

Permeability of Rat Liver Microsomes to Sucrose and Carboxypolyglucose *in vitro**

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ABSTRACT

A study was made of the permeability of the microsomes to C¹⁴-sucrose and to C¹⁴-carboxypolyglucose, a branch-chained glucose polymer with a molecular weight of approximately 50,000. It was concluded that the microsomal membranes are permeable to sucrose on the basis of the following evidence: the volume of distribution of C¹⁴-sucrose was 84 per cent of the total microsomal pellet water; the sucrose unavailable volume, the per cent dry weights of the microsomal pellets, and the optical density of microsomal suspensions were independent of the concentration of sucrose in the suspending medium. It is suggested that the microsomal water which is unavailable to sucrose may be bound to protein and/or ribonucleic acid of the microsomes. The volume of distribution of C¹⁴-carboxypolyglucose was 44 per cent of the total pellet water, and it is considered that the microsomal membranes may be impermeable to this compound. Pretreatment with ribonuclease resulted in small increases in the volumes of distribution of both C¹⁴-sucrose and C¹⁴-carboxypolyglucose.

INTRODUCTION

Palade and Siekevitz (1) have shown by electron microscopy that the microsome fraction isolated from rat liver homogenates is derived from the endoplasmic reticulum, and is composed primarily of membrane-bounded structures, which usually bear small dense particles attached to their outer aspect. These dense particles contain most of the microsomal nucleoprotein. Palade and Siekevitz (1) also reported that, when the microsomes were fixed in hypotonic solutions, the membrane bounded structures appeared to swell; *i.e.*, these vesicles appeared to behave as osmometers. We have reinvestigated the permeability characteristics of the microsomes with two experimental approaches. In one, the optical density of suspensions of microsomes in sucrose solutions of different concentration was used as an index of particle size. This technique has been used successfully in

studies with mitochondria (2) and with red blood cells (3). In the other, the effect of the concentration of sucrose in the suspending medium on the volumes of distribution in microsomal pellets of C¹⁴-sucrose and C¹⁴-carboxypolyglucose was evaluated. The carboxypolyglucose is a branch-chained glucose polymer, with a molecular weight of approximately 50,000. It has been used to measure the extraparticulate space in mitochondrial pellets (4, 5). Our results suggest that the microsomal membranes are permeable to sucrose, but not to carboxypolyglucose.

Materials and Methods

Preparation of Microsomes.—A 10 or 20 per cent homogenate of rat liver was prepared in 0.3 M sucrose and centrifuged for 10 minutes at 15,000 g in the Servall superspeed refrigerated centrifuge. The microsomes were recovered from the resulting supernatant by centrifugation for 45 minutes at 100,000 g in the Spinco preparative ultracentrifuge. Further treatment of the microsomes depended on the type of experiment. All procedures, unless otherwise indicated, were performed at 0–4°C.

Determination of Optical Density.—The microsomes

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were washed once and resuspended in a suitable volume of 0.3 M sucrose. Aliquots of this suspension were diluted with sucrose solutions of different concentration. After 15 minutes, the optical densities of the diluted suspensions were read at 520 m μ in the Coleman model 11 spectrophotometer.

Measurement of Volumes of Distribution of C¹⁴-Carboxypolyglucose and of C¹⁴-Sucrose.—Aliquots of microsomes, prepared as indicated above, were washed once with 0.1 M, 0.3 M, or 0.6 M sucrose. The supernatants were discarded, and the walls of the lusteroid centrifuge tubes were wiped dry with strips of filter paper. Each pellet was resuspended in 0.5 ml. of a sucrose solution of the same concentration as the washing solution and which contained a known concentration of either C¹⁴-carboxypolyglucose or C¹⁴-sucrose. After 10 minutes, the suspensions were centrifuged at 100,000 g for 45 minutes. The supernatant solutions were removed and saved for C¹⁴ determination. The volumes of distribution of the labeled compounds were calculated from the dilution of the label in the supernatant solutions according to the formula $V_m = (C_o/C_s \times V_s) - V_s$, in which V_m = the volume of distribution, C_o = the concentration of C¹⁴ in the solution used for resuspending the microsomes, C_s = the concentration of C¹⁴ in the resulting supernatant, and V_s = the volume of the resuspension solution. The resulting volumes of distribution are then expressed as per cent of the total pellet water. After the walls of the centrifuge tubes were wiped dry, they were placed in a container of dry ice for approximately 15 minutes. The frozen microsomal pellets could then be easily and completely transferred to tared pyrex tubes. The pellets were weighed, dried overnight in a vacuum oven at 105–110°C., and weighed again. The water content of the pellets was estimated from the differences between their wet and dry weights.

In some experiments the microsomes were pretreated with ribonuclease. Unwashed microsomes were resuspended in 0.3 M sucrose which was adjusted to pH 7.4 with 0.03 M tris buffer and which contained ribonuclease, 0.1 mg./ml. The suspensions were incubated at 30°C. for 15 minutes and were then centrifuged at 100,000 g for 45 minutes. After the microsomes were washed once with cold 0.3 M sucrose, the walls of the centrifuge tubes were dried and the pellets were resuspended in 0.5 ml. of cold 0.3 M sucrose, containing either C¹⁴-sucrose or C¹⁴-carboxypolyglucose. The rest of the procedure was the same as in those experiments in which the ribonuclease was not employed.

Determination of C¹⁴ Activity.—Duplicate 0.1 ml. aliquots of each sample were pipetted onto circles of filter paper, 24 mm. in diameter, in cupped nickel planchettes. Each planchette was counted for 5,000 counts in a thin window, gas flow system.

C¹⁴-Labeled Compounds.—The C¹⁴-sucrose used in

these experiments was uniformly labeled. Polyglucose¹ was made radioactive by the addition of C¹⁴-labeled carboxyl groups to the reducing ends of the chains (4, 6).

OBSERVATIONS

Effect of Sucrose Concentration on the Optical Density of Microsomal Suspensions.—In the first series of five experiments, the optical density of microsomes, suspended in sucrose solutions ranging in concentration from 0.025 M to 0.6 M, was used as an index of the volume of the microsomes. An increase in volume of the particulates would be expected to result in a decreased optical density of the suspension. However, the concentration of sucrose in the suspending medium was without effect on the optical density of the microsomal suspensions. If the optical density does, in fact, provide a reliable index of the volume of the microsomes, these data imply that the microsomal membranes are permeable to sucrose. Since the optical density methods for the determination of changes in the volume of small particles are largely empirical in nature, a second, more direct approach to the study of the permeability of the microsomal membranes to sucrose was utilized.

Effect of Sucrose Concentration on the Volumes of Distribution of C¹⁴-Sucrose and of C¹⁴-Carboxypolyglucose in Microsomal Pellets.—The volumes of distribution of C¹⁴-sucrose and of C¹⁴-carboxypolyglucose were 84 and 44 per cent, respectively, of the total pellet water when the microsomes were recovered from suspension in 0.3 M sucrose (Fig. 1 A). The volumes of distribution of these two compounds were not significantly changed when the microsomes were recovered from suspensions in either 0.1 M or 0.6 M sucrose. If the C¹⁴-carboxypolyglucose space is a measure of the extraparticulate space of the microsomal pellet, then the microsomal membranes are permeable to sucrose. Furthermore, the large volume of distribution of sucrose in itself suggests that a major fraction of the intramicrosomal water is available to sucrose. A small volume, 10 to 16 per cent, of the microsomal water was unavailable to sucrose. If this water was sequestered behind membranes which were impermeable to sucrose and permeable

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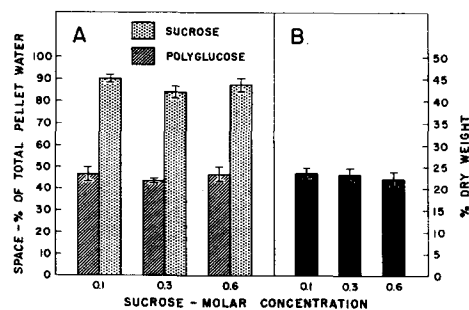


FIG. 1. Effect of concentration of sucrose in the suspending medium on C^{14} -sucrose and C^{14} -carboxypolyglucose spaces (A) and per cent dry weight (B) of microsomal pellets. Mean and standard errors of eight experiments.

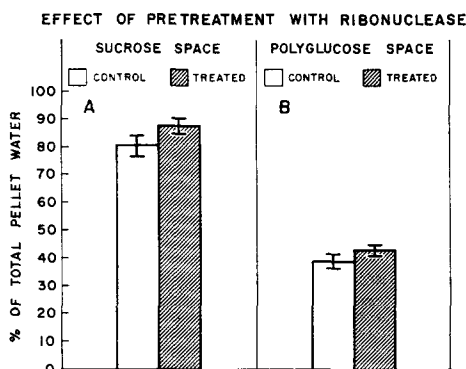


FIG. 2. Effect of pretreatment of microsomes with ribonuclease on C^{14} -sucrose and C^{14} -carboxypolyglucose spaces in microsomal pellets. Mean and standard errors of seven experiments.

to water, then the sucrose unavailable volume should have increased as the concentration of sucrose in the medium was decreased. This was not the case. Alternatively, the fraction of microsomal water which is unavailable to sucrose may be "bound" to some component of the microsomes. A limited attempt was made to test this hypothesis. Since nucleic acids are readily hydrated (7), microsomes were treated with ribonuclease (0.1 mg./ml.) prior to the measurement of the volumes of distribution of C^{14} -carboxypolyglucose and of C^{14} -sucrose. If, in the previous experiments, the sucrose failed to distribute throughout the total pellet water because a significant fraction of the pellet water is "bound" to ribonucleic acid, then the removal of the ribonucleic acid from the microsomes should result in an increase in the volume of distribution of the sucrose. Pretreatment of the mi-

croosomes with ribonuclease resulted in an increase of approximately 10 per cent in both the C^{14} -carboxypolyglucose and C^{14} -sucrose spaces (Fig. 2). These effects were small, but they were found in each of the seven experiments performed and they are statistically significant ($p < 0.01$). The data are compatible with the hypothesis that the microsomal water which is unavailable to sucrose is bound in part to microsomal ribonucleic acid; a more definitive statement cannot be made at this time.

Effect of Sucrose Concentration on the Per Cent Dry Weight of Microsomal Pellets.—The dry weights of the microsomal pellets were also determined in those experiments in which the volumes of distribution of C^{14} -sucrose and C^{14} -carboxypolyglucose were measured. These dry weights were corrected for the weight of the sucrose in the pellets, which was estimated from the sucrose space, and were expressed as per cent of the wet weight of the pellets (per cent dry weight). The corrected per cent dry weight of the microsomal pellets was independent of the concentration of sucrose in the suspending medium (Fig. 1 B). These results also are contrary to what one would expect if the microsomes were behaving as osmometers. For if this were the case, the per cent dry weight of the pellets should have decreased as the microsomes swelled in sucrose solutions of decreasing concentration.

DISCUSSION

Several lines of evidence have been presented which indicate that rat liver microsomes are permeable to sucrose *in vitro*. Thus, 84 per cent of the total microsomal pellet water was available to sucrose, and the concentration of sucrose in the suspending medium was without effect on (1) the sucrose unavailable volume in microsomal pellets, (2) the per cent dry weight of microsomal pellets, and (3) the optical density of microsomal suspensions. These findings are in contradiction to those presented by Palade and Siekevitz (1), that the microsomes appeared swollen after fixation in hypotonic media. It should be pointed out, however, that Palade and Siekevitz isolated the microsomes in 0.88 M sucrose, whereas in the work presented here the microsomes were isolated in 0.3 M sucrose. It is conceivable that under these latter conditions the microsomal membranes were irreversibly damaged, resulting in an increased

permeability. Alternatively, it is also conceivable that fixation in OsO₄ for prolonged periods of time (2 to 20 hours) at 0°C. might have reduced the permeability of the microsomal membranes.

Ten to 16 per cent of the microsomal pellet water was unavailable to sucrose, but evidence was presented that this water was not sequestered behind membranes impermeable to sucrose. Although kinetic studies were not performed, we feel that it is unlikely that these findings are the result of a failure to obtain complete equilibration, for during the period of disequilibrium the volume of the microsomes would be inversely proportional to the external sucrose concentration. That this was not the case is suggested by the constancy of the optical density of microsomal suspensions and of the per cent dry weight of microsomal pellets over a wide range of external sucrose concentrations. A reasonable explanation for the failure of sucrose to distribute throughout the total pellet water is that the sucrose unavailable water is "bound" to microsomal protein and/or ribonucleic acid. Klotz (8) has postulated that proteins are surrounded by a sheath of hydration which may be viewed as an ice-like lattice. A similar concept has been presented by Jacobson *et al.* (7) for deoxyribonucleic acid. The availability of this shell of water to solutes would, presumably, be quite limited. The increase in the volume of distribution of sucrose following treatment of the microsomes with ribonuclease is consistent with the hypothesis that the ribonucleic acid is associated with "bound" water, into which solute cannot diffuse. The increase in the carboxypolyglucose space following treatment with ribonuclease is not so readily explained. One possibility is that the ribonuclease damaged some microsomal membranes, rendering them permeable to the carboxypolyglucose. In keeping with this suggestion is the observation of Palade and Siekevitz (1) that microsomes treated with ribonuclease appeared swollen in electron micrographs.

It was hoped that the volume of distribution of C¹⁴-carboxypolyglucose, because of the large molecular weight of this compound, would provide

a measure of the extraparticulate space in pellets of microsomes. However, its volume of distribution, 44 per cent of the total pellet water, was somewhat larger than has been theoretically calculated for the interspace for packed spheres; *i.e.*, 26 per cent of the total volume (9). Since the microsomes may not be perfectly packed in the pellet, the value obtained for the carboxypolyglucose space may be a reasonable estimate of the interspace. On the other hand, one must also consider the possibility that some microsomal membranes, as a result of damage in the isolation procedure, may be permeable to the carboxypolyglucose.

Although the microsome fraction is derived from the endoplasmic reticulum, it is not possible to extrapolate from the permeability characteristics of the membrane-bounded vesicles of the microsomes *in vitro* to the permeability properties of the membranes of the endoplasmic reticulum *in vivo*. Marked changes in the permeability of these membranes may very well occur in the course of the various procedures involved in the isolation of the microsomes. However, the permeability of the membranous components of the microsomes may be of considerable importance in biochemical studies of these particulates *in vitro*. Molecules smaller than sucrose may penetrate the microsomal membranes readily, but very large molecules may be excluded.

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