METABOLISM AND PHARMACOKINETICS OF HYDROXYETHYLATED RUTOSIDES IN ANIMALS AND MAN

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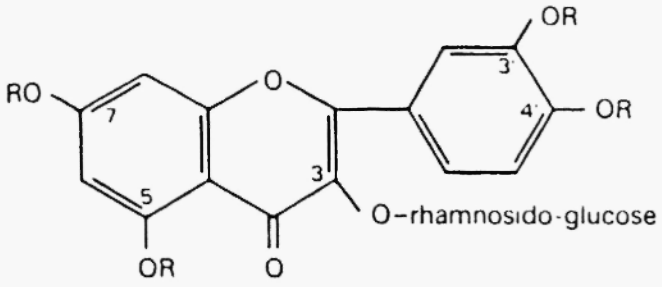
I. INTRODUCTION

Hydroxyethylated rutosides (HRs)* employed in the treatment of vascular disorders /1-3/ are derivatives of the naturally occurring flavonoid, rutin (Fig. 1). Their main pharmacological action has been shown to be on capillary permeability /4-6/. Their general properties have been reviewed previously /7-9/.



R = -H or $-CH_2-CH_2-OH$

Figure 1. Basic chemical formula of HRs. 3'-mono-HR cannot easily be synthesized, accordingly, 7-mono-HR only was available for metabolic studies.

The metabolism of flavonoids in mammals has recently been reviewed /10/. The purpose of the present monograph is a comprehensive presentation of the available data on the metabolism and the kinetics of HRs in animals and man.

The reason why so many studies have been performed in so many different experimental conditions is the fact that, based on the results obtained with rutin, the efficacy of the oral route for the administration of bioflavonoids has been questioned /11,12/. For HRs, it has been argued that their high molecular weight and their low liposolubility would strongly hinder their absorption from the gastrointestinal tract.

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* HRs are the active principles of Venoruton®, Paroven®, Relvene®

Many observations, however, show that HRs are able to exert pharmacological effects after oral administration and it is thus important to consider to what extent pharmacokinetic data support these findings.

II. SUBSTANCE

HRs are not a single substance, but a standardized mixture of hydroxyethylated rutosides (Fig. 1), the composition of which is shown in Table I. They can be administered both orally and intravenously. They will be termed HRs for the present comprehensive summary of their pharmacokinetics and metabolism.

TABLE 1. Composition of HRs as measured by gas chromatography. (This method is based on the separation of silylised aglycones)

7,3′,4′-tri-hydroxyethyl-rutoside	50.1 %	tri-HR
7,4′-di-hydroxyethyl-rutoside	21.9%	di-HR
7,3′-di-hydroxyethyl-rutoside	11.7 %	
3′-mono-hydroxyethyl-rutoside	7.3 %	
5,7,3′,4′-tetra-hydroxyethyl-rutoside	5.1 %	tetra-HR
3,7,3′,4′-tetra-hydroxyethyl-quercetin	3.4 %	
rutin	0.5%	
7-mono-hydroxyethyl-rutoside	traces	mono-HR

III. ANALYTICAL METHODS USED FOR THE DETERMINATION OF THE CONSTITUENTS OF HRs IN BIOLOGICAL FLUIDS

Over the years, a considerable number of analytical methods have been developed to measure blood or urinary concentrations of HRs.

(a) Methods for the quality control of HRs

Many methods useful for monitoring of the pure compounds or their pharmaceutical forms are available for HRs; they permit the separation and identification of the main and secondary components of HRs. Their sensitivity, however, has been found unsatisfactory for biological fluids. They are:

– Thin layer chromatography
– Gas-liquid chromatography with flame ionization detection
– High performance liquid chromatography.

(b) Methods for biological fluids

Many investigations of HRs were performed using ^{14}C-labelled compounds, combining different chromatographic separation procedures. The label was incorporated during the hydroxyethylation of unlabelled rutin. The label, on the hydroxyethyl side chain, has been shown to be stable both *in vitro* and *in vivo*.

The methods developed for the determination of unlabelled components and metabolites of HRs are:

– *Thin layer chromatography.* This method was used by Wienert and Gahlen /13/ for the analysis of urine samples. It was also used for serum and urine by Förster /14/.

– *Circular dchroism.* Such a method was developed by Jung and Voelter /15,16/. The method was neither specific nor sensitive enough to allow the determination of pharmacokinetic parameters for HRs. It should, however, be noted that blood "concentrations" of HRs were observed after both the oral and intravenous administration of the drug. The values being given as "units" per ml, it is not possible to estimate the true concentrations.

– *High performance liquid chromatography.* The two methods described by Kuhnz *et al.* /17,18/, have a detection limit of 1 µg/ml for the tri- and di-ethers (UV-detection) and of 0.1 µg/ml for the tetra-ether (fluorescence detection). After the intravenous injection of 1.5 g of HRs to healthy volunteers, serum concentrations of the tri-ether fell to the detection limit after 2 hours and the concentrations of the tetra-ether after 3 hours. Pharmacokinetic parameters can thus be only estimated and not precisely calculated. A method for the determination of hydroxyethylated quercetins has been described by the same authors /19/.

  The method is not suitable for urine samples because of the concomitant extraction of other hydrophilic compounds which give peaks superimposed on those of HRs constituents.

– *Spectrofluorimetry.* A method has been described by Tan and colleagues /20/ for the determination of tri-HR in urine. Its specificity

can, however, be questioned and it is highly probable that it would measure more than one component of the HRs. Since the intrinsic fluorescence of individual rutoside-aluminium complexes varies, it is not certain that the method would lead to a reliable evaluation of HRs levels in urine. The sensitivity of the method is not high enough to allow the measurement of HRs in plasma after oral administration.

IV. *IN VITRO* STUDIES

(a) Protein binding

Protein binding was measured by Hackett *et al.* /21/ both after the administration of ^{14}C-HRs and by adding ^{14}C-HRs to samples of human plasma. Equilibrium dialysis showed that about 30% of the radioactivity was bound to plasma proteins. In the same study, it was shown that radioactivity was wholly located in plasma over the entire experimental period as radiochemical and chromatographic examination of lyophilized blood cells indicated no uptake of HRs or its metabolites.

Bauer-Staeb and Niebes /22/ measured the binding of rutin, mono-HR, di-HR, tri-HR and tetra-HR to serum proteins by equilibrium dialysis. The binding diminished progressively with the substitution of phenolic groups by hydroxyethyl groups from 70% for rutin, to 5.4% for tetra-HR. The binding was found to be reversible.

(b) Perfused rat liver

Förster *et al.* /23/ studied the behaviour of tri-HR and tetra-HR in a perfused rat liver preparation. They found that the liver takes up tetra-HR more slowly than tri-HR. During the period of perfusion, tri-HR was converted to a metabolite; both substances being found in high concentration in the bile. Tetra-HR was found in lower amounts. The decrease in tri-HR concentration in the perfusion solution remained constant during the whole liver perfusion and the metabolite concentration increased steadily. The concentration of tetra-HR, however, decreased mainly during the first hour and there was no significant change afterwards. These results are in good agreement with *in vivo* studies performed by Griffiths and his coworkers in bile cannulated animals as described later in this review.

V. BLOOD, PLASMA, SERUM CONCENTRATIONS

Although the metabolism and excretion pattern of HRs have been extensively studied in animals including mice, rats, rabbits and monkeys, little information is available on classical pharmacokinetic parameters.

(a) Mono-HR

Mouse – Decline in blood concentrations of ^{14}C following injection of mono-HR was rapid, and paralleled accumulation of ^{14}C in the liver and kidneys /24/.

(b) Tri-HR and tetra-HR

Mouse – When tri-HR was administered by intravenous injection the blood concentrations decreased very rapidly for the first 30 minutes, until almost baseline values were reached. This decline paralleled accumulation of ^{14}C in the liver and kidneys /24/.

Rat – Using narcotised rats, Förster and Ziege /25/ investigated the gastrointestinal absorption and first-pass metabolism of tri-HR and tetra-HR by the determination of arterioportal concentration differences for these substances. Following intestinal perfusion, the two substances appeared in the portal vein as observed by thin layer-fluorescence chromatography. In some of the animals a fluorescing metabolite was detected in the blood during the absorption of tri-HR but it was not identified.

Man – When Venoruton® was administered orally (4000 mg) and intravenously (1500 mg) to healthy volunteers and tri-HR and tetra-HR were measured by circular dichroism, it was not possible to determine quantitatively the amounts of these two compounds in serum /15,16/. It can nevertheless be concluded from these studies that after intravenous administration of HRs, the two substances disappeared rapidly from the serum, since after 1-2 hours base line values were obtained, whereas after oral administration the dichroistic perturbation lasted usually for more than 12 hours.

When HRs were given as an intravenous bolus (1500 mg) and tri-HR and tetra-HR were measured by HPLC, fluorescence detection showed a

rapid disappearance of these compounds in serum /17,18/. It has been postulated that this short half-life (1-2 hours) is representative of the distribution phase rather than of the elimination phase /26/.

When Venoruton® was infused during 6 hours to healthy volunteers at 3 doses (1680, 3360 and 6720 mg) and the serum concentrations of tri-HR and tetra-HR were measured by thin layer chromatography /14/, it was possible to measure these two compounds in serum. From these concentration-time curves it appears that at the doses used for this study the two compounds follow non-linear kinetics. The concentrations at the end of the infusion were not proportional to the dose and the steady-state was not reached at 6 hours for the higher dose whereas this seemed to be the case for the lower doses.

(c) HRs

Mouse – When ^{14}C-HRs were given intraperitoneally, a peak was observed around 30 min after the injection; the blood levels of total radioactivity seemed to decrease in a biphasic manner /27/.

Dog – When ^{14}C-HRs were administered to dogs /28/, radioactivity was measured in plasma for more than 48 hrs. No pharmacokinetic parameters can be derived from this data. The results, however, are compatible with extensive enterohepatic cycling as indicated by the numerous secondary peaks of radioactivity observed in plasma as a function of time during the elimination phase.

Man – Following oral administration of ^{14}C-HRs, maximal radioactivity concentrations in plasma were obtained between 1 and 9 hours /21,29,30/. Although not very high at the peak, radioactivity could be measured over more than 120 hours after a single dose. The decline in radioactivity was clearly bi-exponential /21/. The apparent half-life of elimination, however, was not calculated due to the numerous secondary peaks observed in these volunteers.

From this data it is difficult to establish if these low serum concentration-time profiles are due to distribution of the drug in tissues acting as "deep compartments" or to extensive enterohepatic cycling of the drug. As we shall discuss in a later section of the review, the second hypothesis seems highly probable. The secondary peaks of radioactivity found in human serum support this hypothesis.

In one study /31/, 900 mg HRs were given to healthy volunteers. A spectrophotometric method indicated maximal plasma concentrations of about 5 μg/ml 8 hours after the administration of the drug. These results must, however, be analyzed with caution in view of their very fragmentary publication.

VI. URINARY EXCRETION

(a) Mono-HR

Mouse – After the intravenous administration of ^{14}C-mono-HR about 25% of the radioactivity was recovered in urine, half of it as the unchanged compound and half of it as the glucuronide /24/.

Rat – Following intravenous administration of ^{14}C-mon-HR, the urinary excretion amounted to between 35 and 55% of the dose. Mono-HR represented about three quarters of the urinary radioactivity, in addition to other metabolites. One of these metabolites is probably a glucuronide conjugate of another unidentified derivative /32/.

Intravenous and intraperitoneal administration of mono-HR to cannulated rats confirmed these findings /32/.

Following oral administration of ^{14}C-mono-HR, 5 to 31% of the dose was recovered in urine both in bile duct cannulated and non-cannulated animals /33/.

(b) Di-HR

Rat – Following intravenous and intraperitoneal administration of ^{14}C-di-HR to bile duct cannulated animals, between 5 and 15% of the radioactivity was recovered in the urine. Most of the excreted material was the unchanged glycoside, but two glucuronides of di-HR were also detected. In addition, an unidentified substance was detected /32/. In non-cannulated rats only between 1 and 2% of the administered dose was recovered in urine /32/.

Following oral administration, about 10% of the radioactivity was recovered in urine both in bile cannulated and non-cannulated rats /33/.

(c) Tri-HR

Mouse – After the intravenous administration of ^{14}C-tri-HR, about

20% of the radioactivity was excreted in urine. The compound was excreted both unchanged (about one half) and as the 5-O-glucuronide /24/.

Rat – Following intravenous and intraperitoneal administration of ^{14}C-tri-HR, the average level of ^{14}C in urine did not exceed 20% of the dose. Liquid scintillation counting of the chromatograms showed that most of the radioactivity was at the R_f of the glycoside, a small fraction was associated with a metabolite. This metabolite is probably the glucuronide of the unchanged compound /32/. These observations were repeated in bile duct cannulated rats /32/.

Following oral administration only about 2% of the dose was recovered in the urine both in bile duct cannulated and non-cannulated animals /33/.

Rabbit, Rhesus monkeys – An intravenous dose of ^{14}C-tri-HR was recovered as less than 20% in the urine. In the rabbit most of the radioactivity was as the unchanged glycoside. In the monkey the separation of the radioactivity was hindered by the presence of material which caused considerable streaking on the chromatograms /32/.

After an oral dose, only about 1 to 2% of the dose was detected in the urine of both rabbits and monkeys /33/.

Man – In early experiments, HRs were administered to healthy volunteers. Urine was collected after an intravenous dose of 500 mg or an oral dose of 3600 mg. The samples were analysed by thin layer chromatography by a method specific for tri-HR /13/. The authors of this study concluded that HRs are not absorbed from the gastrointestinal tract. It must be considered, however, that the method was probably unable to detect conjugates of HRs and that, as a consequence, the results of this study must be analysed with caution.

When tri-HR (500 mg) was administered orally to healthy volunteers, urine was collected and analysed by a spectrofluorimetric method. A mean recovery of about 8.6% of the administered dose was obtained over a urine collection period of 120 hours /34/. The terminal apparent half-life of elimination, determined by the Sigma-minus method (amount to be excreted) was 18.3 hours with a range of 13.5 to 25.7 hours.

When HRs were infused over 6 hours to healthy volunteers and tri-HR was measured in urine over the infusion period, the relative urinary

excretion of this compound was greater at higher than at lower doses /14/. The urinary excretion of tri-HR represented about 10% of the dose for 1680 mg, and it was increased to about 40% for 6720 mg. The reasons for this particular behaviour cannot be found in this set of experiments. It is, however, possible that the renal handling of tri-HR is saturable. This explanation would be in accordance with findings in serum during the same experiment. Similar results were reported for tetra-HR as seen below /14/.

(d) Tetra-HR

Rat – After intravenous administration of ^{14}C-tetra-HR, an average of about 60% of the administered ^{14}C-activity was recovered in urine. The urinary excretion was maximal in the first 24 hrs, with less than 1% being voided in the urine in the subsequent 72 hrs. Chromatographic and radiochemical analysis of the urine confirmed that the glycoside was excreted essentially unchanged /35/.

After oral administration to bile cannulated and non-cannulated animals, about 2% of the administered radioactivity was found in urine.

Rhesus monkey – Following intravenous administration of ^{14}C-tetra-HR to monkeys, about 55% of the dose was excreted in urine, mainly as the unchanged glycoside, with small amounts of the aglycone also being present /35/.

Following oral administration of the compound, about 1% of the dose was recovered in urine /33/.

Man – Following a 6 hour infusion of HRs to healthy volunteers, the urinary excretion of tetra-HR was considerable /14/. As observed with tri-HR there was no linear relationship between the infused dose and the 6 hour urinary excretion. This is also possibly the consequence of a saturable handling of the drug.

(e) HRs

Man – When ^{14}C-HRs (300 mg) were administered orally to healthy volunteers, between 3 to 6% of the administered ^{14}C was excreted in urine over 120 hours /21, 30/. When the urinary excretion rate is plotted versus time, it is possible to determine an "overall apparent half-life of elimination of radioactivity". This parameter seemed to vary from

subject to subject between 10 and 25 hours. This finding is in good agreement with the value found for tri-HR by Ritschel and Kaul /34/. This apparent half-life of elimination was relatively constant when the same subject received the drug on two occasions /21/.

In addition, the fact that in this study, the second administration of ^{14}C-HRs (following chronic intake of 900 mg of Venoruton® per day for eight weeks) did not result in any change in urinary ^{14}C-excretion, is indicative of the fact that HRs are not influencing their own metabolism either by enzyme inhibition or induction. These results indicate also that in man, adaptive changes in the intestinal microflora are not important in promoting the catabolism of HRs. The same results have been obtained when tri-HR was chronically administered to rats /33/. In contrast, following the serial administration of mono-Hr to rats, it was found that there was an increased catabolism of this compound to ring scission products /33/.

(f) Rutin

Rat – Following intraperitoneal administration of rutin to rats (bile cannulated or not), it was not possible to detect rutin, rutin conjugates or quercetin in urine. The urine samples were found to contain phenolic acid metabolites not present in control urine /32/.

VII. BILIARY EXCRETION AND FAECAL ELIMINATION

(a) Mono-HR

Mouse – Following intravenous administration of ^{14}C-mono-HR, about 65% of the radioactivity was found in the faeces as 7-mono-O-(β-hydroxyethyl)-quercetin /24/.

In studies with bile duct cannulated mice receiving an intravenous injection of ^{14}C-mono-HR, about 70% of the radioactivity was found in the bile, two thirds as unchanged mono-HR and one third as a glucuronide /24/.

Rat – Intravenous and intraperitoneal administration of ^{14}C-mono-HR to bile cannulated rats led to a biliary excretion ranging from 64 to 96% of the dose. The unchanged compound represented only about one sixth of the biliary radioactivity. A major metabolite was a glucuronide of either mono-HR or of one of its metabolites /32/.

When the drug was given to non-cannulated rats, only 13 to 29% of the dose were measured in the faeces. Identification of the faecal metabolites was rendered difficult by the presence of other components, which were also extracted by methanol from all samples of faeces. It was, nevertheless, possible to identify the glycoside and the aglycone in this material /32/.

After oral administration to bile cannulated animals, about 20% of the dose was measured in the bile and in non-cannulated rats, the faecal excretion being between 39 and 71% /33/.

(b) Di-HR

Rat – Following intravenous and intraperitoneal administration of ^{14}C-di-HR to bile duct cannulated rats, the level of excretion of glycoside material in bile was about 70%, i.e. at least five times that found in urine. A metabolite, probably the 3′-O-glucuronide, was the major component in bile /32/.

When di-HR was given intraperitoneally to non-cannulated rats, the methanolic extracts of faeces contained 63% of the dose as the aglycone /32/.

Following oral administration to bile cannulated rats, about 10% of the radioactivity was recovered in bile and 85% of the radioactivity was found in the faeces of non-cannulated animals /33/.

(c) Tri-HR

Mouse – Following the intravenous administration of ^{14}C-tri-HR, about 70% of the radioactivity was found in faeces as tri-O-(β-hydroxyethyl) quercetin /24/.

Intravenous administration to bile duct cannulated mice also led to a 70% excretion of the radioactivity, but as unchanged tri-HR for about three quarters and as the 5-O-glucuronide for one quarter /24/. The significance of these results is discussed below.

Rat – After intravenous administration of tri-HR to the biliary cannulated rat, it was found that over a 24 h period, 67% of the radioactivity was voided in the bile and 20% in the urine /36, 37/. These results were confirmed in a second set of experiments performed in non-cannulated rats, where it was shown that the urinary excretion was predominantly of the glycoside, with only small amounts of the glucuronide conjugates /32/. In addition, $^{14}CO_2$ in expired air was measured

and statistically significant counts were found in only one of the experiments /32/.

After intraperitoneal administration of tri-HR to bile duct cannulated rats, 77% of the radioactivity was found in the bile and 12% in the urine /32/. Chromatographic analysis of the bile revealed the presence of the glycoside and a metabolite. This metabolite, which represented about one fifth of the biliary radioactivity, has been identified as the 5-O-glucuronide of tri-HR /32/. Similar results were obtained after the intravenous administration of this substance. These results are in good agreement with the results of Förster *et al.* /23/ obtained with tri-HR in the perfused rat liver preparation.

After oral administration, about 10% of the dose was excreted via the bile in cannulated animals and about 75% was excreted in the faeces of non-cannulated animals /33/. It is interesting to note that under the same conditions 11% of the administered dose was excreted in bile but only 2% in urine over a period of 48 h /37/. The difference in the ratios of urinary to biliary excretion measured after intravenous (0.29) and oral (0.17) administration of tri-HR was interpreted by Griffiths and Barrow /37/ as an indication that urinary excretion of tri-HR is of significance only where a high blood level is achieved (i.e. by direct introduction of tri-HR into the vascular system), resulting in an overload of the hepatic excretion mechanism. Under conditions of slower uptake, viz. involving passage of the glycoside across the intestinal mucosa, it was postulated that the major route of excretion of absorbed tri-HR is via the hepatic route.

Rabbit – After intravenous administration of tri-HR to rabbits, 48% of the dose was found in the faeces and 12% in the urine /32/.

Rhesus monkey – The intravenous administration of tri-HR to two male rhesus monkeys resulted in elimination of 55% of the dose in the faeces but only 14% in the urine /32/. From these results it was concluded that it appears probable that the pattern of excretion of tri-HR in the primates (including man) is similar to that observed in the rat and that selective excretion of absorbed tri-HR occurs via the bile. It seems evident, therefore, that failure to detect tri-HR in urine should not be accepted as evidence of non-absorption /37/.

After oral administration, about 70% of the radioactivity was found in faeces /33/.

(d) Tetra-HR

Rat – After intravenous and intraperitoneal administration of ^{14}C-tetra-HR, about 15 to 25% of the radioactivity was found in the faeces. The liquid scintillation counting of the paper chromatograms showed that the major part of the radioactivity was located at the R_f of the aglycone /35/.

Following intravenous administration of tetra-HR to rats with cannulated bile duct, about 30% of the ^{14}C was excreted in the bile. Liquid scintillation counting showed that, on the contrary to the findings in faeces, almost all the radioactivity was located at the R_f of the glycoside, and only a small portion at the R_f of the aglycone /35/. This finding is discussed in a later part of this review; it confirms the findings of Förster *et al.* /23/ in the perfused rat liver.

After oral administration, less than 1% of the dose was found in bile of cannulated animals and about 60% in the faeces of non-cannulated animals /33/.

Rhesus monkey – Following intravenous administration of ^{14}C-tetra-HR, about 25% of the radioactivity is recovered in the bile, mainly as the unchanged glycoside /35/.

(e) Rutin

Following the intraperitoneal administration of rutin to bile duct cannulated rats, no compound showing the spectral or chromatographic properties of rutin or quercetin was detected. Other substances were, however, present in trace amounts. In faeces of non-cannulated rats, a compound having a R_f value similar to quercetin was seen /32/.

The results obtained with rutin both in urine and in bile could be explained by the low water solubility of the compound such that after the intraperitoneal injection of a dose (partly as a suspension), the glycoside precipitates at the site of injection with subsequent slow uptake into the blood /32/.

Recently, Brown and Griffiths /38/ demonstrated that both quercetin and rutin can be transformed into their 3′-O-methyl-ethers prior to their excretion in bile. This metabolic pathway could be of importance in protecting the body against the mutagenic action of quercetin. Although the quercetin glycoside, rutin is reported to be non-mutagenic in the Salmonella/microsomal assay, it is known that rutin may be

hydrolysed to quercetin by the intestinal microflora. At the present time it is still difficult to extrapolate these findings to the fate of HRs in man. Methylation is probably only of relevance for compounds which are not hydroxyethylated in the 3′ position and it has recently been shown that 7-mono-HR is excreted in the bile of the rat as 3′-O-methyl-mono-HR and 3′-O-methyl-mono-HR glucuronide (Brown and Griffiths, unpublished observation). Independently of these findings, it has been shown that HRs are devoid of any mutagenic properties in different mutagenicity tests and, moreover, HRs have not shown any transforming potential in an *in vitro* carcinogenicity test (Cortat, personal communication).

VIII. ENTEROHEPATIC CYCLING

Plastic cannulae were implanted in the common bile duct of 2 male Wistar rats under nembutal anaesthesia /39/. A cannula connected the common bile duct of rat 1 to the distal portion of the common bile duct of rat 2 such that the bile from rat 1 was delivered into the duodenum of rat 2. A second cannula was also established in the proximal end of the bile duct of rat 2, such that bile from this animal could be collected, the bile duct being ligated between the two cannulae. Under these conditions biliary metabolites arising in the liver of rat 1 are transferred to the intestine of rat 2. Determination of these metabolites in the bile and urine of rat 2 provides a measure of their reabsorption in a first enterohepatic cycle.

Tri-HR, tetra-HR and mono-HR were administered by injection in the caudal vein of rat 1. The total values of ^{14}C recovered from bile, urine and faeces of rats No. 2 indicated that biliary metabolites representing minimal mean values of 54%, 27% and 64% of the three rutosides administered to rats No. 1 were eliminated in the bile of those animals and subsequently transferred to the lumen of rats No. 2. Summation of the ^{14}C bile and urine levels in respect of each No. 2 rat indicated that respectively about 5%, 2% and 10% of the administered tri-HR, tetra-HR and mono-HR were recovered. This represented a reabsorption from the intestine of 9%, 7% and 15% of the total biliary excreted metabolites.

These experiments, where the nature of the main metabolites was also assessed, indicate that a significant level of enterohepatic cycling occurs in rats receiving hydroxyethylrutosides.

IX. INTESTINAL MICROFLORA

(a) Stability of the hydroxyethyl side chains

In a series of experiments in which hydroxyethyl-rutosides labelled with ^{14}C in the α and the β positions of their hydroxyethyl side chains were orally administered to rats /40/, the expired CO_2 was trapped, but no significant levels of radioactivity were found indicating that the intestinal microflora, at least *in situ*, is unable to degrade the side chains of hydroxyethylrutosides to $^{14}CO_2$.

(b) Ring fission

Early metabolic investigations on orally administered naturally occurring flavonoid compounds showed that their metabolites were largely ring fission products. Urinary excretion of these metabolites was considered to provide a measure of flavonoid absorption from the gastrointestinal tract. However, it was subsequently shown that these phenolic ring fission products were formed in the lumen of the intestine by the gut microflora /41-43/.

Three methods have been used to show that these metabolites are of microfloral origin:

1) The demonstration that metabolite formation is reduced in animals dosed with antibiotics.

2) The demonstration that these metabolites can be formed by incubating the specific flavonoid with the intestinal microflora *in vitro*.

3) The demonstration that after the administration of flavonoids to germ-free animals in which (although conjugate excretion is elevated, showing the absorption of flavonoids), the excretion of ring fission products is completely suppressed.

The latter method has been used for HRs /43/. Rats of the Wistar strain, which had been bred under germ-free conditions and from which the normal intestinal flora had been shown, by bacteriological testing, to be absent, were maintained in isolation on a sterilized diet. Sterilized rutin, tri-HR and tetra-HR were administered orally to these animals. The urine and faeces were collected for 5 days, extracted and examined by two-dimensional thin layer chromatography.

The recovery of unchanged tri-HR and tetra-HR from the faeces of germ-free animals indicates that their glycoside linkage is stable to secretions of the alimentary tract.

From the microfloral studies /33, 43/ performed with HRs, it appears that in contrast to naturally occurring flavonoids, the hydroxyethylrutosides show considerable resistance to bacterial ring fission, for fission products have been detected only in respect of the mono-HR, which has been shown to give rise to fragments similar to those formed following the ingestion of rutin. This suggests that hydroxyethylation at position 4′ alone may be sufficient to prevent further degradation /33/.

The detection of large amounts of unchanged rutin in the faeces of germ-free animals is noteworthy, as it has been reported that low recoveries of this compound are usual in the presence of normal microflora /43/. It has been shown that rutin gives the same phenolic metabolites from the B ring of the flavonoid molecule as mono-HR /33/.

(c) Conversion to the aglycone

As the conversion of HRs to their aglycones results in considerable insolubilization of the administered compound, it was important to establish whether hydrolysis occurs in the upper alimentary tract (viz. on exposure to gastric juice) or lower in the intestine (i.e. in the presence of the intestinal microflora), which is known to be responsible for the metabolic hydrolysis of certain glycosides.

The following results have been obtained by Griffiths and his collaborators /40/:

1) Conversion of the mono-, tri- and tetra-HRs to their aglycones in the rat is reduced by the administration of an oral antibiotic (viz. neomycin).

2) Incubation of mono-, tri- and tetra-HRs with the intestinal microflora resulted in aglycone formation *in vitro*.

3) Conversion of the tri- and tetra-HRs to their aglycones does not occur in the germ-free rat.

4) 24 h after dosing normal rats with tri-HR, *post mortem* examination showed that although the glycoside was present in the stomach, no aglycone was detectable in the organ although both were present in the caecum and ileum.

It was, therefore, concluded that hydrolysis of the glycoside is effected only by the microflora and after entry of the glycoside into the intestine.

X. TISSUE DISTRIBUTION

The tissue distribution of mono-HR and tri-HR was measured in mice both by autoradiography and by direct counting of the $^{14}CO_2$ produced by combustion of tissue samples /24, 44/.

Decline in blood concentrations of ^{14}C following injection of either compound was rapid (see previous sections), and paralleled accumulation of ^{14}C in the liver and kidneys. The period of greatest decline in liver-^{14}C also corresponded to the period of maximal biliary excretion. In addition to the liver and kidney, ^{14}C was detected in the more vascularized tissues namely spleen and lung. In none of the experiments was ^{14}C detected in peritoneal fat, in bone samples and in the brain, which shows that HRs do not cross the blood-brain barrier /24/.

Mono-HR and tri-HR were also administered to pregnant mice on approximately the 18th day of pregnancy /24/. Radioactivity in the foetuses and the amniotic fluid was negligible.

XI. SKIN PENETRATION

The skin penetration of HRs was measured using a repeated stripping technique of the corneum with adhesive tape after the topical application of a gel and an ointment to healthy volunteers /45, 46/. The results of these studies indicated that HRs penetrate the skin and that most probably HRs can be absorbed when applied topically.

XII. SYSTEMIC AVAILABILITY

The superficial analysis of urinary excretion data could lead to the conclusion that HRs are not, or are very poorly absorbed from the gastrointestinal tract. We have presented some reasons (e.g. nonlinear hepatic handling, nonlinear renal clearance) why it may not be as easy as suspected to draw definitive conclusions.

From the available data it thus appears that HRs are absorbed from the gastrointestinal tract, but that the extent of systemic availability cannot be calculated with precision from the available results. It seems clear, however, that the systemic availability of HRs is less than 1 but that it is different for each of the compounds.

XIII. CONCLUSIONS

It is evident from the studies surveyed in this review that, although extensive information is available on the metabolism of the HRs in relation to both metabolite identification and quantitative excretion data, rather more limited data have been obtained on the pharmacokinetics of these compounds. The detection of HR glycosides and their glucuronides, following oral administration of HRs, in urine and bile of the rat /33/ and in the urine of man /20, 21/ presents unequivocal evidence of HR absorption from the gastrointestinal tract but the low HR plasma levels reported in several species /21, 28, 30/ suggest either that absorption from the gastrointestinal tract is slow or that the efficient hepatic extraction, demonstrated in the rat /32, 39/ restricts the attainment of higher HR plasma levels. Evaluation of this problem in man is made difficult by the absence of quantitative data on the biliary excretion of HRs in human bile for, although tri-HR-glucuronides have been detected in human bile using a T-tube drainage technique /47/, no quantitative data for human bile levels is currently available.

It has long been appreciated that naturally-occurring flavonols, e.g. rutin /12/ and quercetin /48/ are poorly absorbed from the gastrointestinal tract of the rat and man and Gugler, Leschnik & Dengler /48/ concluded that less than 1% of an oral dose of quercetin was absorbed from the human gut over a period of 9 h. Another potential problem associated with the use of rutin which is overcome by hydroxyethylation is its low water solubility. As a matter of fact, Pfeifer and colleagues /49/ showed that rutin, administered as soluble salts, precipitated out and formed concretions with associated suppurative inflammation in the liver bile duct and renal channels of newborn mice, rats, hamsters and newborn or premature babies following parenteral dosage. Such results have not been observed with the administration of HRs even at high intravenous doses. The relatively high recoveries (14-20%) of orally administered HRs from the bile and urine of the rat /33/ indicate therefore that chemical modification of flavonols by hydroxyethylation facilitates their absorption from the gastrointestinal tract. Although it is sometimes thought that flavonoid compounds generally are poorly absorbed from the gastrointestinal tract, certain flavonoids, including those of the *flavanol* group have been found to be readily absorbed from the mammalian gut. Over 64% of the naturally-occurring flavanol, (+)-catechin /51/ and 63% of 3-O-methyl (+)-catechin have been accounted for as flavanol conjugates in the bile and urine of orally-dosed

biliary-cannulated animals /52, 53/, whilst the flavanone, naringin after oral administration to the rat gave rise to biliary and urinary metabolites corresponding to an absorption of circa 15% /50/. Since these three categories of flavonoids, viz. flavonols, flavanones and flavanols differ mainly in the degree of hydrogenation of the O-heterocyclic ring, it is probable that the oxidation level of the O-ring is a major determinant of the absorption characteristics of flavonoid compounds. These findings also indicate that wide variation in the absorption characteristics following structural modification of the various classes of flavonoid compound should be anticipated and that future research needs not only to be directed towards the development of flavonoid compounds with favourable pharmacological profiles but additionally study is required of the molecular, structural factors which determine pharmacokinetic behaviour (including absorption and distribution) of active flavonoids.

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