SEDIMENTATION PROPERTIES OF RAT LIVER MITOCHONDRIA

Effects of Cortisone Treatment

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ABSTRACT

A prediction of the velocity of sedimentation of rat liver mitochondria in sucrose gradients is made on the basis of recent measurements of the size of isolated mitochondria suspended in sucrose medium and the model proposed by Bentzel and Solomon to describe the osmotic behavior of mitochondria. The experimentally observed velocity is extremely close to the predicted value and confirms by a different approach the estimate of mitochondrial volume made by Baudhuin and Berthet on the basis of electron microscopic measurements. Because cortisone treatment of rats is known to result in a marked increase in mitochondrial size as observed under the electron microscope, mitochondria were co-isolated from livers of control and cortisone-treated animals, and the sedimentation behavior of the mixtures was examined by sucrose density gradient centrifugation. Mitochondria from cortisone-treated animals were found to sediment 1.4 times as rapidly as those from control animals, indicating that their increased size cannot entirely be due to an increased imbibition of fluid from the surrounding sucrose medium, and that the change in size must at least in part be due to a change in content of nondiffusible mitochondrial components. Although the increase in sedimentation velocity of mitochondria from cortisone-treated animals is striking, it is less than that predicted solely on the basis of their size relative to that of control mitochondria. It is concluded that the increases in mitochondrial size and content of nondiffusible components produced by cortisone treatment are accompanied by alterations in mitochondrial composition as well.

INTRODUCTION

Treatment of rats with cortisone is known to produce profound changes in a number of functions of liver mitochondria when tested in vitro. Thus defects in oxygen consumption and oxidative phosphorylation, as well as other abnormalities in mitochondrial function, have been reported over the past few years (1–5). In 1955 Lowe et al. reported a decrease in the number of mitochondria per liver cell in cortisone-treated rats (6), and more recently a combined biochemical and morphological study by Kimberg et al. has suggested not only that cortisone treatment of rats is associated with changes in mitochondrial function, but that treatment with hormone for as little as 6 days results in striking morphological modifications in mitochondria observed in tissue sections under the electron microscope (4). Thus, for example, treatment of rats with 5 mg cortisone acetate daily for 6 days

was shown to produce a three- to fourfold increase in the volume of the average liver mitochondrion. Although there is an increase in mitochondrial size, there is a concomitant reduction in the number of mitochondria per cell, the total mitochondrial material per cell remaining approximately constant. The authors interpreted their findings as indicating either enlargement of some mitochondria with loss of others or fusion of preexisting mitochondria to form larger units.

Although morphometric analysis suggested that the mitochondrial enlargement reflected an increase in all of the morphologic components rather than mitochondrial "swelling" due to imbibition of fluid from the cytoplasm, the authors emphasized that estimations of this type, based upon morphological observations under the electron microscope, can be difficult (4, 7). The technique itself is laborious and involves a number of statistical assumptions, and, even in careful studies, the number of mitochondria which can be so examined is small. Furthermore the possibility of changes in mitochondrial size and shape arising during fixation and preparation of tissues for electron microscopy makes it desirable to have an independent measure of mitochondrial size.

The purpose of the present report is twofold. First, to describe a method for the estimation of mitochondrial size based on the determination of the velocity of sedimentation of mitochondria in a sucrose density gradient, and, second, to apply this technique to determine the effects of cortisone treatment on the sedimentation properties of rat liver mitochondria. Although the sedimentation properties of mitochondria have been considered in detail from a theoretical point of view (8) and although a number of investigators have employed sedimentation velocity techniques to determine the size distribution of normal mitochondria (9–12), until recently there has been little agreement about a satisfactory model for their osmotic behavior. While previous sucrose density gradient studies of the "sedimentation-equilibirum" or "isopycnic" type (13, 14) have been of great value in yielding information on the average density of the hydrated nondiffusible components of mitochondria, the principal effects to be investigated in the present study were those on mitochondrial size rather than composition, and hence it was thought that one might obtain more useful information from non-equilibrium determinations in which measurements of sedimentation *velocity* were made. The results to be presented show, first, that the experimentally observed sedimentation velocity of rat liver mitochondria is in excellent agreement with that predicted from electron microscopic measurements and recent detailed studies of the osmotic properties of isolated mitochondria, and, second, that treatment of rats with cortisone results in an enlargement of liver mitochondria which is not due to simple swelling but rather represents at least in part a true increase in their nondiffusible components.

MATERIALS AND METHODS

Materials

All experiments were performed with male albino Sherman (Columbia strain) rats weighing between 125 and 150 g and maintained on standard laboratory food. L-leucine-^{14}C was obtained from the New England Nuclear Corp., Boston, Mass. Cortisone acetate (Cortone acetate), 25 mg/ml in saline suspension, was purchased from Merck, Sharp and Dohme, division of Merck and Co., Inc., West Point, Pa.

Treatment of Animals

9 days before sacrifice 12 rats were divided into two groups of six animals each. One group received an intraperitoneal injection at 9 a.m. of 0.5 ml of a neutral solution of uniformly labeled L-leucine-^{14}C (250 mCi/mmole) containing 50 μCi/ml in 0.15 M NaCl. This dose was repeated at 5 p.m. and at the same times on the following day (i.e., each animal received a total of 100 μCi of leucine-^{14}C). The animals in the other group were also injected but received only 0.5 ml 0.15 M NaCl each time. Beginning 7 days before sacrifice and on each day thereafter three radioactive and three nonradioactive animals received 5 mg cortisone acetate in 0.2 ml volume subcutaneously and the remaining ("control") animals received instead 0.2 ml 0.15 M NaCl solution subcutaneously. These injections were continued daily including the day prior to sacrifice. By the time the animals were sacrificed six animals had thus received seven daily injections of cortisone and six had received only saline. Of the six animals in each group, three had previously received leucine-^{14}C and three had not.

Isolation of Mitochondria

Rats were stunned by a blow to the head, exsanguinated, and the livers were immediately removed and chilled in iced 0.25 M sucrose solution. The following four mixtures of liver were prepared: (*a*) ½ liver from each of the nonradioactive control animals + ⅛ of a liver from each of the radioactive

cortisone-treated animals; (*b*) ½ liver from each of the nonradioactive cortisone-treated animals + ⅛ of a liver from each of the radioactive control animals; (*c*) ½ of the remaining portion of each of the livers of the radioactive control animals; (*d*) ½ of the remaining portion of each of the livers of the radioactive cortisone-treated animals.

The livers in each of these four mixtures were homogenized together in 4 volumes of 0.25 M sucrose in a hand-driven Dounce-type glass homogenizer with 20 strokes of a loosely fitting pestle (Blaessig Glass Co., Rochester, N.Y.), and all subsequent steps were carried out at 0°–4°C unless otherwise stated. The homogenates were decanted and diluted with equal volumes of 0.25 M sucrose to produce 10% homogenates of liver. 80 ml of each of the four homogenates (each corresponding to a total of 8 g of liver) was centrifuged in two portions at 600 g_{max} for 10 min in a Sorvall RC2-B refrigerated centrifuge (Ivan Sorvall Inc., Norwalk, Conn.). The supernatants were then withdrawn by pipette to within 2–3 cm of the surface of each pellet. The pellets were discarded and the supernatant material was centrifuged at 8500 g_{max} for 10 min. The supernatant material from this second centrifugation was discarded, like pellets were combined and resuspended with a stirring rod in 40 ml 0.25 M sucrose solution, and the mitochondria were resedimented at 8500 g_{max} for 10 min. The supernatant material was discarded, and the pellets were resuspended in 40 ml 0.25 M sucrose and resedimented for a total of five washes.

Each of the four final pellets was resuspended in 10 ml 0.25 M sucrose solution by means of a small Dounce-type hand-driven glass homogenizer with 10 gentle strokes of a tightly fitting pestle. The resulting suspensions thus contained mitochondria corresponding to 8 g of original liver in a volume of 10 ml.

Sucrose Density Gradient Analyses

EQUILIBRIUM SEDIMENTATION STUDIES: ½ ml of a suspension of washed mitochondria containing 3.0–3.5 mg mitochondrial protein in 0.25 M sucrose was layered onto a 34-ml 1.0–2.0 M linear sucrose gradient and centrifuged at 27,000 rpm in an SW 27 rotor for 20 hr at 4°C in a Beckman L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.). After centrifugation the tube was punctured, 40-drop samples were collected, and the optical density of each sample was determined at 520 mμ.

SEDIMENTATION VELOCITY DETERMINATIONS: 1 ml of a diluted suspension of washed mitochondria containing 3.5–4.5 mg mitochondrial protein in 0.25 M sucrose was layered over a 34-ml 0.5–1.5 M linear sucrose gradient, centrifuged at 3000 rpm in a Beckman SW 27 rotor for 75 min at 4°C, and stopped with the brake.[1] After centrifugation the tube was punctured and the optical density of the effluent at 520 mμ was monitored by means of a Gilford recording spectrophotometer equipped with a flow-through cell (Gilford Instrument Laboratories Inc., Oberlin, Ohio). 40-drop samples of effluent were collected in conical centrifuge tubes, and 0.5 ml of a solution of bovine serum albumin (2 mg/ml) was added to each. The contents of the tubes were mixed, and the protein was precipitated by the addition of 2 ml of 10% trichloroacetic acid. After standing for 20 min the precipitates were spun down, drained, and dissolved in 0.5 ml concentrated formic acid, and the resulting solutions were transferred to scintillation vials by means of two 5-ml samples of Bray's solution (15). Radioactivity was subsequently determined in a Packard liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.). Counting efficiency was 57%.

PROTEIN DETERMINATIONS were performed by a modification of the Folin-Ciocalteu method (16).

RESULTS

Equilibrium Sedimentation of Mitochondria from Livers of Normal Rats

½ ml of a suspension of mitochondria in 0.25 M sucrose (containing 3.2 mg mitochondrial protein) was layered over a 34-ml 1.0–2.0 M linear sucrose density gradient and the gradient was centrifuged at 27,000 rpm for 20 hr as described under Methods. Centrifugation under these conditions resulted in a tightly packed band of mitochondria at a level in the tube corresponding to a sucrose concentration of 1.66 M and a density of 1.215 g-cm^{-3} (Fig. 1). The value 1.215 g-cm^{-3} is in excellent agreement with the value reported earlier by Thomson and Mikuta (17) and was not found to be significantly different in mitochondria from cortisone-treated animals. It can be thought of as the average density of the hydrated nondiffusible components of the mitochondrion and will be of use in subsequent calculations.[2]

[1] Under the conditions of centrifugation employed the maximal angle between the wall of the centrifuge tube and a line of force acting on a mitochondrion was less than 8°.

[2] For mitochondria suspended in 1.66 M sucrose solution the volume of the osmotically active sucrose-inaccessible water space is negligibly small, and their effective density is determined by the density of their hydrated components; the remaining aqueous compartment of mitochondria is freely permeable to the surrounding sucrose medium (see Discussion).

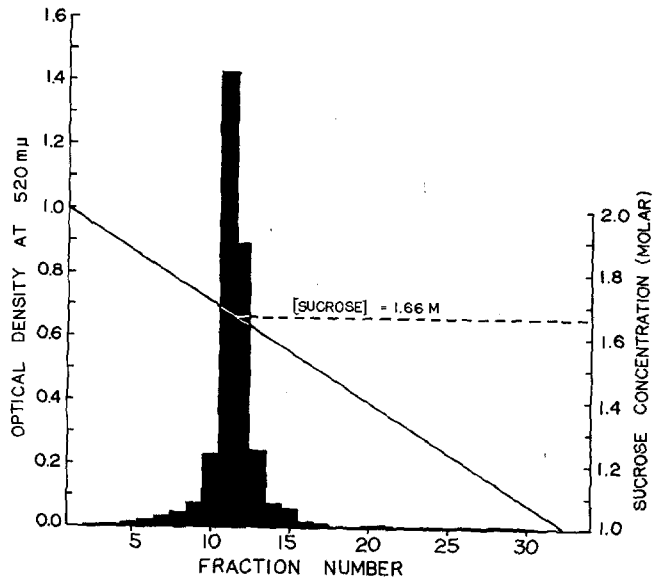

FIGURE 1 Equilibrium sedimentation of mitochondria in a linear 1.0–2.0 M sucrose density gradient. The experimental procedure is described under Methods. The solid line shows the concentration of sucrose in the gradient. Mitochondria centrifuged under these conditions for 20 hr form a tightly packed band at a position corresponding to a sucrose concentration of 1.66 M and a specific gravity of 1.215 (dashed line).

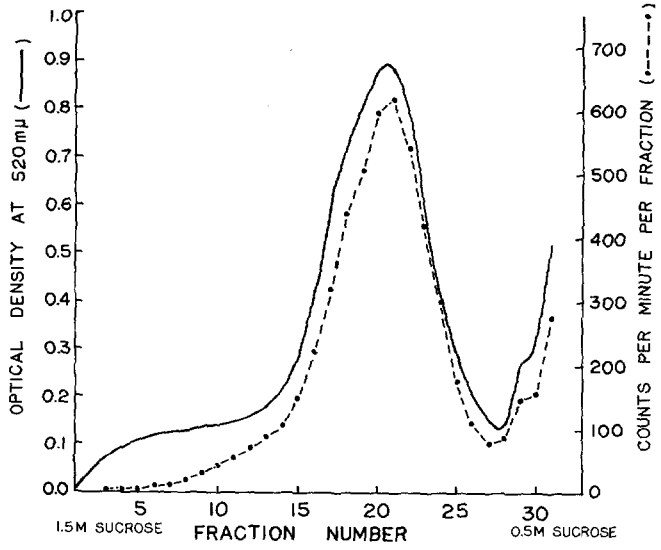

FIGURE 2 Velocity sedimentation of normal rat liver mitochondria from animals which had previously received 100 μCi of leucine-^{14}C (preparation (*c*) under Methods). After centrifugation for 75 min in a linear 0.5–1.5 M sucrose density gradient the optical density at 520 mμ and radioactivity of the effluent from the gradient were monitored as described under Methods. The positions of the optical density (solid line) and radioactivity (dashed line) peaks are seen to coincide.

Sedimentation Velocity of Mitochondria from Livers of Normal Rats

In sedimentation velocity experiments 1.0 ml of a mitochondrial suspension from normal rats which had previously received radioactive leucine was layered over a 0.5–1.5 M linear sucrose density gradient and the gradient was centrifuged at 3000 rpm in a Beckman SW 27 rotor for 75 min as described under Methods. Fig. 2 shows a typical pattern of the effluent from such a gradient. Under these conditions the distance moved by the mitochondrial peak was found to be 2.38 ± 0.09 cm (mean ± 1 SE for six such determinations). The position of the radioactive peak is seen to coincide exactly with that of the optical density peak.

Comparison of Sedimentation Velocity of Mitochondria from Livers of Control and Cortisone-Treated Rats

Preliminary experiments demonstrated that mitochondria from the livers of cortisone-treated

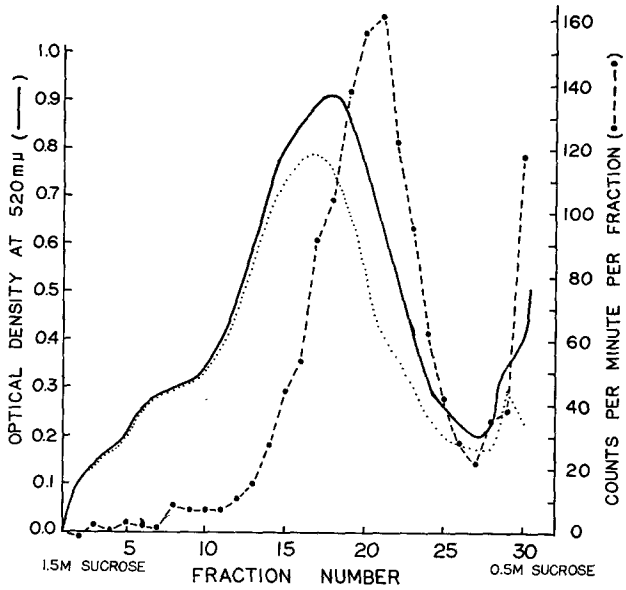

FIGURE 3 Sucrose density gradient analysis of mitochondria co-isolated from a mixture of radioactive liver from control animals and nonradioactive liver from cortisone-treated animals (preparation (*b*) under Methods). The *solid line* shows the optical density of the effluent at 520 mμ, and the *dashed line* the radioactivity present in the effluent. The dashed line corresponds to the position of mitochondria from normal animals. The *dotted line* shows the position of mitochondria from cortisone-treated animals and was obtained by subtracting from the solid line the (relatively low) optical density contributed by the radioactive mitochondria from the control animals. The correction for the optical density contributed by the radioactive mitochondria was obtained by multiplying the radioactivity present in each fraction by the ratio of optical density to radioactivity present in a preparation of radioactive mitochondria from normal animals (preparation (*c*) under Methods). Note that the mitochondria from cortisone-treated animals (corresponding to the dotted line) sediment more rapidly than do those from normal animals (corresponding to the dashed line).

animals consistently sedimented with a greater velocity than did those from livers of control rats, and that the position of the radioactive peak again coincided with that of the optical density peak. Because of the possibility that such differences might be due to slight variations in the handling of the mitochondria from the two groups, "mixing experiments" of the following two types were performed: (1) Radioactive mitochondria from livers of control animals were co-isolated with nonradioactive mitochondria from livers of cortisone-treated animals and examined by sucrose density gradient centrifugation as described under Methods. The quantity of radioactive liver was small in comparison to that of nonradioactive liver, and hence the optical density peak itself could be used as an approximate marker for the mitochondria from the cortisone-treated rats. (2) In the reverse experiment, radioactive mitochondria from cortisone-treated animals were co-isolated with nonradioactive mitochondria from control animals, and the mixtures were similarly examined by sucrose density gradient analysis.

Fig. 3. shows a sucrose density gradient analysis of a mixture of mitochondria in the first type of experiment. In contrast to Fig. 2 it is seen that there is a striking displacement of the optical density peak (primarily contributed by mitochondria from the cortisone-treated animals—see above) from the radioactivity peak (contributed only by mitochondria from control animals), indicating that mitochondria from the livers of the cortisone-treated rats sediment more rapidly than do those from livers of normal animals. The dotted curve shows the distribution of nonradioactive mitochondria after correction for the optical density contributed by the radioactive mitochondria (see legend). The ratio of the distances moved during the 75 min centrifugation is 1.4. In the reverse experiment (Fig. 4), the radioactivity

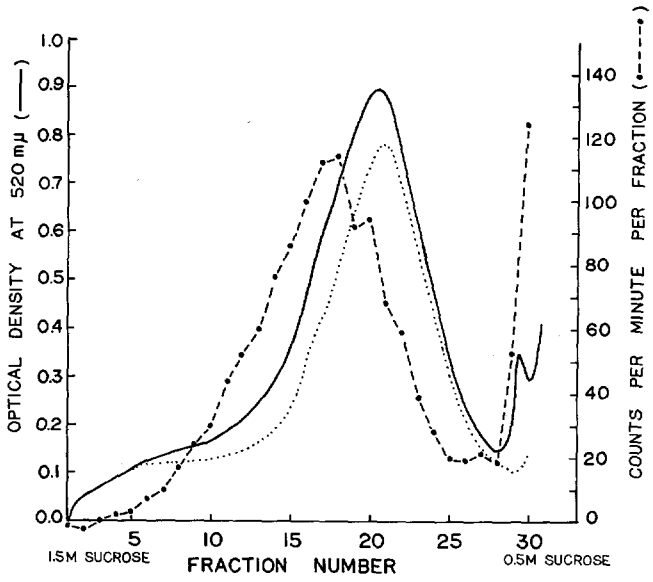

FIGURE 4 Sucrose density gradient analysis of mitochondria co-isolated from a mixture of radioactive liver from cortisone-treated animals and nonradioactive liver from control animals (preparation (*a*) under Methods). The *solid line* again shows the optical density, and the *dashed line* the radioactivity of the effluent. The *dotted line* again shows the optical density after correction for the optical density contributed to the solid line by the radioactive mitochondria (as determined from preparation (*d*)—see legend to Fig. 3). Note that the positions of the dashed and dotted lines are reversed from those shown in Fig. 3; the mitochondria from cortisone-treated animals are again seen to sediment faster than those from control animals.

peak (now contributed by mitochondria from the cortisone-treated rats) moves more rapidly than the optical density peak (due to mitochondria from the normal animals). The absolute positions of the peaks from mitochondria from control and from cortisone-treated animals are, however, the same as in Fig. 3, the mitochondria from cortisone-treated rats again having sedimented 1.4 times as far as the mitochondria from control animals.

DISCUSSION

Calculation of Predicted Sedimentation Velocity of Mitochondria

The rate of sedimentation of a spherical particle[3] of radius r in a centrifugal field in which the sedimenting force is balanced by the force due to the viscous drag of the medium is

$$\frac{dR}{dt} = \frac{\omega^2 R \mu}{6\pi\eta r}, \qquad \text{Equation 1}$$

where ω is the angular velocity of the centrifuge rotor, η is the viscosity of the medium, R is the distance of the particle from the center of the rotor, and μ is the effective mass of the particle. The effective mass of a mitochondrion is equal to its own mass minus the mass of sucrose medium which it displaces. Malamed and Recknagel have shown that mitochondria may be considered to contain two aqueous compartments, one of which is freely accessible to both sucrose and water and one of which is accessible to water but not to sucrose (20). More recently de Duve (11) and Bentzel and Solomon (21) have shown that only part of the water in the sucrose-inaccessible compartment is osmotically active, the rest presumably being "nonsolvent" water of hydration of mitochondrial macromolecules.

The effective mass of a mitochondrion suspended in sucrose solution is determined by the mass of its nondiffusible components (both soluble and insoluble), the mass of the water in the aqueous phase from which sucrose is excluded, and the mass of the external sucrose solution which these components displace. Under these conditions:

$$\mu = V_m[\rho_m - \rho_o] + V_o[\rho_w - \rho_o], \qquad \text{Equation 2}$$

where V_m is the volume per mitochondrion occupied by mitochondrial framework, mitochondrial solutes (other than sucrose), and nonsolvent water, V_o is the volume per mitochondrion of "sucrose-inaccessible" water other than nonsolvent water, ρ_m is the average density of the hydrated nondiffusible mitochondrial components,

[3] Examination of mitochondria by the technique of Baudhuin and Berthet shows a mean axial ratio after isolation in 0.25 M sucrose of 1.1 (18). For axial ratios of less than approximately 3 the frictional coefficients for ellipsoids of revolution are within 10% of those for spheres of equal volume (19).

ρ_w is the density of water, and ρ_o is the density of the surrounding sucrose medium. The second term in Equation 2 is negative and contributes a buoyant force during centrifugation.

Bentzel and Solomon (21) have shown that, per milliliter of mitochondria in 272 milliosomolar sucrose medium, the combined volume of mitochondrial framework, solutes exclusive of sucrose, and nonsolvent water is 0.404 ml, and the volume of osmotically active water is 0.107 ml.[4] Baudhuin and Berthet have recently shown that mitochondria isolated in 0.25 M sucrose have an average volume of 2.9×10^{-13} cm^3 (18). From these data it can be calculated that for mitochondria in 0.25 M sucrose:

$V_m = 0.404 \times 2.9 \times 10^{-13} = 1.17 \times 10^{-13}$ cm^3, and

$V_o = 0.107 \times 2.9 \times 10^{-13} = 0.31 \times 10^{-13}$ cm^3.

Since V_o behaves as an ideal osmometer (21), one may employ the above value to calculate values for V_o over the range of external sucrose concentrations which obtain during the sedimentation velocity determination:

Sucrose	$V_o \times 10^{13}$
M	cm^3
0.50	0.16
0.66	0.12
0.83	0.09

V_m may be taken to be independent of the molarity of the external sucrose solution, and indeed the only compartment of the mitochondrion whose volume is expected to undergo a change with a change in the external sucrose concentration is that which contains that portion of the sucrose-inaccessible water which is osmotically active.[5] It is seen that upon the transfer of mitochondria from 0.25 M sucrose to 0.5 M sucrose V_o decreases by $(0.31-0.16) \times 10^{-13} = 0.15 \times 10^{-13}$ cm^3. In still higher concentrations of sucrose subsequent changes in volume are small, and their effect upon the radius of the mitochondrion is negligible. From these figures one may calculate the radius of the average mitochondrion suspended in sucrose solutions of concentrations between 0.5 M and 0.83 M to be

$$\left(\frac{3}{4\pi} \times 2.8 \times 10^{-13}\right)^{1/3} = 4.1 \times 10^{-5} \text{ cm.}$$

In order to calculate the predicted sedimentation behavior of mitochondria in a sucrose density gradient, Equations 1 and 2 may be combined to yield:

$$\frac{dR}{dt} = \frac{\omega^2}{6\pi r} \cdot \frac{R\{V_m[\rho_m - \rho_o] + V_o[1 - \rho_o]\}}{\eta}.$$

For concentrations of sucrose in which r is very nearly constant (see above), one may replace $\frac{\omega^2}{6\pi r}$ by K and write:

$$\frac{dR}{dt} = K\frac{R\mu}{\eta} = Kq, \qquad \text{Equation 3}$$

where $q = \frac{R\mu}{\eta}$. For a sedimentation experiment in which the movement of the mitochondria is restricted to the upper 1/3 of the centrifuge tube (corresponding to a range of sucrose concentrations from 0.5 M to 0.83 M), one may compute representative values of q as follows:

R[6]	Sucrose	η[7]	$\mu \times 10^{13}$	$q \times 10^{12}$
cm	M	g-cm^{-1}sec^{-1}	g	cm^2-sec
9.4	0.50	0.0265	0.161	5.70
10.5	0.66	0.0329	0.136	4.35
11.6	0.83	0.0420	0.110	3.04

It can be seen that over this range of sucrose concentrations introducing the approximation that q is linear with R leads to a maximal error in q of less than 0.5%. Setting $R_o \equiv$ value of R at $t = 0$, and $s \equiv R - R_o =$ distance moved by a

[4] These data are for mitochondria at 25°C; very similar values for total mitochondrial water (22) and for the distribution of water between sucrose-accessible and sucrose-inaccessible spaces (20) have been observed at 0–4°C, the temperature at which the present studies were performed.

[5] Malamed and Recknagel (20) and, more recently, Bentzel and Solomon (21) have demonstrated that the sucrose-*accessible* water volume remains very nearly constant over a wide range of external sucrose concentrations.

[6] Beckman ultracentrifuge manual.

[7] From values tabulated in de Duve et al. (8).

mitochondrion in time t, one obtains $q = q_o - ks$, where $q_o = 5.70 \times 10^{-12}$ cm²-sec, and $k = 1.21 \times 10^{-12}$ cm-sec. Equation 3 becomes

$$\frac{dR}{dt} = \frac{ds}{dt} = K(q_o - ks),$$

which, when integrated with the boundary condition that $s = 0$ at $t = 0$, yields

$$\ln\left[1 - \frac{ks}{q_o}\right] = -kKt.$$

Substituting the above values for q_o and k and using $K = 1.28 \times 10^8$ cm^{-1}-sec^{-2} (corresponding to a revolution frequency of 3000 min^{-1}), one calculates that, at $t = 75$ min, $s = 2.36$ cm. The observed value for s (see Results, above) was 2.38 ± 0.09 cm.

Many detailed studies of the sedimentation properties of isolated mitochondria have employed techniques in which mitochondria are sedimented to equilibrium in various types of density gradients rather than those in which the velocity of sedimentation is studied (13, 14). This has been due to both the fact that heterogeneity of mitochondrial size leads to a certain "spread" in the sedimenting peak in "velocity experiments" and the difficulty in choosing a satisfactory model for the variation during centrifugation of the size and density of mitochondria because of conflicting reports on their osmotic behavior.[8] A number of studies of mitochondrial sedimentation velocity have, however, been made previously and the results have been analyzed according to different models for the osmotic behavior of mitochondria in sucrose solutions (9–11). The present mathematical treatment of the sedimentation velocity of mitochondria is based upon a recent detailed study of the osmotic behavior of mitochondria by Bentzel and Solomon (21) and, in particular, was made possible by the availability of a useful technique for the determination of the size of mitochondria isolated in sucrose medium (18, 23). The good agreement between the predicted and the observed sedimentation rates of mitochondria reported in the present study is gratifying and, in particular, provides evidence that the electron microscopic technique of Baudhuin and Berthet permits an estimate of mitochondrial size which corresponds closely to that of nonfixed mitochondria suspended in sucrose medium.

[8] See de Duve et al. for an extensive review of the subject (8).

Swelling Versus Generalized Enlargement of Mitochondria in Livers of Cortisone-Treated Rats

Electron micrographs have shown that rat liver mitochondria from both control and cortisone-treated animals after isolation in 0.25 M sucrose are very nearly spherical (4, 18) and that mitochondria from cortisone-treated animals are larger than those from control animals (4). Two extreme possibilities arise as to interpretation of the increase in mitochondrial size associated with cortisone treatment: (*a*) The mitochondria in the livers of cortisone-treated animals are similar in composition to those in normal liver but are "packaged" in larger units; (*b*) the increase in size of mitochondria from cortisone-treated rats reflects an enlargement due only to swelling. In this latter case the mitochondria isolated in 0.25 M sucrose from cortisone-treated animals would be assumed to be larger than normal rat liver mitochondria in 0.25 M sucrose by virtue of having imbibed a greater quantity of water or of sucrose solution.

These two possibilities lead to opposite predictions of the effect of cortisone treatment on the sedimentation behavior of mitochondria as can be shown by the following simple calculation. Consider a sphere of radius r, density ρ, and volume V. In a medium of density ρ_o and viscosity η, and in a centrifugal field G, such a sphere will achieve a sedimentation velocity v given by $6\pi\eta vr = G \cdot \frac{4}{3}\pi r^3 (\rho - \rho_o)$. Similarly, for another, larger sphere of volume V', $6\pi\eta v'r' = G \cdot \frac{4}{3}\pi r'^3 (\rho' - \rho_o)$, and hence

$$\frac{v'r'}{vr} = \frac{r'^3(\rho' - \rho_o)}{r^3(\rho - \rho_o)}. \qquad \text{Equation 4}$$

First consider the case where V' is larger than V by virtue of an increase in total mitochondrial material without a change in relative composition. Under these conditions $\rho' = \rho$, and Equation 4 reduces to

$$\frac{v'}{v} = \left(\frac{r'}{r}\right)^2.$$

In this case the sedimentation velocity of the larger sphere will be *greater* than that of the smaller

one. Next consider the case where V again enlarges to V' but does so only by imbibition of medium. Clearly the net sedimenting force on the mitochondrion does not change. Thus:

$$\tfrac{4}{3}\pi r^3(\rho - \rho_o)G = \tfrac{4}{3}\pi r'^3(\rho' - \rho_o)G, \quad \text{and}$$
$$r^3(\rho - \rho_o) = r'^3(\rho' - \rho_o).$$

Substituting this result in Eq. 4, one obtains that, in this case,

$$\frac{v'}{v} = \frac{r}{r'},$$

and hence that the sedimentation velocity of the larger sphere will be *less* than that of the smaller one.[9] The fact that the observed ratio (1.4—see Results) is greater than 1 shows that the increase in mitochondrial volume reflects at least in part a true increase in the amount of nondiffusible material contained per mitochondrion and rules out the possibility that the observed enlargement is entirely due to mitochondrial swelling.

The size of native mitochondria *in situ* is unknown. Estimates of mitochondrial size, moreover, differ markedly according to the particular techniques of measurement employed (7, 18, 24, 25). Nevertheless, examination of both sections of whole liver and of pellets of isolated mitochondria have led to a similar estimate of a three- to fourfold increase in the volume of the average liver mitochondrion after cortisone treatment. Calculations based on the data of Kimberg et al. (4) show that cortisone treatment resulted in a 3.2-fold increase in the volume of the average mitochondrion as measured in liver sections. If this increase does not involve a change in mitochondrial composition, one would predict that mitochondria from cortisone-treated animals would sediment *faster* than those from control animals by a factor of the order of $(3.2)^{2/3} = 2.2$. The fact that the increase in sedimentation velocity of the mitochondria from cortisone-treated rats is less than what is predicted on the basis of their size and the assumption that their composition is the same as that of normal mitochondria suggests that there may indeed be actual alterations in composition such as changes in water content or changes in relative lipid and protein content. Such changes would not be surprising in view of the striking derangements which are observed when a number of biochemical functions of such mitochondria are examined in vitro (1–5).

The authors would like to thank Miss Elizabeth Tolentino and Miss Elaine Gershon for their skillful technical assistance.

This work was supported in part by United States Public Health Service Grants GM-15022 and AM-07625.

Received for publication 6 November 1969, and in revised form 27 January 1970.

[9] If the mitochondrion enlarges by the imbibition of water alone instead of the sucrose-containing medium in which it is suspended, the ratio v'/v will clearly be still less.

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