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# Myoinositol Uptake by Rat Hepatocytes *In Vitro*

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**Abstract** □ Myoinositol uptake by rat hepatocytes *in vitro* was studied. Adult rat hepatocytes were prepared by digestion of the perfused liver with collagenase. Cell suspensions were incubated with tritium-labeled myoinositol in pH 7.4 Krebs bicarbonate solution containing 1% gelatin at 37°. $^{14}$C-Carbon-labeled polyethylene glycol was used as a marker of adherent extracellular fluid volume. Myoinositol uptake was demonstrable after 5 min of incubation, but no intracellular concentration in excess of that in the incubation medium was observed after 60 min of incubation. Uptake saturation over a wide myoinositol concentration range could not be demonstrated. Neither the omission of sodium ions nor the inclusion of ouabain suppressed the distribution ratio significantly. Metabolic inhibitors and lower temperatures also showed no effect. Hexoses, phlorizin or mannitol, exerted no observable effect on myoinositol uptake. The results indicated that myoinositol uptake by rat hepatocytes is probably a passive process.

**Keyphrases** □ Myoinositol—uptake by hepatocytes, *in vitro*, rats, pharmacokinetics □ Hepatocytes—myoinositol uptake, pharmacokinetics, *in vitro*, rats □ Liver—myoinositol uptake, *in vitro*, pharmacokinetics, rats

The liver plays a predominant role in lipid metabolism. Under normal conditions, the influx of nonesterified fatty acids into the liver from the serum is counterbalanced by the formation of lipoprotein, which is transported back to the blood. The whole process requires myoinositol. In myoinositol-deficient animals, fatty livers often can be observed since the rate of fatty acid transport to the liver from the adipose tissue exceeds the capacity of the liver to mobilize the lipid and to transport it back to the plasma (1, 2). The administration of myoinositol to myoinositol-deficient animals often can alleviate the fatty liver condition.

## BACKGROUND

Myoinositol has long been considered to be a lipotropic agent because of its ability to prevent and remove fat deposits in the liver (3, 4). This lipotropic action is thought to be due to stimulation of hepatic transferable phosphatidylinositol synthesis. Once formed, the phosphorylated myoinositol can be utilized in β-lipoprotein production in the endoplasmic reticulum. The newly synthesized β-lipoprotein is transported from the liver to the blood (4). In addition, exogenous myoinositol inhibits any further deposition of either liver triglycerols or cholesterol and causes the removal of the lipids deposited during the myoinositol-deficiency period (5–7).

Although there is much evidence that myoinositol is transported into the small intestine (8), kidney (9–12), central nervous system (13–16), and crystalline lens (17–19) *via* a specific carrier-mediated system, hepatic uptake of myoinositol has not been studied in spite of its well-known lipotropic activity. Hauser (11) reported that the radioactive myoinositol distribution ratio in liver slices is less than one-half of the radioactivity in the incubation medium, while the hepatic myoinositol concentration *in vivo* is similar to that of plasma; higher hepatic than blood myoinositol concentrations also were observed (20, 21).

In recent years, the use of isolated hepatocytes for various biochemical and pharmacological studies has gained wide acceptance (22). Studies with isolated liver cells can clarify certain molecular aspects of the transport system, such as binding and metabolism, while cellular integrity is maintained.

The purpose of this study was to investigate hepatic myoinositol uptake using liver cells freshly isolated from normal adult rats.

## EXPERIMENTAL

Male rats[1], 200–300 g, were housed in groups in wire-bottomed cages in an air-conditioned room (23°). They received regular laboratory chow and water without restriction.

The methods of liver perfusion and cell isolation were described previously (23, 24). Cell viability was determined by the exclusion of 0.2% trypan blue from the cells and was frequently checked by incubating the cells with α-aminoisobutyric acid, which is actively accumulated by the hepatocytes (23). Cell pellets, following the final wash of the isolation procedure, were resuspended in 10 volumes of pH 7.4 Krebs–Ringer bicarbonate buffer containing 1% gelatin.

Uptake studies were performed by placing 5 ml of the final cell suspension (50–80 mg wet weight/ml of suspension) into 25-ml plastic erlenmeyer flasks. Under control conditions, the cell suspensions contained a trace amount of [2-$^3$H(N)]-myoinositol[2] (3 μCi/nmole/100 ml of suspension) with or without 0.1 μmole of nonlabeled myoinositol/ml. In

[1] Charles River Breeding Laboratory, Wilmington, Mass.
[2] New England Nuclear Corp., Boston, Mass.



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**Table I—Time Course of Myoinositol Uptake as the Distribution Ratio between Intracellular Fluid and Medium [a]**

| Minutes | Nonlabeled Myoinositol (*M*) | |
|---|---|---|
| | 0 | $1 \times 10^{-4}$ |
| 5 | 0.66 ± 0.04 | 0.92 ± 0.08 |
| 15 | 0.83 ± 0.09 | 0.89 ± 0.11 |
| 30 | 0.90 ± 0.09 | 0.96 ± 0.18 |
| 45 | 1.24 ± 0.20 | — |
| 60 | 1.10 ± 0.16 | 1.06 ± 0.04 |
| 90 | 1.14 ± 0.20 | 0.91 ± 0.14 |

[a] Hepatocytes were incubated at 37°. Incubations were terminated at different times. Values are means ± *SD* ($n = 4$).

experiments with a sodium-free medium, tromethamine buffer[3] was used and sodium chloride was replaced by tromethamine chloride (23, 24). The flasks were shaken in a metabolic shaker at 37° under an atmosphere of 95% $O_2$–5% $CO_2$ for various times.

At the end of the incubation period, 1 ml of the cell suspension was removed, placed into a preweighed microcentrifuge tube[4], and centrifuged for 30 sec in a microcentrifuge[5]. The supernate was removed, and the tube was weighed. The cell pellets were resuspended in 150 μl of distilled water, transferred into an 8-ml counting vial, and digested at room temperature with 0.5 ml of tissue solubilizer[6] for 16–24 hr or until a clear solution was obtained. Absolute methanol, 250 μl, was added with mixing, and then 5 ml of scintillation cocktail[7] was added.

An aliquot of the supernate was placed into an 8-ml scintillation vial containing 6 ml of scintillation fluid[8]. The extracellular aqueous volume was determined by incubating hepatocytes in the presence of $^{14}C$-polyethylene glycol[2] for varying times. The $^{14}C$- and $^{3}H$-radioactivity was measured in a liquid scintillation counter[9] using methods for double-isotope counting that correct for quenching and $^{14}C$-activity appearing in the $^{3}H$-channel. Results were corrected on this basis to account for extracellular myoinositol trapping. The distribution ratio ($I/M$) is referred to as the ratio of intracellular to medium (or extracellular) concentrations.

The extent of macromolecular myoinositol binding was estimated by the following procedures. At the end of 5 and 60 min of incubation, 10 ml of cell suspension was centrifuged at 1000 rpm[10] for 1 min; the supernate was removed by pipet. The cell pellets were washed with 10 volumes of ice-cold Krebs bicarbonate buffer, resuspended in an equal volume of 0.05 *M* phosphate buffer (pH 7.2), and sonicated. Then 0.5 ml of the cell extract was placed on a 0.9 × 8-cm column[11], equilibrated with 0.05 *M* phosphate buffer (pH 7.2), and eluted with the same buffer. One-half-milliliter fractions were collected and counted in 6 ml of scintillation fluid.

Formation of myoinositol metabolites during the transport process was tested by TLC using an acetone–water (9:1) solvent system (25). The sonicated solution was deproteinized with five volumes of absolute

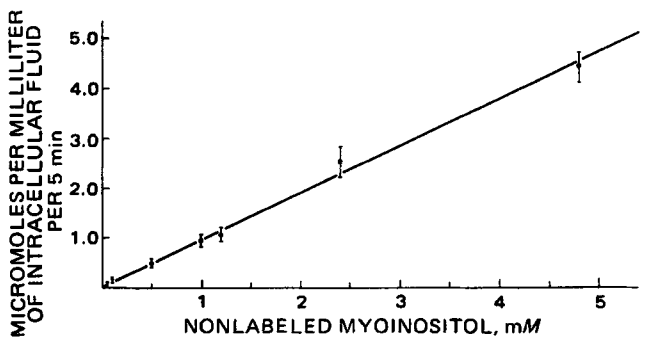


**Figure 1**—*Effect of myoinositol concentration on 5-min in vitro myoinositol uptake into hepatocytes. Data are expressed as means* ± SD. *Each point is the mean of four determinations.*

[3] Tris(hydroxymethyl)aminomethane, Sigma Chemical Co., St. Louis, Mo.
[4] Brinkmann Instruments, Westbury, N.Y.
[5] Centrifuge 3200, Brinkmann Instruments, Westbury, N.Y.
[6] Unisol, Isolab, Akron, Ohio.
[7] Unisol-complement, Isolab, Akron, Ohio.
[8] Scintisol, Isolab, Akron, Ohio.
[9] Model 3375, Packard Instrument Co., Chicago, Ill.
[10] Sorval GLC-1, Dupont Instruments, Newtown, Conn.
[11] Bio-Gel P-2, Bio-Rad Laboratories, Richmond, Calif.

**Table II—Effect of Inhibitors Added *In Vitro* on Myoinositol Uptake [a]**

| Inhibitor | Concentration, *M* | Percent of Control |
|---|---|---|
| None | — | 100.0 ± 6.1 [b] |
| Sodium free | — | 98.0 ± 12.2 |
| Ouabain | $1 \times 10^{-4}$ | 103.3 ± 2.1 |
| 2,4-Dinitrophenol | $1 \times 10^{-4}$ | 107.7 ± 25.5 |
| Potassium cyanide | $1 \times 10^{-3}$ | 95.1 ± 6.1 |
| | $5 \times 10^{-3}$ | 100.0 ± 6.1 |
| *N*-Ethylmaleimide | $1 \times 10^{-4}$ | 93.9 ± 2.4 |

[a] Hepatocytes were incubated at 37° for 30 min in the presence of various inhibitors or the omission of sodium. [b] Each value is expressed as the mean ± *SD* from three to four determinations. All values were not significantly different from the control ($p > 0.05$).

methanol. The methanol solution was concentrated under an air stream and placed on the instant thin-layer plate[12] (5 × 20 cm). After development, the nonsprayed chromatogram was cut into strips (1 cm wide), and the radioactivity of each strip was measured by counting it with 6 ml of scintillation fluid. Authentic myoinositol was cochromatographed with all samples tested.

## RESULTS

When isolated liver cells were incubated under normal conditions with radioactive myoinositol, the initial uptake rate was rapid. Uptake was demonstrable after 5 min of incubation; however, the entry rate decreased over time (Table I). Equilibrium was reached after 30 min of incubation, and further incubation did not increase the intracellular myoinositol concentration. When 0.1 m*M* of nonlabeled myoinositol was added to the incubation medium, the initial entry rate increased significantly while the distribution ratio remained unchanged after prolonged incubation.

The concentration dependence of myoinositol uptake also was investigated. There was no evidence of uptake saturation over a wide myoinositol concentration range (0.1–4.8 m*M*) (Fig. 1).

To test whether hepatic myoinositol uptake is energy dependent, isolated hypatocytes were incubated in the presence of various metabolic inhibitors. Myoinositol uptake was not altered significantly by such inhibitors (Table II).

The specificity of myoinositol uptake by isolated liver cells also was examined. The addition of glucose or galactose (5 m*M*) did not appreciably affect the distribution ratio (Table III). Similarly, phlorizin (0.1 m*M*) or mannitol (5 m*M*) produced no measurable effect on myoinositol uptake.

The extent of myoinositol and tissue binding is depicted in Fig. 2.

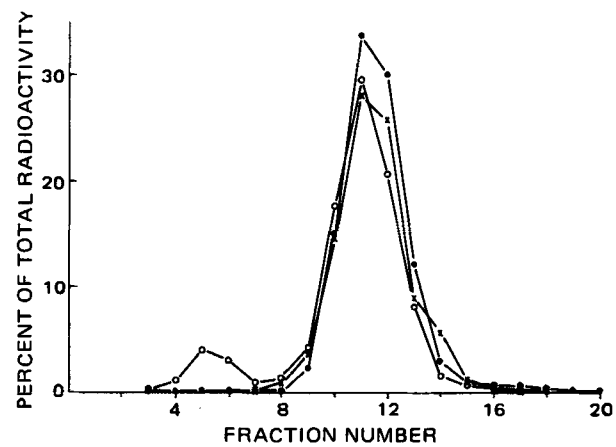


**Figure 2**—*Chromatographic identification of free and bound myoinositol. Radioactivity found in the aqueous hepatocyte extract after 5 and 60 min of incubation with labeled myoinositol was chromatographed on 0.9 × 8-cm Bio-Gel P-2 columns equilibrated with 0.05* M *phosphate buffer (pH 7.2) and eluted with the same solvent. Authentic $^{3}H$-myoinositol was cochromatographed as a control. Key:* ●, *myoinositol standard;* ×, *after 5 min; and* ○, *after 60 min.*

[12] Gelman Instrument Co., Ann Arbor, Mich.

**Table III—Effects of Sugars and Analogs on the Myoinositol Uptake [a]**

| Addition | Concentration, $M$ | $I/M$ [b] | Percent of Control |
|---|---|---|---|
| None | — | 0.91 ± 0.04 | 100.0 ± 4.4 |
| Glucose | $5 \times 10^{-3}$ | 0.95 ± 0.22 | 104.4 ± 24.2 |
| Galactose | $5 \times 10^{-3}$ | 0.79 ± 0.19 | 86.8 ± 20.9 |
| Mannitol | $5 \times 10^{-3}$ | 0.91 ± 0.07 | 100.0 ± 7.7 |
| Phlorizin | $1 \times 10^{-4}$ | 0.81 ± 0.17 | 89.0 ± 18.7 |

[a] Hepatocytes were incubated with $1 \times 10^{-4}$ $M$ myoinositol at 37° for 30 min in the presence of various agents. [b] Distribution ratio between intracellular and medium concentration. Each value is the mean of four determinations with the standard deviation. All values were not significantly different from the control ($p > 0.05$).

Cellular extracts were chromatographed on small columns. Only a small portion of the transported molecules was associated with the cellular macromolecules while most myoinositol existed in a free state. Some metabolic myoinositol alterations apparently took place during the experimental period (Fig. 3).

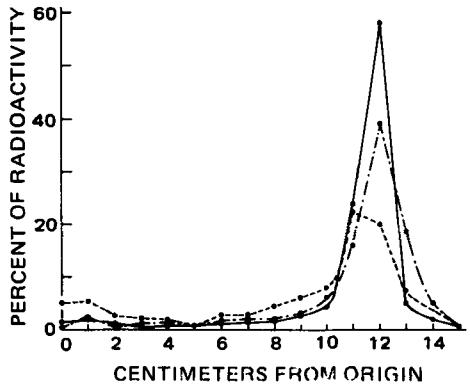


**Figure 3**—*Chromatographic identification of myoinositol and its metabolites. The aqueous hepatocyte extracts after 20 (●---●) and 30 (●---●) min of incubation with labeled myoinositol were chromatographed on thin-layer plates with an acetone–water (9:1) solvent system. Authentic myoinositol was cochromatographed as a control (●—●).*

## DISCUSSION

Although numerous studies demonstrated that isolated rat liver cells can transport various substances into the cellular compartment by a specific carrier-mediated process (22–24), the present study indicated that myoinositol probably enters the hepatocytes by a nonenergy-requiring, noncarrier-mediated process. The nature of hepatic myoinositol uptake appeared to be different from the transport systems for myoinositol in various biological systems (8–19, 26–31).

The failure of hepatocytes to establish a concentration gradient after prolonged incubation demonstrated that hepatic myoinositol uptake is not cumulative. Although demonstrable amounts of intracellular myoinositol were observed after 5 min of incubation, the distribution ratio ($I/M$) rose slowly with time and reached unity after 30 min of incubation. The $I/M$ ratio remained around one throughout the experimental period. Addition of nonlabeled myoinositol (0.1 m$M$) did not change the distribution ratio significantly. The absence of saturation kinetics (Fig. 2) in the present study suggested a passive diffusion process.

Myoinositol uptake also was not dependent on metabolic energy. As shown in Table II, inclusion of an uncoupling agent (2,4-dinitrophenol), a cellular respiration poison (potassium cyanide), or sulfhydryl blocking agents (iodoacetic acid and $N$-ethylmaleimide) in the incubation medium did not cause any significant inhibition. Likewise, ouabain, a "sodium-pump" inhibitor, also showed no effect. Replacement of sodium ions also did not inhibit cellular myoinositol uptake.

The competitive inhibition of hexoses and phlorizin on the transport of myoinositol has been reported in various biological systems (8, 10, 13, 16, 18). The present experiment (Table III), which demonstrated that hepatic myoinositol uptake was not inhibited by monosaccharides or a sugar-carrier inhibitor (phlorizin), also strongly suggests that hepatic myoinositol uptake is not a carrier-mediated process.

Although myoinositol plays an important role in lipoprotein formation in experimental animals (5–7), the present study could not demonstrate significant myoinositol binding to hepatocyte macromolecules, nor was myoinositol metabolized significantly during the experimental periods. There are similar findings in the choroid plexus (13, 16) and the crystalline lens (17).

The results obtained with ouabain, sodium-free medium, metabolic inhibitors, and a lack of saturation all lead to the suggestion that myoinositol uptake into the hepatic parenchymal cells is probably by a passive mechanism.

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