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*Research Article*

# The Effect of Parenterally Administered Cyclodextrins on Cholesterol Levels in the Rat

**Henderik W. Frijlink,[1,4] Anko C. Eissens,[1] Nanco R. Hefting,[1] Klaas Poelstra,[3] Coenraad F. Lerk,[1] and Dirk K. F. Meijer[2]**

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The inclusion complex formation of intravenously administered hydroxypropyl-β-cyclodextrin and β-cyclodextrin with endogenous lipids was studied. We tested the hypothesis that complex formation of endogenous cholesterol with cyclodextrins in the bloodstream leads to extraction of cholesterol from the large lipoprotein particles. The relatively small cholesterol–cyclodextrin complexes then leave the bloodstream via capillary pores, and dissociation of the complex in the extravascular compartment finally causes redistribution of cholesterol from blood to tissue. This hypothesis is supported by the following experimental findings. Intravenous administration of cyclodextrins led to a transient decrease in plasma cholesterol levels in a dose-dependent manner, and *in vitro* cholesterol–cyclodextrin complexes passed dialysis membranes with a molecular weight cutoff of 6000–8000. Further, cyclodextrins increased protein binding of the steroidal drug spironolactone, probably through removal of cholesterol from plasma protein binding sites. Finally, extravascular redistribution was directly demonstrated in histological studies of the kidneys. Glomerular filtration of the cholesterol–cyclodextrin complex is followed by dissociation of the complex in the ultrafiltrate, resulting in cholesterol accumulation in the proximal tubule cells. The cholesterol-β-cyclodextrin complex has a limited aqueous solubility. Crystallization of this complex in renal tissue might explain the nephrotoxicity of parenterally administered β-cyclodextrin. The absence of such crystallization might explain the lower nephrotoxicity of hydroxypropyl-β-cyclodextrin after intravenous administration.

KEY WORDS: β-cyclodextrin; hydroxypropyl-β-cyclodextrin; intravenous administration; cholesterol; cholesterol–cyclodextrin complex; nephrotoxicity.

## INTRODUCTION

This study addresses the complexation behavior of intravenously administered β-cyclodextrin and of hydroxypropyl-β-cyclodextrin and the effects of cyclodextrins on the behavior of the complexed endogenous lipids are described. After intravenous administration in rats β-cyclodextrin and hydroxypropyl-β-cyclodextrin were rapidly distributed over the extracellular fluids and were subsequently eliminated from the body by glomerular filtration (1). The pharmacokinetics of hydroxypropyl-β-cyclodextrins were linear up to a dose of about 200 mg/kg, whereas the elimination rate of β-cyclodextrin decreased when the dose exceeded 100 mg/kg. For both cyclodextrins, over 90% of the dose was recovered unchanged in urine within 24 hr after administration.

Since cyclodextrins are known to form inclusion complexes with many lipophilic molecules (2,3), it is likely that the cyclodextrin molecules form a complex with various endogenous lipids, thereby changing the distribution of these lipids. Carpenter *et al.* (4) intravenously administered hydroxypropyl-β-cyclodextrin to a patient, suffering chronic hypervitaminosis A, thereby improving the patient's situation. Further dimethyl-β-cyclodextrin was shown to increase the toxicity of retinoic acid when administered simultaneously with the retinoic acid (5). However, when administered after the retinoic acid the cyclodextrin decreased toxicity. Frank *et al.* (6) reported the formation of crystals in the kidney after parenteral administration of β-cyclodextrin. Nephrotoxicity has been reported after relatively high parenteral doses of cyclodextrins (6–8), hydroxypropyl-β-cyclodextrin was shown to be less nephrotoxic (9,10). However, none of these studies addressed the complex formation of cyclodextrins with endogenous lipids or its potential link to renal toxicity; this possibility was only mentioned in a recent review (11). The implications of this complex formation for the nephrotoxicity of cyclodextrins were investigated in this study.

[1] Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands.

[2] Department of Pharmacology and Therapeutics, University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands.

[3] Department of Pathology, University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands.

[4] To whom correspondence should be addressed.



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## MATERIALS AND METHODS

### Materials

β-Cyclodextrin was kindly supplied by AVEBE, Veendam, The Netherlands. The 2-hydroxypropyl-β-cyclodextrin was a gift from Prof. Szejtli, Chinoin, Budapest, Hungary. The average molar degree of substitution of the cyclodextrin molecule was 2.7. Sodium 4-(4-hydroxy-1-naphthylazo)-1-naphthalene sulfonate (HNS) was a gift from Dr. Matsui, Shimane University, Japan. The physiological saline used to prepare the injections was sterile and pyrogen free. Heparin was obtained from Leo Pharmaceutical Products, Weesp, The Netherlands. Cholesterol, cholesteryl-oleate, spironolactone, and bovine serum albumin (98%) were obtained from Sigma, St. Louis, MO. All other chemicals used were of analytical grade.

### Instruments

The UV/Vis absorption measurements were performed on a Philips PU 8720 UV/VIS scanning spectrophotometer. Infrared spectroscopy measurements were performed on a Philips PU 9706 infrared spectrophotometer.

The HPLC system consisted of a Perkin–Elmer Series 10 liquid chromatograph and a Waters Model 510 pump, a Promis autosampler, and a Waters Associates Model 441 absorbance detector (254 nm) or a Schoeffel Spectroflow Monitor SF 770 (202 nm).

### Complex Stability Constants

Complexation between the cyclodextrins and cholesterol was studied by the phase-solubility method (12). To an aqueous suspension of cholesterol, increasing amounts of cyclodextrin were added. The suspensions were shaken until equilibrium was reached (8 days, 20 ± 1°C). The solutions were filtered (0.2 μm) and the concentration of dissolved cholesterol was determined by HPLC.

The complex stability constant between the cyclodextrins and cholesterol or cholesteryl-oleate was determined according to the spectral displacement technique (13). Phenolphthalein was used as chromophoric ligand for the determination at pH 10.8 and HNS for the determination at pH 6.4. The method was performed as described previously (14). To prepare solutions of cholesterol and cholesteryl-oleate, methanol was used. The final aqueous solutions contained 4.7% methanol.

### Identification of the Precipitates from Plasma

When β-cyclodextrin was added to rat plasma, at a final concentration exceeding 1.0 mg/ml, the white precipitate formed (at 20 ± 1°C) was isolated by centrifugation and washed several times with ice-cold water and ethanol. TLC was performed on the aqueous solution of this precipitate and on the diethyl-ether extract of this solution, with benzene:ethyl acetate (9:1) and Silicagel $F_{254}$ (Merck, Darmstadt, FRG). To visualize the sterols the plates were sprayed with sulfuric acid (96%) and examined under exposure to UV (254 nm) light (15). Infrared spectroscopy was performed by preparing KBr discs of the dried diethyl-ether extract of the aqueous solution. The ether-wash method described previously (16) was used to confirm true complex formation. The occurrence of β-cyclodextrin in the precipitate was shown by the method of Vikmon (17).

### Protein Binding

Plasma or a 5% bovine serum albumin solution in Krebs-bicarbonate buffer (pH 7.4) was spiked with 6.0 μ*M* spironolactone and various concentrations of β-cyclodextrin (Table II). Spironolactone protein binding was determined by ultrafiltration using the Amicon MPS-1 filtration system (Amicon Corp., Danvers, MA) at 37°C. The spironolactone concentrations in the ultrafiltrate were measured with HPLC.

To 100 μl ultrafiltrate, 2.0 ml citric acid phosphate buffer (pH 4.1) containing 50 ng diazepam (internal standard) and 5.0 ml dichloromethane were added. After mixing and centrifugation the aqueous layer was removed. The tube was placed in liquid nitrogen for 15 sec, and the dichloromethane layer transferred to a centrifuge tube. The solvent was evaporated and the residue dissolved in 200 μl of the mobile phase. A volume of 100 μl was injected onto the column. The mobile phase was acetonitrile:water (35:65) with 1% acetic acid, used at a flow rate of 2.0 ml/min. The analytical column was a Novapack $C_{18}$ reversed-phase column (Waters Associates) used with a Chrompack RP guard column. The effluent was monitored at 254 nm. A calibration graph was prepared by spiking ultrafiltration samples with known amounts of spironolactone.

### Membrane Passage

Plasma was spiked with varying amounts of hydroxypropyl-β-cyclodextrin (Table III). Spectrapor membranes (molecular weight cutoff, 6000–8000) were used to dialyze 10 ml plasma against 60 ml saline for 16 hr. The β-cyclodextrin concentration in the saline was determined according to the method of Vikmon (17) and the total cholesterol concentration was determined by HPLC.

### Cholesterol Analysis

The analysis of cholesterol was based on the HPLC method described by Duncan *et al.* (18). Free cholesterol in plasma was measured by adding 900 μl methanol to 100 μl plasma. After mixing and centrifugation 100 μl of the clear supernatant was injected onto the column.

Total cholesterol was determined after hydrolysis of the cholesteryl-esters. To 50 μl plasma, 1.0 ml of ethanolic potassium hydroxide (6 ml 33% potassium hydroxide in water diluted to 100 ml with absolute ethanol) was added. For the analysis of 2.0 ml urine samples, 6.0 ml of the ethanolic potassium hydroxide and 4.0 ml water were used. The mixtures were incubated at 55°C for 15 min. After cooling to room temperature 1.0 ml water and 4.0 ml petroleum ether 40-60 were added. After vortexing for 30 sec 3.0 ml of the petroleum ether was transferred to a centrifuge tube. The solvent was evaporated and the residue dissolved in 250 μl methanol, of which 50 μl was injected onto the Novapack $C_{18}$ reversed-phase column (Waters Associates), with a Chrompack RP guard column. The mobile phase was acetonitrile:isopropanol (65:35) for free cholesterol determina-

tions and acetonitrile:isopropanol (61:39) for total cholesterol. The effluent was monitored at 202 nm. In every analysis a calibration curve containing known amounts of cholesterol in absolute ethanol was measured simultaneously.

### Cyclodextrin Analysis

The cyclodextrin concentrations were determined by the HPLC method described in earlier publications (1,19).

### Cholesterol Plasma Levels

Male Wistar rats (280–427 g) were used in this study. The jugular vein of the rats was permanently cannulated according to the method described by Steffens (20). The rats were operated at least 1 week before the experiments. The experiments were started between 10 and 11 AM. During the experiment the rats were placed in metabolic cages. The cyclodextrin doses were dissolved in 1.0 ml saline (for the 200 mg/kg β-cyclodextrin dose 1.5 ml was used) and saline was used as reference. The solutions were administered intravenously through the cannula. Blood samples of 250 μl were taken 30, 15, and 1 min before administration, and 15, 30, 60, 90, 120, 150, and 180 min after administration. Blood samples were immediately placed in ice and plasma samples prepared by centrifugation (1800*g*) were stored frozen until analysis. Urine samples were collected in a vessel placed in ice, from 0 to 24 hr after administration. The changes in cholesterol level from that in the first sample ( − 30 min) were compared using Student's *t* test. Differences were considered significant if $P < 0.05$.

### Histological Studies

To male Wistar rats (300–370 g) doses of 200 and 500 mg/kg β-cyclodextrin and hydroxypropyl-β-cyclodextrin, respectively, were administered intravenously (tail vein). The 200 mg/kg administrations were dissolved in 1.5 ml saline; the 500 mg/kg administrations, in 4.0 ml saline. An injection of saline was used as reference. The infusion rate was 1.5 ml/min. The rats were placed in a metabolic cage and the urine was sampled over two periods, 0–24 and 24–48 hr after administration. After 48 hr the rats were killed by a blow on the head. The kidneys were removed and immediately frozen in freon ( − 96°C). Morphological examination of kidneys was performed upon cryostat sections (4 μm), after staining with hematoxylin and eosin. Cholesterol and its esters were demonstrated in these kidneys by staining of 4 μm cryostat sections with sulfuric iodine according to the Okamoto method (21). Briefly, sections were fixed in 4% formalin (10 min) and subsequently covered with 1 ml sulfuric iodine solution (10 ml sulfuric acid, 96%, and 20 ml ethanol, 95%, with 153 m*M* iodine and 84 m*M* potassium iodide). Immediately afterward sections were examined under the light microscope. In an *in vitro* control experiment the cyclodextrins did not react with the sulfuric iodine solution, nor did they interfere with the reaction of cholesterol with the sulfuric iodine solution.

## RESULTS AND DISCUSSION

### Identification and Isolation of the Complexes Formed in Plasma

Cyclodextrin complexes are difficult to isolate because of their high solubility in body fluids and rapid dissociation if extracted with organic solvents. Also, many endogenous lipids may complex with cyclodextrins. Most interesting was the observation that after addition to plasma of β-cyclodextrin to final concentrations exceeding 1.0 mg/ml (up to a concentration of 15.0 mg/ml), white precipitates were formed after approximately 60 min. The presence of β-cyclodextrin in the isolated precipitate was demonstrated by the method of Vikmon (17). Further, the TLC analysis and infrared spectroscopy demonstrated that the diethyl-ether extract, of the aqueous solution of the precipitate, contained cholesterol and cholesteryl esters. The aqueous solution of the complex gave only one spot at the origin of the chromatogram, although the presence of cholesterol in this solution was shown by HPLC. This result suggests that the isolated precipitate was a true cyclodextrin complex, since the β-cyclodextrin complex of cholesterol is insoluble in the organic mobile phase and thus should not elute. True complex formation was also proved with the ether-wash method, in which no cholesterol was dissolved in the diethyl-ether. This is explained by the fact that, without water in the system, the cholesterol–cyclodextrin complex does not dissolve in the apolar diethyl-ether, whereas pure cholesterol should have dissolved. The TLC chromatogram of the aqueous solution after extraction did show only a small spot at the start, which is β-cyclodextrin since this substance is not extracted to the organic layer.

### Complexation of Cyclodextrins with Cholesterol and with Cholesteryl Oleate

The phase-solubility diagrams of cholesterol with β-cyclodextrin and hydroxypropyl-β-cyclodextrin are presented in Fig. 1. For β-cyclodextrin a $B_S$-type diagram (12) was found. In the first part the solubility of the cholesterol is increased, but the solubility of the complex is limited and precipitation occurs at higher β-cyclodextrin concentrations. For the hydroxypropyl-β-cyclodextrin, an $A_p$-type diagram was found. The complex did not precipitate, indicating a large solubility of the cholesterol-hydroxypropyl-β-cyclodextrin complex. The complex stability constants could not be calculated from the phase-solubility diagrams, as described previously (19), because the aqueous solubility of the

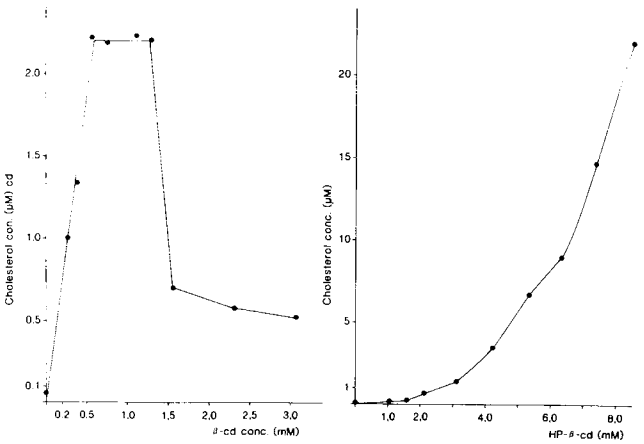


Fig. 1. Phase-solubility diagrams of cholesterol with β-cyclodextrin (left) and hydroxypropyl-β-cyclodextrin (right).

pure cholesterol is very low and its determination is difficult and likely to be disturbed by self-association (22).

Therefore the complex stability constants were determined by the spectral displacement technique (Table I). The stability constants at pH 6.4 of cholesterol with β-cyclodextrin and with hydroxypropyl-β-cyclodextrin are identical. However, at pH 10.8 the stability constant of the β-cyclodextrin complex is much larger. Bergeron (23) pointed out that charge and hydration of the hydroxyl groups of cyclodextrins and of the guest molecules might be of importance for the complex formation. An increase in the complex stability constant at higher pH is associated with a hydroxyl group of the guest molecule placed near the rim of the cyclodextrin cavity. At higher pH, the interaction with the cyclodextrin hydroxyl groups stabilizes the complex. The smaller increase in complex stability constant observed for the complex with hydroxypropyl-β-cyclodextrin may result from the hydroxyl groups being partially hydroxypropylated and consequently unable to interact with the cholesterol hydroxyl group.

The complex stability constants of cholesteryl oleate with both cyclodextrins was taken to be indicative for the complexation of the cholesteryl esters present in plasma. The measurement at pH 10.8 was not performed for the oleate ester because of its instability at this pH. The complex stability constant of cholesteryl oleate with β-cyclodextrin is higher than that of the complex with hydroxypropyl-β-cyclodextrin. Since the hydroxyl group of the cholesterol is placed near the rim of the cyclodextrin molecule, this difference in complex stability could be due to steric hindrance between the oleate chain and the hydroxypropyl groups of the hydroxypropyl-β-cyclodextrin molecule.

### Effect of Cyclodextrins on Protein Binding of Drugs and on Membrane Transport of Plasma Components

Spironolactone and cholesterol have a steroidal structure in common and the drug is known to exhibit considerable plasma protein binding (24,25). The complex stability constant between spironolactone and β-cyclodextrin is 24000 $M^{-1}$ (26). The plasma protein binding of spironolactone was expected to decrease, upon the addition of increasing amounts of β-cyclodextrin to the samples; however, the opposite was observed (Table II). The protein binding of spironolactone, in whole plasma, increased considerably when the cyclodextrin concentration was increased, whereas in albumin solutions the expected decrease in protein binding was observed. This phenomenon might be explained by the fact that cyclodextrin removes cholesterol from plasma proteins thereby increasing the number of binding sites for spironolactone. At the same time, spironolactone is displaced by cholesterol from the cyclodextrins and becomes available for binding to the plasma proteins (the complex stability constants are of the same order of magnitude but the cholesterol concentration is much higher than the spironolactone concentration, resulting in an almost complete displacement).

The results of the membrane passage studies also demonstrated the complexation of hydroxypropyl-β-cyclodextrin with cholesterol and its esters (Table III). In plasma, cholesterol and cholesteryl esters are bound in lipoproteins and in chylomicrons, particles that are too large to pass the dialysis membrane used, while the free cholesterol fraction in plasma (the driving force) is negligibly small. Therefore no cholesterol was detectable in the saline compartment when no cyclodextrin was added to the plasma, as previously reported by Quafordt and Hilderman (27). However, when hydroxypropyl-β-cyclodextrin was added to the plasma, cholesterol concentrations were detectable in the saline. Apparently the cholesterol–cyclodextrin complex is small enough to pass through the membrane pores. The cholesterol concentrations in the aqueous layer are rather small compared with the cyclodextrin concentrations. This is a result of the fact that the degree of complexation is, besides the complex stability constant, also determined by the concentrations of both cyclodextrin and guest molecule. In plasma the cholesterol concentration is high, resulting in a high degree of complexation. Once the complex has entered the saline compartment with a low cholesterol concentration, the complex dissociates and the liberated cholesterol will diffuse back through the membrane to bind again to

**Table II.** The Effect of β-Cyclodextrin on the Protein Binding of Spironolactone (6.0 μ*M*) in Plasma and in 5% BSA Solution; Mean ± SD ($n$ = 4)

| Solvent | β-Cyclodextrin conc. (μ*M*) | Unbound fraction of spironolactone (%) |
|---|---|---|
| Plasma | — | 1.73 ± 0.02 |
| Plasma | 727 | 1.27 ± 0.67 |
| Plasma | 7270 | 0.16 ± 0.04 |
| BSA | — | 8.23 ± 1.16 |
| BSA | 727 | 10.56 ± 4.46 |
| BSA | 7270 | 18.50 ± 2.34 |

**Table I.** Complex Stability Constants ($K_c$) of Cholesterol and Cholesteryl Oleate with Cyclodextrins

| Complex | $K_c$ ($M^{-1}$) pH 6.4 | pH 10.8 |
|---|---|---|
| Cholesterol-β-cyclodextrin | 17,000 | 34,000 |
| Cholesterol-hp-β-cyclodextrin | 19,000 | 23,000 |
| Cholesteryl-oleate-β-cyclodextrin | 16,000 | |
| Cholesteryl-oleate-hp-β-cyclodextrin | 7,500 | |

**Table III.** The Effect of Hydroxypropyl-β-Cyclodextrin on the Passage of Cholesterol Through Dialysis Membranes

| Initial cyclodextrin conc. in plasma (m*M*) | Final cyclodextrin conc. in saline (m*M*) | Final cholesterol conc. in saline (μ*M*) |
|---|---|---|
| — | — | 0.0 |
| 37.8 | 5.4 | 21.3 |
| 75.6 | 10.8 | 35.6 |

lipoproteins or chylomicrons. TLC analysis of the ethereal extract of the saline layer revealed that beside cholesterol no significant amounts of other plasma components were transported through the membrane by the cyclodextrin.

The hypothesis of complexation of cholesterol and of cholesteryl esters by cyclodextrins in plasma is further supported by the following considerations. Among the many lipophilic molecules, forming inclusion complexes with cyclodextrins, steroids are among those with the highest stability constants with β- and with hydroxypropyl-β-cyclodextrin (3,11). Furthermore, of all plasma steroids, cholesterol and cholesteryl esters are generally present at concentrations 1000 to 100,000 times higher than most others. Our results also agree with the observation of Irie *et al.* (28) that *in vitro* cyclodextrins were able to remove cholesterol from the membranes of red blood cells.

### Influence of Cyclodextrins on Cholesterol Levels *in Vivo*

Intravenous cyclodextrin injections were always administered between 10 and 11 AM, because from 10 AM to 3 PM the cholesterol level is most stable in rats (29,30). The inter- and intraindividual variation in cholesterol plasma levels of the rats is rather large. The levels of nonesterified cholesterol and total cholesterol in the blank plasma samples varied between 180–320 μg/ml and 600–950 μg/ml, respectively. Therefore the cholesterol levels in the first sample, taken 30 min before the cyclodextrin injection was used as the control for each animal.

The line of the reference solution (saline) shows a small initial decrease in the nonesterified (free) cholesterol level (Fig. 2). As expected, the free cholesterol levels, found before the administration of β- or hydroxypropyl-β-cyclodextrin (−15 and −1 min), did not differ from the control. However, after administration of cyclodextrins significantly lower levels were measured (Fig. 2). The hydroxypropyl-β-cyclodextrin caused a dose-dependent decrease in the free cholesterol level in plasma. The level at 120 min after administration of the 200 mg/kg dose rather deviated from the entire pattern. An explanation for this deviation was not found and further research is required on this point. It was found that 2 hr after intravenous administration, most of the hydroxypropyl-β-cyclodextrin was excreted in urine (1). In good agreement with this finding, the cholesterol levels in plasma started to return to normal levels at this time.

A 200 mg/kg dose of β-cyclodextrin also caused a significant decrease in the plasma level of nonesterified cholesterol (Fig. 3). The longer duration of the effect than that after hydroxypropyl-β-cyclodextrin, is consistent with the longer half-life of β-cyclodextrin after a 200 mg/kg administration (1). On the other hand, the decrease was significantly smaller than after 200 mg/kg hydroxypropyl-β-cyclodextrin. This difference is probably due to the similar complex stability constants of β-cyclodextrin with cholesterol and with cholesteryl esters, whereas the stability constant of hydroxypropyl-β-cyclodextrin with cholesterol is higher than that with the esters (Table I). Indeed the opposite effect is observed for total cholesterol levels (Fig. 4). As expected, total cholesterol plasma levels were more strongly decreased by β-cyclodextrin than by hydroxypropyl-β-cyclodextrin. The ratio of the decrease in free and esterified cholesterol level, after β-cyclodextrin administration, is 1:2.6, which correlates well with the ratio of the plasma concentrations of free and esterified cholesterol, 1:3.4. This is in good agreement with the fact that the complex stability constants of β-cyclodextrin with cholesterol and cholesteryl esters are similar.

Since after intravenous administration most of the cyclodextrin was excreted in urine (1), it was first assumed that the decrease in plasma cholesterol levels was caused by urinary excretion of the complex. However, the increase in urine cholesterol excretion (micrograms) (Table IV) was too small to account for the decrease in cholesterol plasma levels. Therefore, the decrease in plasma cholesterol effected by the cyclodextrins should be due to a redistribution phenomenon.

In blood, cholesterol and its esters largely occur in lipoproteins and in chylomicrons; there are no significant levels of nonbound cholesterol and cholesterol rapidly exchanges between different lipoproteins and cellular plasma membranes (27,31,32). The main barrier in transport of cho-

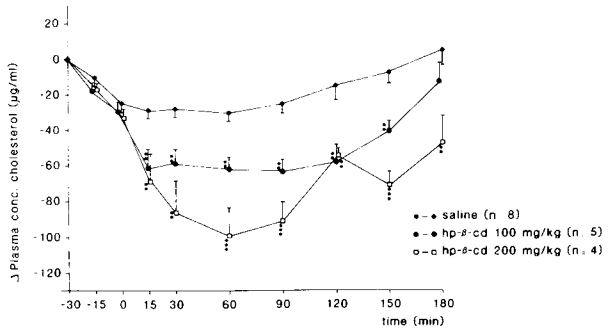


Fig. 2. The effect of intravenous hydroxypropyl-β-cyclodextrin administration on the plasma concentration of free cholesterol. The changes in cholesterol level (mean ± SE) compared with the first sample (time = −30 min) are plotted against time. The injections were given at time = 0 min. (*) $P < 0.05$, compared with the reference (saline). (**) $P < 0.01$, compared with the reference (saline). (***) $P < 0.001$, compared with the reference (saline).

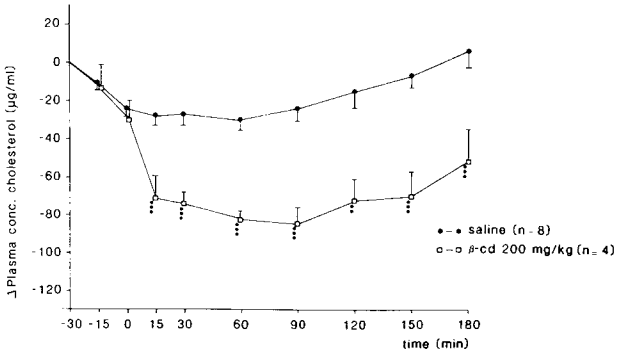


Fig. 3. The effect of intravenous β-cyclodextrin administration on the plasma concentration of free cholesterol. The changes in cholesterol level (mean ± SE) compared with the first sample (time = −30 min) are plotted against time. The injections were given at time = 0 min. (*) $P < 0.05$, compared with the reference (saline). (**) $P < 0.01$, compared with the reference (saline). (***) $P < 0.001$, compared with the reference (saline).

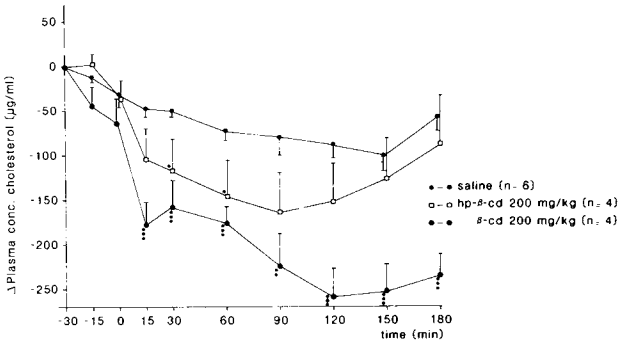


**Fig. 4.** The effect of intravenous cyclodextrin administration on the plasma concentration of total cholesterol. The changes in cholesterol level (mean ± SE) compared with the first sample (time = −30 min) are plotted against time. The injections were given at time = 0 min. (*) $P < 0.05$, compared with the reference (saline). (**) $P < 0.01$, compared with the reference (saline). (***) $P < 0.001$, compared with the reference (saline).

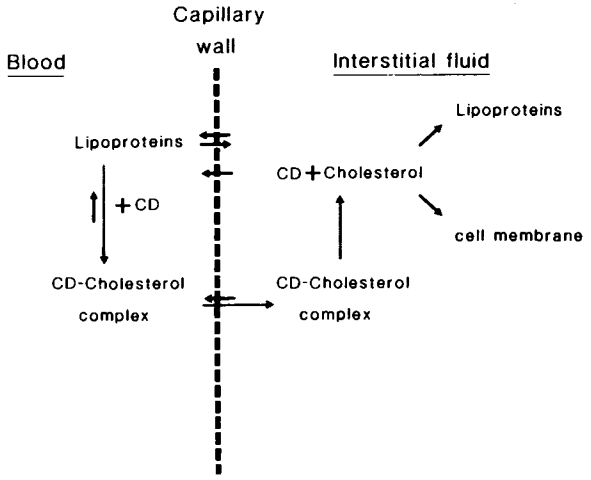


Fig. 5. Suggested model of the mechanism by which cyclodextrins decrease the plasma cholesterol level.

lesterol and its esters from the intravascular to the extravascular compartment is the vascular endothelium. The endothelium is thought to transport low-density lipoproteins (the main carrier of cholesterol: 60–70%) by a transcytosis pathway, whereas receptor-mediated endocytosis of low-density lipoproteins is used for the supply of cholesterol to the cell itself. The transport of lipoproteins through intercellular spaces of junctions of capillary or arterial endothelium into the extravascular compartment is limited (31,33,34). In blood, high concentrations of cholesterol are available; therefore most of the cyclodextrin forms a complex with cholesterol, which can be transported rapidly from the intravascular to the extravascular compartment. In the capillary wall aqueous pores exist which represent sufficient area to provide for very rapid exchange of small molecules (MW <10,000), such as β- and hydroxypropyl-β-cyclodextrin (1) and, simultaneously, is cholesterol complex.

The processes thought to occur after intravenous administration of cyclodextrins are depicted in Fig. 5. The increased transport rate of the cholesterol cyclodextrin complex increases the apparent volume of distribution for cholesterol and explains the decrease in plasma cholesterol levels after cyclodextrin injection. The observed effects of cyclodextrins on plasma cholesterol levels might consequently be described as a redistribution phenomenon, in which the cyclodextrin functions as a carrier for cholesterol and its esters.

**Table IV.** Urinary Cholesterol Excretion in the First 24 hr After the Different Intravenous Cyclodextrin Administrations; Mean ± SD ($n = 4$)

| Administration | Volume of urine (ml) | Cholesterol excreted in urine (μg) |
|---|---|---|
| Saline | 15.2 ± 2.2 | 99.7 ± 25.9 |
| 100 mg/kg hp-β-cyclodextrin | 14.1 ± 2.7 | 171.6 ± 57.0 |
| 200 mg/kg hp-β-cyclodextrin | 14.3 ± 2.3 | 198.8 ± 47.0 |
| 200 mg/kg β-cyclodextrin | 31.4 ± 4.3 | 337.3 ± 123.0 |

## Accumulation of Cholesterol in Renal Tissue and Renal Toxicity

The above-described mechanism also accounts for the small increase in urinary cholesterol excretion after cyclodextrin administration. The cholesterol–cyclodextrin complex can be filtered by the glomerular basement membrane and enter the primary urine. By entering the primary urine the environment of the complex changes from one with a high cholesterol concentration (blood) to one with a low cholesterol concentration (primary urine), leading to a decrease in the driving force for complex formation. The complex dissociates and the free cholesterol enters the cells of the proximal convoluted tubule or the loop of Henle. Since most of the administered cyclodextrin was found to be excreted through the kidney (1), an equivalent amount of cholesterol or cholesteryl esters is delivered in this way to kidney tissue.

To verify this mechanism, histological studies were performed. Over 89% of the administered cyclodextrin was excreted into the urine within 48 hr, except for the 500 mg/kg β-cyclodextrin dose, of which only 48% was recovered in urine. In the kidneys only trace amounts of cyclodextrin were recovered (<3%) except for the 500 mg/kg β-cyclodextrin dose, of which 14% was recovered in the kidneys.

No kidney damage could be observed in cryostat sections of saline-treated rats, after staining with hematoxylin and eosin. In contrast, rats receiving β-cyclodextrin demonstrated, 48 hr after administration, severe kidney damage at the light microscopical level, related to the administered dose. Although glomeruli appeared normal, many of the tubuli were diluted with a disrupted cellular structure, blurred cell nuclei, and large cytoplasmatic vacuoles. These observations agree with the observations of Frank *et al.* (6). In contrast, the administration of hydroxypropyl-β-cyclodextrin did not cause severe kidney damage; only an increase in cytoplasmatic vacuoles was observed in the tubuli. Figures 6, 7, and 8 show kidney cryostat sections stained with sulfuric iodine. The dark reaction products indicate the presence of cholesterol or its esters. As demonstrated in Fig. 7 dark intracellular staining can be observed in tubuli of β-cyclodextrin-injected rats, whereas only slight cholesterol

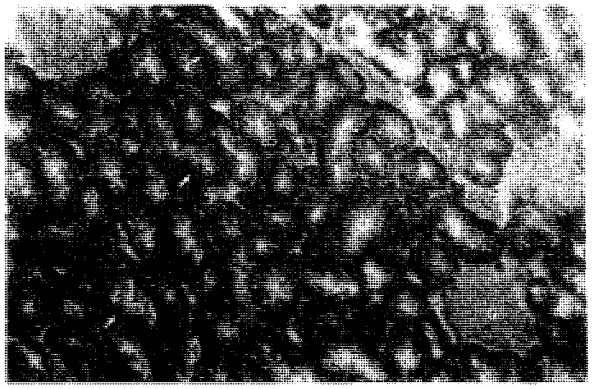

Fig. 6. Kidney cryostat section of saline-treated rat, stained for cholesterol and cholesteryl esters according to the Okamoto method. A slight dark staining predominantly along the basal site of the tubuli (arrows) can be observed. G = glomerulus. (40x; reduced 45% for reproduction).

staining is observed in tubuli of saline treated rats (Fig. 6). Increased amounts of cholesterol were also observed in the loop of Henle in β-cyclodextrin-injected rats. In contrast, rats receiving hydroxypropyl-β-cyclodextrin showed less tubular staining for cholesterol and its esters as compared with β-cyclodextrin-treated rats, although increased staining for cholesterol was observed in these kidneys as compared with saline-treated rats. The increase in cholesterol staining appeared to be related to the administered dose of both β-cyclodextrin and hydroxypropyl-β-cyclodextrin.

The observed damage to the kidney tissue might explain the prolonged elimination half-life after intravenous administration of 200 mg/kg β-cyclodextrin found earlier (1). In electron microscopic studies Frank *et al.* (6) found microcrystals in the proximal tubules after administration of β-cyclodextrin. The occurrence of these crystals was directly related to the nephrotoxicity observed. The authors assumed these crystals to be pure cyclodextrin since their occurrence was dose dependent (6). However, considering the above described accumulation of cholesterol and the low solubility of the cholesterol-β-cyclodextrin complex, these crystals are more likely to consist of complexes formed be-

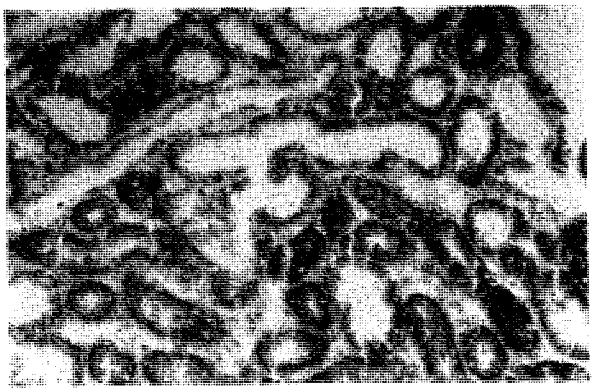

Fig. 7. Kidney cryostat section, stained for cholesterol and cholesteryl esters according to the Okamoto method, 48 hr after administration of 500 mg/kg β-cyclodextrin. Note the considerable increase in tubular staining in these kidneys as compared with Fig. 6. (40x; reduced 45% for reproduction).

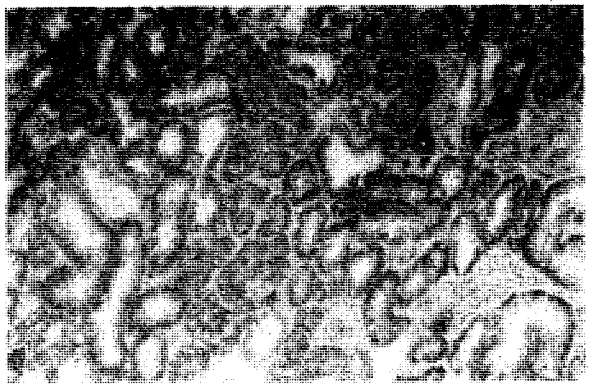

Fig. 8. Kidney cryostat, section stained for cholesterol and its esters according to the Okamoto method, 48 hr after administration of 500 mg/kg hydroxypropyl-β-cyclodextrin. Tubular staining for cholesterol is increased as compared with saline-treated rats (Fig. 6) but less staining is observed compared with β-cyclodextrin-treated rats (Fig. 7). G = glomerulus. (40x; reduced 45% for reproduction).

tween the cholesterol or cholesteryl esters and the small amount of β-cyclodextrin which remains in the kidney. The much lesser nephrotoxicity of hydroxypropyl-β-cyclodextrin as compared with β-cyclodextrin described earlier (9,10) can also be explained now, by the lower tendency of cholesterol-hydroxypropyl-β-cyclodextrin complex to crystallize. Both cyclodextrins carry cholesterol to the kidney but only β-cyclodextrin forms insoluble complexes with it (Fig. 1), and it is these intracellular crystals that are the main cause of cellular damage in the kidney.

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