Volumes of distribution of hemoglobin, of [^{14}C]carboxypolyglucose and of [^{14}C]sucrose in pellets of rat-liver mitochondria

In order to determine the concentration of various solutes within the mitochondria, it is usually necessary to estimate and make an appropriate correction for the interspace of the mitochondrial pellet. The [^{131}I]albumin space[1] has been used as a measure of the extraparticulate volume and yields values of about 18 % of the total pellet volume. However, it was concluded that a significant fraction of the iodinated albumin was bound by the mitochondria. WERKHEISER AND BARTLEY[2] used the volume of distribution of [^{14}C]carboxypolyglucose, a branch-chained glucose polymer with a molecular weight of approximately 50,000, as a measure of the interspace in mitochondrial pellets. They reported this space to be 19–22 % of the total pellet water. In subsequent work, AMOORE AND BARTLEY[3] found carboxypolyglucose spaces ranging from 25–54 % of the total pellet water. These investigators[2,3] have also reported that a large fraction of the intramitochondrial water is available to sucrose. These latter observations have been confirmed by MALAMED AND RECKNAGEL[4]. Since polyglucose is not readily available, we have sought some other compound which could be used to estimate the extraparticulate volume of mitochondrial pellets. Thus, a comparison was made of the volumes of distribution of three large molecular weight compounds, hemoglobin, [^{14}C]carboxypolyglucose and [^{14}C]carboxydextran, in pellets of rat-liver mitochondria. The [^{14}C]sucrose space was also measured.

Mitochondria were prepared as previously described[5] from 72 g rat liver in 0.3 *M* sucrose. The mitochondria were washed once, resuspended in cold 0.3 *M* sucrose and divided equally among six tared, heavy-walled, 12 ml, Pyrex centrifuge tubes. After centrifugation at 25,000 × *g* for 10 min in the Servall Superspeed refrigerated centrifuge, the supernatants were discarded and the walls of the tubes were wiped dry with strips of filter paper. The mitochondria were resuspended in 0.5 ml 0.3 *M* sucrose containing known concentrations of [^{14}C]carboxypolyglucose, [^{14}C]carboxydextran, hemoglobin* or uniformly labeled [^{14}C]sucrose. The tubes were then centrifuged at 25,000 × *g* for 10 min. The supernatants were removed for analysis, and the walls of the tubes were again wiped dry with filter paper. All the above procedures were carried out in the cold. After the mitochondrial pellets were weighed, they were dried overnight in a vacuum oven at 105–110°. The volume of distribution of each compound was calculated from its dilution in the supernatant. The water content of the mitochondrial pellets was estimated from the differences between their wet and dry weights. Hemoglobin was estimated by the benzidine method of BING AND BAKER[6] as modified by HAM[7]. The polyglucose and dextran were made radioactive by the addition of [^{14}C]carboxyl groups to the reducing ends of the chains[2,8]. The dextran** was not homogeneous, but was a mixture of macromolecular material having an average molecular weight approximating that of serum albumin; the molecular weights ranged from 25,000 to 200,000.

In two experiments the volume of distribution of hemoglobin in the mitochondrial

* Purified (twice recrystallized) bovine hemoglobin, supplied by the Nutritional Biochemicals Corp.

** An aliquot of a 6 % (w/v) dextran solution in 0.9 % NaCl (Plavolex, Wyeth), used for intravenous infusion, was dialyzed against running tap water for 3 days to remove the salt and preservative.

pellets varied between 4,000 and 10,000 times the total pellet water. It was concluded that the hemoglobin was extensively adsorbed to the mitochondria, and no further experiments were performed with this substance. The volumes of distribution of [^{14}C]carboxypolyglucose, of [^{14}C]carboxydextran and of [^{14}C]sucrose are summarized in Table I. The sucrose and carboxypolyglucose spaces were 61.0 % and 29.8 %, respectively, of the total pellet water. These values are in good agreement with those obtained by WERKHEISER AND BARTLEY[2] and AMOORE AND BARTLEY[3]. The volume of distribution of the carboxydextran was identical to that of the carboxypolyglucose, indicating that these two compounds can be used interchangeably.

TABLE I

VOLUMES OF DISTRIBUTION OF [^{14}C]SUCROSE, [^{14}C]CARBOXYPOLYGLUCOSE AND [^{14}C]CARBOXYDEXTRAN IN PELLETS OF RAT-LIVER MITOCHONDRIA

The volumes of distribution are expressed as % of total pellet water.

	Sucrose	*Carboxy-polyglucose*	*Carboxydextran*
Mean	61.0	29.8	29.9
S.E.	6.24	1.37	1.58
No. of samples	6*	10**	8**

* 3 experiments. ** 4 experiments.

There is, presently, no absolute measure of the extraparticulate space of mitochondrial pellets. However, the reasons which suggest that the volumes of distribution of carboxypolyglucose and carboxydextran may be used as an index of the extraparticulate space are summarized as follows: (a) these compounds have high molecular weights; (b) their volumes of distribution are smaller than those of other, smaller molecular weight compounds which have been tested[2,3]; (c) the volumes of distribution of carboxypolyglucose and carboxydextran are of the order of magnitude which one would expect theoretically[9].

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