GAMMA-CAMERA STUDY OF THE HEPATO-BILIARY EXCRETION OF ^{131}I-THYROXINE-GLUCURONIDE AND ^{131}I-ROSE BENGAL IN THE RAT

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The hepatic uptake and biliary excretion of ^{131}I-rose bengal and ^{131}I-T_4-glucuronide in the rat were studied using a gamma camera. For rose bengal, peak hepatic concentration was reached within 10 min of injection, the hepatic disappearance half-time was 39 min, and 55% of the dose was excreted in the bile at 1 hr. For T_4-glucuronide, these values were 5 min, 13 min and 70%, respectively. The liver seemed to handle T_4-glucuronide similarly to rose bengal, but three times faster.

A similar study of ^{131}I-T_4 kinetics was unsatisfactory because of high level of vascular activity.

A major pathway of thyroxine (T_4) metabolism is its conjugation to glucuronic acid in the liver and excretion in the bile (*1,2*). In general glucuronides are more extensively excreted in the bile than the parent compounds (*3*) and a recent report from this laboratory showed that in the rat intravenously administered T_4-glucuronide was rapidly and almost completely excreted in the bile (*4*).

The present study describes the hepatic uptake and release and biliary excretion of ^{131}I-labeled T_4-glucuronide in rats, using a gamma camera and digital computer and compares these data with those obtained using ^{131}I-labeled rose bengal, a halogenated fluorescein derivative, which is known to be extensively excreted in the bile (*3,5,6*). The labeled compounds will henceforth be called T_4-glucuronide, T_4, and rose bengal. Both rose bengal (*3,5*) and T_4-glucuronide (*4*) are excreted unchanged in the bile.

MATERIALS AND METHODS

Male Sprague-Dawley rats*, weighing 280–300 gm, were maintained on a modified diet 41 B. Iodine-131-labeled L-T_4 (30.7 mCi/mg) and rose bengal (67.8 μCi/mg) were obtained from the Radiochemical Centre, Buckinghamshire, England.

Preparation of T_4-glucuronide. Three rats were anesthetized with pentobarbital 60 mg/kg intraperitoneally. Tracheostomy was performed and the common bile duct of each was cannulated with a 20-cm length of polyethylene tubing with an internal diameter of 0.5 mm (PP 30, Portex Ltd., Hythe, Kent). The animals were kept warm by a 60-watt light bulb 30 cm overhead. Each rat then received 300 μCi (9.8 μg) T_4 into a jugular vein. Bile was collected for 6 hr and was applied in multiple 0.1-ml aliquots to Whatman No. 3 filter paper. Ascending chromatograms were developed in 1-butanol:1,4-dioxan:2 *N* NH_4OH (4:1:5) for 18 hr. Autoradiographs were prepared on Kodak Kodirex x-ray film. The middle of the main heavy band in the glucuronide region was eluted with 100 ml 0.02 *N* NH_4OH. The eluate was lyophilized and the residue dissolved in 1.8 ml 0.9% NaCl. Sixty μCi were recovered. Since the amount of administered T_4 was far in excess of endogenous circulating T_4 (serum PBI of six rats was 2.7 $\pm$ 0.12 μg/100 ml; mean $\pm$ sem), the specific activity of the eluate was taken as that of the administered T_4. Rechromatography showed a single homogeneous radioactive spot and two-dimensional chromatography first for 18 hr in the above solvent and then for 30 hr in tertiary amyl alcohol saturated with 2 *N* NH_4OH (*7*) showed that 92% of the activity was present in one dense spot. No attempt was made to identify three minor contaminants. Similarly prepared ^{125}I-T_4-glucuronide was almost completely hydrolyzed by bovine liver β-glucuronidase with the appearance of material having the same R_f as free T_4; 1,4-saccharolactone, a

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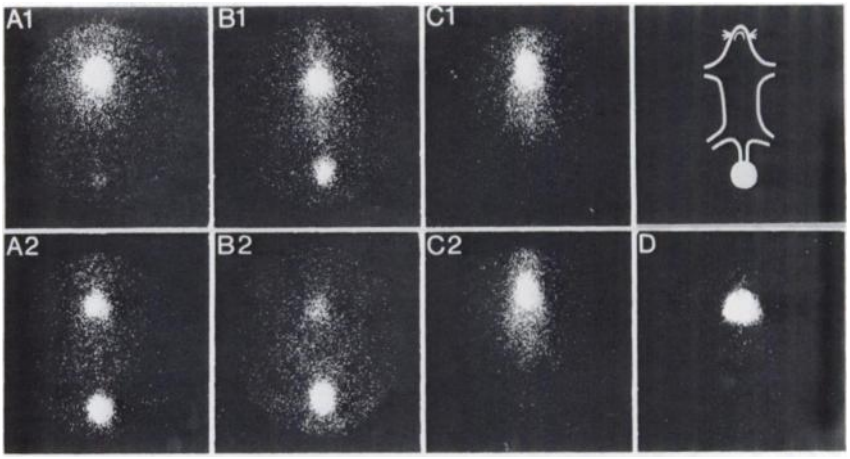

FIG. 1. Polaroid images of distribution of ^{131}I-rose bengal (A), ^{131}I-T_4-glucuronide (B), ^{131}I-T_4 (C), and ^{99m}Tc-sulfur colloid (D). Upper row (1) were obtained 9 min and lower row (2) 60 min after injection. Biliary activity is shown in lower half of each image. Outline of rat and bile collection tube is shown in upper right panel.

specific inhibitor of β-glucuronidase activity, completely inhibited the reaction (*4*).

Gamma camera and computer operation. Each rat was studied individually. About 30 min after tracheostomy and bile duct cannulation, the rat was given another 40 mg/kg of pentobarbital subcutaneously and was pinned onto a shoe-box with bile draining into a tube at the end of the box. Fifteen microcuries of T_4-glucuronide (0.72 mμmoles, equivalent to 0.7 μg T_4), or rose bengal (22.8 mμmoles), 22 μg, or 50 μCi T_4 (2.75 mμmoles, 2.14 μg) was injected into a jugular vein and the animal was immediately placed under the gamma camera. Two rats were studied with each compound.

The gamma camera* was linked to a dedicated digital computer†. Data acquisition started at the moment of injection. The activity distribution during 110 sec was stored in one 4K of the computer's 8K memory using the data break facility. At the end of each acquisition period the integrated activity distribution was displayed on a monitor screen and stored on magnetic tape and the 4K of memory was cleared for the acquisition of the new distribution. These procedures took 10 sec so that data acquisition started at 2-min intervals. Acquisition was stopped about 70 min after injection. Polaroid pictures of the isotope distribution were occasionally taken; exposures of 10,000 counts were used.

After each study a phantom containing a duplicate sample of the dose was placed in a similar position below the camera and three activity distributions were acquired. The field uniformity was then determined by placing about 1 mCi of ^{87m}Sr in a disk of uniform thickness on the face of the camera. The apparent distribution of activity was acquired for about 5 min and was used to compute the factors to correct for any spatial nonuniformity of response. These data were stored on the magnetic tape for use during analysis.

TABLE 1. SUMMARY OF RESULTS

		Rose bengal		T_4-glucuronide	
		1	2	1	2
	Peak (% dose)	89	79	68	69
Hepatic activity	Time to peak (min)	10	8	3	4.5
	Disappearance half-time (min)	39.5	38.5	16.5	11.0
Biliary excretion at 60 min	(% dose)	55	55	65	75
Liver blood volume	(% total blood volume)	46	47	48	41

For analysis the activity distributions were successively displayed on the monitor screen until the hepatic and biliary areas had become clearly visible. A cursor spot was then used to outline rectangular fields covering these areas and usually an additional area in the lower abdomen, representing vascular activity, for quantitative analysis. The activity distributions were next analyzed in their order of acquisition, firstly by correcting each distribution for any nonuniform camera response and then by computing the number of counts in the areas selected. The sequential counts in each area were listed on a teletype. The mean activity in the phantom and in a background area were similarly analyzed. After background subtraction the counts in each area were expressed as a percentage of the dose given.

The main problem in the analysis of the data was the delineation of the liver. When T_4 was used, ac-

* Nuclear Enterprises Mk IV scinticamera.

† Digital Equipment Corp. PDP8-I.

tivity in the heart and major vessels was high and clear visual separation of the liver was impossible. However, rose bengal and T_4-glucuronide were rapidly taken up by the liver and the apparent hepatic distribution of ^{131}I coincided with that of 50 μCi ^{99m}Tc-sulfur colloid injected intravenously 30 min before the rat was placed underneath the camera. The accuracy of the hepatic uptake values was still limited because only a rectangular area, which would include some vascular activity, could be used to outline the irregular shape of the liver with our present equipment.

At the end of each study the bile volume was measured and an aliquot of it and of the dose administered were assayed in a well scintillation counter. The fraction of the dose excreted was compared to the value obtained from the computer analysis. Since the tube containing bile was 10–12 cm below the rat, it was necessary to apply a factor of 1.1 to the computer values to correct for geometrical effects.

RESULTS

Polaroid images of the distribution of activity 9 and 60 min after injection are shown in Fig. 1. For rose bengal (Fig. 1A) and T_4-glucuronide (Fig. 1B), concentrations of activity in both the liver and bile collection vessel were clearly visible. Comparison of A1 and B1 at 9 min and A2 and B2 at 60 min shows qualitatively that T_4-glucuronide was handled more rapidly by the liver than rose bengal. When T_4 was given, however, the whole rat was visible and the "liver area" extended upwards to include the heart and great vessels, most likely because of a higher level of blood activity (see Fig. 1C). Also no activity was detectable in the bile collection vessel. For comparison, the liver of the same rat, given ^{99m}Tc-sulfur colloid 30 min previously, is shown. This clearly indicates that the region of high activity in C1 and C2 was due to both hepatic and cardiovascular activity and that the two were not separable. The ^{99m}Tc activity, however, closely matched the presumed liver areas in rats given T_4-glucuronide or rose bengal. Thus the rate of handling of T_4 was much slower than that of T_4-glucuronide or rose bengal.

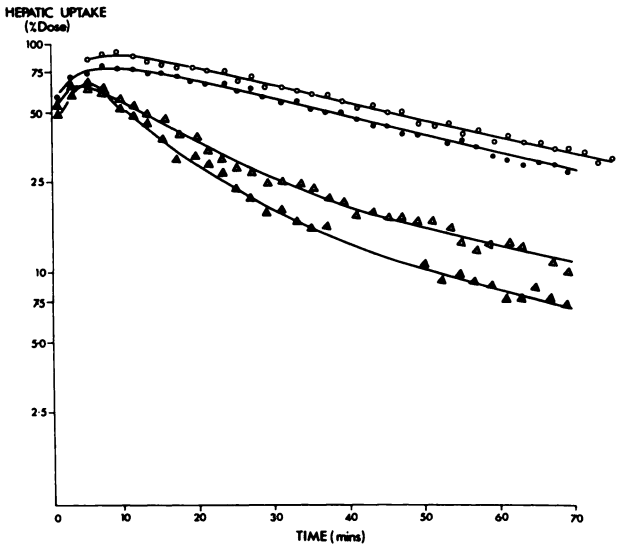

FIG. 2. Hepatic uptake of ^{131}I-rose bengal (o, •) and ^{131}I-T_4-glucuronide (△,▲). Missing T_4-glucuronide data in one rat were due to technical fault in computer.

Qualitative analysis of hepatic and bile activity was feasible with T_4-glucuronide and rose bengal and the change in hepatic activity with time is shown in Fig. 2. With rose bengal the hepatic activity reached a peak (89 and 79% of the dose) at 8–10 min and then fell monoexponentially with disappearance half times of 38.5 and 39.5 min (Table 1). The best-fit regression lines relating the logarithm of percent dose in the liver to time were determined by the method of least squares ($r = 0.997$ and 0.996). With T_4-glucuronide, peak hepatic activity (68 and 69%) was reached within 5 min and the disappearance curves were more complex. Comparative analysis with exponential, biexponential, and power law functions showed that they could best be described by an exponential variation of the form

$$(\text{uptake} - K) = Ce^{-\lambda t},$$

where t is the time following the peak activity and K, C, and λ are constants. For both curves, when K was taken as 7, i.e., when there was a constant contribution of 7% to the uptake curve, the fall in hepatic activity was monoexponential. Figure 3 shows the best-fit regression lines obtained by the method of least squares, using this relationship; disappearance half-times were 11.0 and 16.5 min ($r = 0.998$ and 0.995; Table 1).

The biliary activities are shown in Fig. 4. Those for rose bengal increased approximately linearly, 55% being excreted at 1 hr. Those for T_4-glucuronide rose with time toward asymptotic values, 75 and 65% being excreted at 1 hr.

The changes in the activity distributions of T_4 were not analyzed in detail because it was impossible to isolate the hepatic activity. However, it is perhaps worth noting that the activity in the combined cardiovascular and hepatic regions declined monoexponentially with disappearance half-times of 110 and 230 min; 4.2 and 1.0% of the dose was excreted in the bile at 1 hr.

DISCUSSION

The present study shows that the gamma camera may usefully be applied to the study of the kinetics

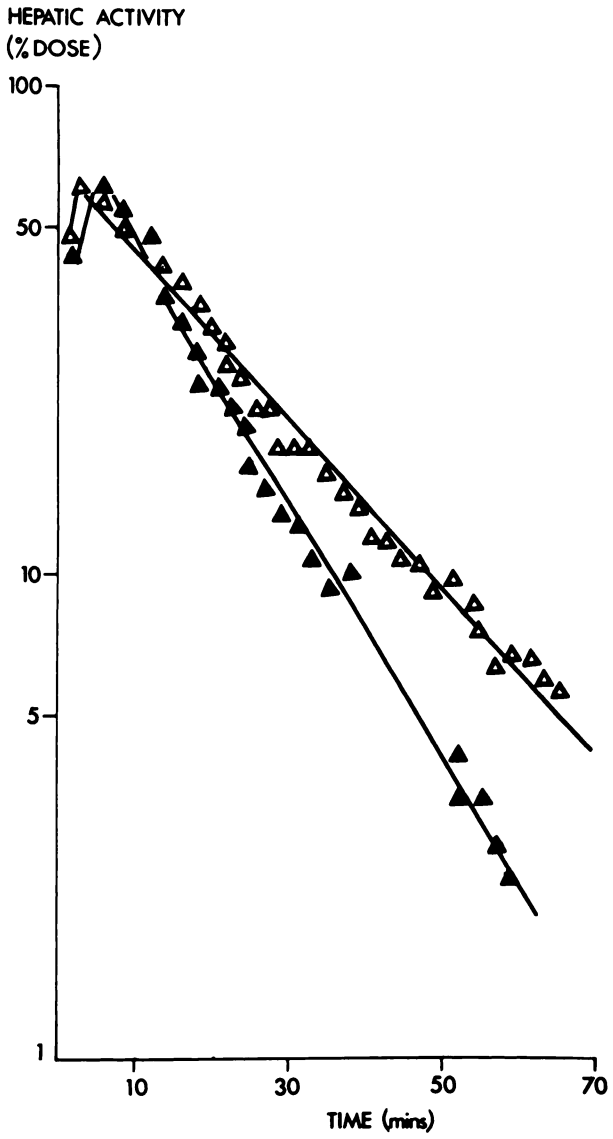

FIG. 3. Hepatic uptake of T_4-glucuronide after subtraction of constant contribution of 7% from data shown in Fig. 2.

of labeled materials in small animals. The principal limitation is separation of anatomical sites of interest. If two or more organs, each with activity, overlap, the technique may not be useful. This situation occurred in the T_4 studies, where the cardiovascular and hepatic activities were not separable.

Our data with rose bengal agree well with those obtained by Meurman (*5*). He observed a peak hepatic uptake of 77–80% of the dose at 8–12 min, a biliary excretion of 59% at 60 min and an hepatic disappearance half-time of 38.5 min, all similar to our values (cf., Table 1). In Meurman's experiments the hepatogram curve flattened after about 70 min with a new disappearance half-time of 53 min (*5*). This was attributed to reflux of rose bengal from liver to blood and could be explained by a model in which the liver has bound and unbound compartments, the latter being in equilibrium with the blood. The model predicts that the blood disappearance curve should be biexponential and the hepatogram triexponential. Meurman's results (*5*) agree with these predictions and, although a change in the hepatogram slope was not apparent during the 70 min of our observations, our data agree closely with Meurman's (*5*). The same model was used by Turco, et al (*6*) to explain rose bengal kinetics in normal man and in patients with Laennec's cirrhosis.

The hepatograms obtained with T_4-glucuronide could not be described by a monoexponential slope but by a monoexponential decay superimposed on a constant level of activity in the liver. However, they could also be explained by the presence of slowly changing activity in addition to the rapidly changing activity that was observed. The hepatic uptake curves of T_4-glucuronide (Fig. 2) agreed remarkably with those of rose bengal obtained by Meurman (*5*) over a longer period, if the time scale of the latter were divided by 3, suggesting that this hypothesis might be valid. Thus it would appear that T_4-glucuronide was handled similarly to rose bengal by the liver, but 3 times faster; initial hepatic disappearance half-

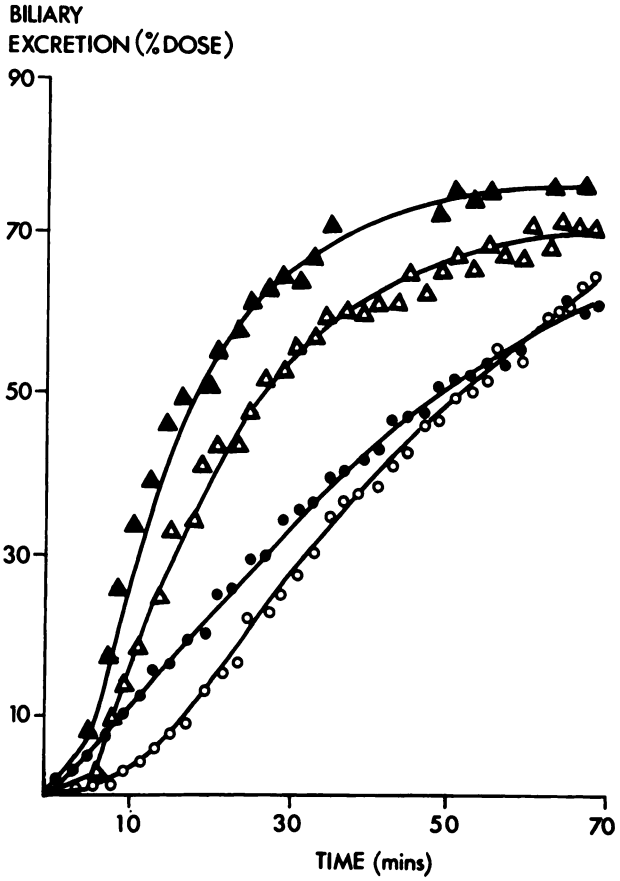

FIG. 4. Biliary excretion of ^{131}I-rose bengal (o, •) and T_4-glucuronide (△,▲).

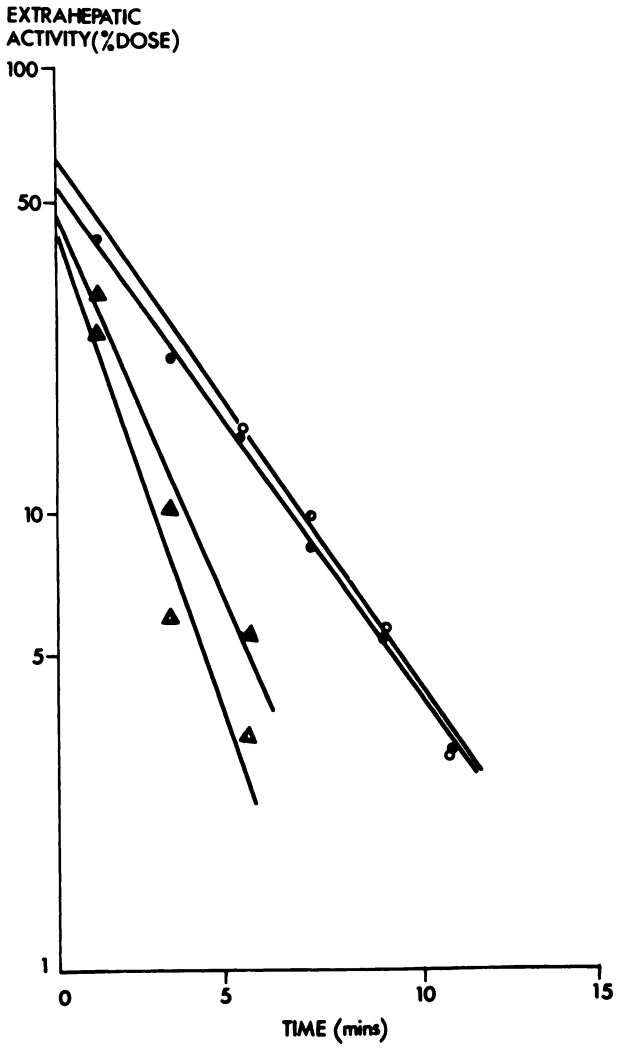

FIG. 5. Extrahepatic activity following injection of ^{131}I-rose bengal (o, •) and ^{131}I-T_4-glucuronide (△,▲). Points were obtained by subtracting ascending portion of hepatic uptake curve from descending portion extrapolated back to 0 time.

times were 13 min for T_4-glucuronide and 39 min for rose bengal.

The fractional liver blood volumes, determined by analysis of the hepatogram curves according to Lowenstein (*8*), were similar whether rose bengal or T_4-glucuronide was used. The monoexponential hepatic disappearance curves were extrapolated back to 0 time, the intercept on the ordinate, E_0, representing the total dose given. Subtraction of the total hepatic activity, before it reached a peak, from this line gave a curve depicting the change in extrahepatic activity with time. The best-fit straight lines obtained by the method of least squares for the change in extrahepatic activity of rose bengal and T_4-glucuronide are shown in Fig. 5. When these lines were extrapolated back to 0 time, the intercept on the ordinate, U_0, represented extrahepatic activity at the moment of injection. E_0-U_0 denoted hepatic activity

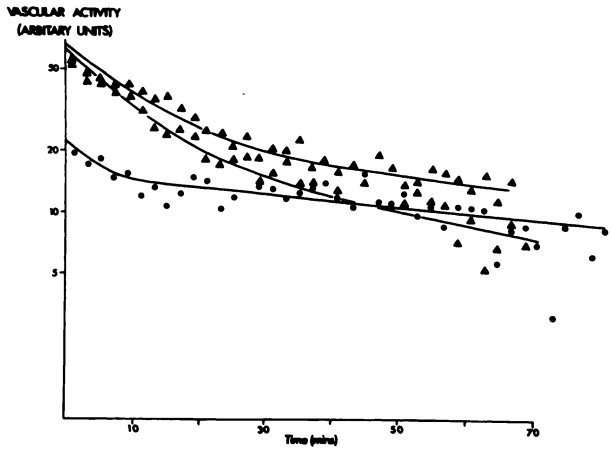

FIG. 6. Vascular activity in lower abdominal region following injection of ^{131}I-rose bengal (•) and ^{131}I-T_4-glucuronide (△,▲).

at the instant of injection before hepatic cellular uptake could have taken place and therefore represented hepatic vascular activity. Hence $(E_0-U_0)/E_0$ corresponded to the liver blood volume as a fraction of total blood volume (*8*). Values of 46 and 47% were obtained with rose bengal and of 48 and 41% with T_4-glucuronide (Table 1).

O'Reilly, et al (*9*) have proposed a different model to explain the kinetics of the biliary excretion of the acidic azo dye, amaranth. In this model reflux occurred between the bound and unbound compartments in the liver but not between the liver and blood. This resulted in a monoexponential blood disappearance curve and a triexponential hepatogram. Since the blood disappearance curve of rose bengal was biexponential (*5*), this model could not describe rose bengal kinetics. Figure 6 shows the change in blood activity in the two T_4-glucuronide studies and in one of the rose bengal studies obtained by quantitative analysis of an area in the lower abdomen of each rat. Although these curves are not accurate, it is clear that none were monoexponential and that the T_4-glucuronide and rose bengal curves were roughly the same. This would seem to rule out the possibility that the model, which explained amaranth kinetics (*9*), was applicable to T_4-glucuronide. We therefore suggest that the liver eliminates T_4-glucuronide into the bile in the same way as it dose rose bengal but 3 times faster.

The rapid hepatic elimination of T_4-glucuronide may be important physiologically. A recent report (*10*) showed that in the rat enterally administered T_4 was excreted in the bile, mostly as T_4-glucuronide with little entering the systemic circulation. It seems likely that T_4 in the bowel is absorbed as a glucuronic acid conjugate in vitro as shown by Herz, et al (*11*) and is then rapidly excreted into the bile.

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