivity in the two components, respectively. The rate constants were 0.85 ± 0.17 and 0.16 ± 0.01 min^{-1} for components 1 and 2, respectively.

Figure 2 shows autoradiographs from cross sections of three calves removed and frozen 15 sec, 4 min, and 20 min following injection of a bolus of ^{85}Kr. Inspection of the resolved components of the clearance curve in Fig. 1 indicates that radioactivity remaining in the calf at 20 min is responsible for component 3. The 20-min autoradiograph in Fig. 2 shows that nearly all of the radioactivity is located in the marrow of the tibia with additional small deposits in soft tissue. Components 1 and 2 occur at earlier times indicating that they reflect washout from soft tissue, predominantly the muscle of the

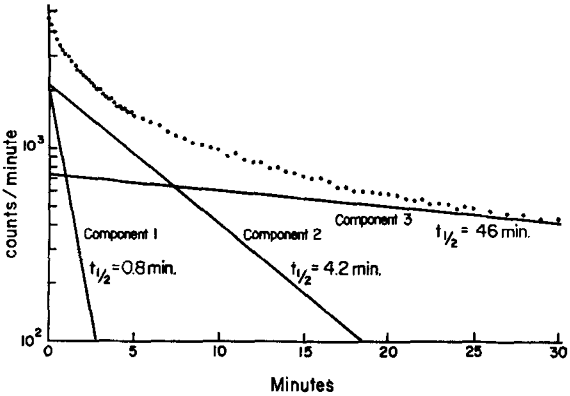

FIG. 1. Semilogarithmic plot of the first 30 min of a 60-min ^{85}Kr disappearance curve (background subtracted) from dog calf muscle and bone after intra-arterial injection at zero time. The curve was resolved into three mono-exponential components as described in Methods.

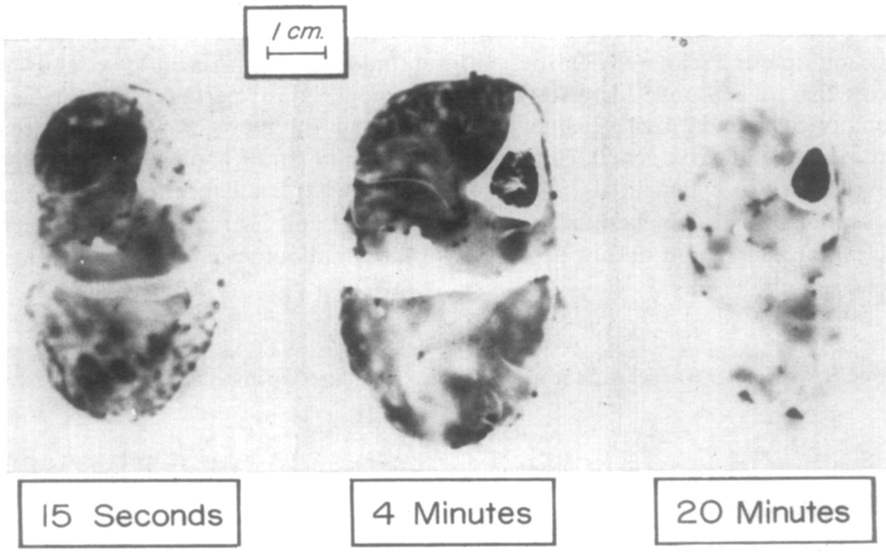

FIG. 2. Autoradiographs made from cross sections of dog calf muscles rapidly frozen 15 sec, 4 min, and 20 min after intra-arterial bolus injection of ^{85}Kr. Autoradiograph film exposure times were 22, 72, and 336 hr respectively.

calf. Note the variation in the density of the 15-sec and 4-min autoradiographs, indicating various rates of washout of ^{85}Kr.

DISCUSSION

Several investigators have proposed that the multi-exponential nature of inert gas clearance from muscle is caused by removal of the gas from multiple parallel compartments with varying flow to volume ratios (Kjellmer *et al.*, 1967; Paradise *et al.*, 1971; Thompson *et al.*, 1959). Sejrsen and Tonneson (1968) argue against this possibility on the basis of their observation that small local deposits of ^{133}Xe cleared multi-exponentially. They suggested that the small local deposits should clear mono-exponentially. They predicted that in such a small area, diffusion of gas would prevent the development of large concentration gradients which would occur if different flow to volume ratios were to exist so close to each other. Although their local deposits were only 0.5 mm deep, they were approximately 10 mm in diameter. This is a large enough area to encompass several different densities on the autoradiograph in Fig. 2. Thus, it appears that concentration gradients may exist within the local deposits observed in Sejrsen and Tonneson's experiments (1968). If this is the case, multi-exponential clearance of inert gas for the local deposits could be caused by heterogeneity of flow. This finding does not rule out a causal role for counter current exchange in the shape of inert gas clearance curves of muscle. We would only suggest that a significant fraction of the shape is due to heterogeneity of flow.

We do not have reason to believe that the variability of the density of the autoradiographs in Fig. 2 is an artifact. Mistakes in preparation of the tissue, for example, slow freezing following blood vessel clamping, allowing the tissue to thaw at a later time, or poor polishing of the surface of the tissue slice, would allow ^{85}Kr to become more equally distributed throughout the slice and/or reduce the resolution of the autoradiograph which would obscure any concentration differences.

The multiple and scattered densities of the autoradiographs taken at 15 sec and 4 min indicate that no anatomic correlates (parallel homogeneously perfused compartments) of components 1 and 2 exist. Kjellmer *et al.* (1967) and Van Liew (1967) point out that a multi-exponential curve generated by a large number of parallel compartments may be resolved into two exponentials. This is apparently what has happened in the experiments reported here. The parameters of components 1 and 2 serve only to characterize the clearance curve and do not provide information about specific compartments of skeletal muscle.

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