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COMPARISON OF IN VITRO DRUG METABOLISM BY LUNG, LIVER, AND KIDNEY OF SEVERAL COMMON LABORATORY SPECIES

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ABSTRACT

Comparative studies of *in vitro* drug metabolism by hepatic and extrahepatic tissues have been complicated by the use of a single experimental tissue, few animal species, and variable experimental conditions. In an attempt to minimize these complications, liver, lung and kidney from rat, mouse, rabbit, hamster, and guinea pig were assayed for standard microsomal and soluble fraction enzymes involved in drug biotransformation. For all species, liver was the most active organ. Kidney and lung activities were usually 15%–40% of those found in liver, with kidney slightly more active than lung. No single species demonstrated total superiority in its drug-metabolizing ability, although hamster showed a large number of instances of greatest activity. The rat was a surprisingly poor representative of drug-metabolizing ability; it was superior to the other four species in less than 25% of the instances studied. All species appeared to N-demethylate aminopyrine equally except for high pulmonary and nearly absent renal activities in rabbit and high hepatic activity in hamster. Rat had the lowest level of cytochrome P-450 and low activity of NADPH-cytochrome *c* reductase. UDP-glucuronyltransferase activity toward the acceptors *p*-nitrophenol and *o*-aminophenol was higher in hamster and rabbit than other species. Guinea pig appeared to have the most active soluble fraction enzymes. Mouse lung and kidney had glutathione S-aryltransferase activities 10-fold greater than any other species and comparable to liver activity from rabbit and hamster.

It has been known for some time that the levels of mixed-function oxidase (MFO[1]) activity are generally highest in mammalian liver, whereas those in kidney, skin, lung, intestine, adrenal, and other organs are lower but measurable (1–4). Recently, however, work in our laboratory has shown that the specific activities of several MFO systems of rabbit lung are equal to or slightly higher than the corresponding activities in liver (5, 6). On the other hand, isolated published reports have suggested that the levels of extrahepatic MFO activity in species other than the rabbit may be quite low relative to liver (7–9). Comparative studies of *in vitro* drug metabolism by hepatic and extrahepatic organs of experimental animals have been complicated by the fact that investigators have commonly utilized only a single extrahepatic tissue and two or three animal species. Therefore, in attempting to obtain comparative information for these parameters it has been necessary to extrapolate from values obtained in one laboratory under one set of conditions to those obtained in another laboratory under another and usually different set of conditions. In an attempt to minimize this complication we have studied the ability of microsomal and supernatant fractions of lung, liver, and kidney from rat, mouse, hamster, rabbit, and guinea pig to metabolize a variety of drug substrates.

[1] The abbreviations used are: MFO, mixed-function oxidase; PNP, *p*-nitrophenol; OAP, *o*-aminophenol; PABA, *p*-aminobenzoic acid.

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Methods

Animals. Adult males of the following species of common laboratory animals were used in all experiments: New Zealand rabbits (2.5 kg), Hartley guinea pigs

(300 g), Sprague-Dawley rats (200 g), Syrian Golden hamsters (100 g), and CDF_1 mice (25 g). Hamsters and rabbits were obtained from the National Institutes of Health breeding colony and all other animals came from the Mammalian Genetics and Animal Production Unit. Each of the 4–8 separate determinations was conducted on a separate pool of tissue, each pool containing organs from 3–40 individual animals, depending on the species. Animals were allowed food and water *ad lib.* and were killed by cervical dislocation.

Tissue Preparation. Organs to be studied were excised, rinsed in cold 0.25 M sucrose containing 50 mM Tris-HCl (pH 7.4), blotted dry, and weighed. All subsequent operations were conducted at 4°C. Tissue was pooled to obtain 3–4 g of liver, at least 6 g of lung, and 4–5 g of kidney. Tissue was minced into small pieces with scissors and then homogenized in 3 volumes of cold 0.25 M sucrose in a glass Potter-type homogenizer with a motor-driven Teflon pestle (A. H. Thomas Co., Philadelphia, Pa., size C). The homogenate was then centrifuged at 9,000*g* for 20 min. After discarding the floating fat layer the 9,000*g* supernatant fraction was further centrifuged for 60 min at 105,000*g*. The resulting supernatant fraction was collected and the microsomal pellet resuspended in 0.25 M sucrose (pH 7.4) using a glass-Teflon homogenizer. Protein concentration was determined by the method of Lowry *et al.* (10). The microsomes were then diluted to a concentration of 3 mg of protein per ml and the soluble fraction diluted to concentration of 6 mg of protein per ml.

Enzyme Assays. Activities of microsomal and soluble enzymes were determined by aerobic incubation at 37°C. Preliminary experiments with each animal species established that all incubations reported herein were conducted under zero-order conditions with respect to cofactor and substrate concentrations and were linear with respect to incubation time and enzyme concentration.

Incubation mixtures for mixed function oxidation consisted of an NADPH-generating system (1 mM NADP, 10 mM glucose 6-phosphate, 5 mM $MgCl_2$, and 2 units of glucose 6-phosphate dehydrogenase), Tris-HCl buffer (100 mM, pH 7.4), microsomal protein (1 mg/ml), and either aminopyrine (25 mM), aniline hydrochloride (5 mM), or biphenyl (15 mM) in a final volume of 3.0 ml. Activity of aminopyrine N-demethylase was determined by following the production of formaldehyde by the Nash procedure (11) using the method of Cochin and Axelrod (12). Biphenyl hydroxylase activity was measured by following the production of 4-hydroxybiphenyl fluorometrically using the method of Creaven *et al.* (13). Aniline hydroxylase activity was determined by the method of Imai *et al.* (14) in which *p*-aminophenol production is monitored.

Incubation mixtures for UDP-glucuronyltransferase activity consisted of Tris-HCl buffer (0.83 mM, pH 7.4), $MgCl_2$ [1.0 mM *p*-nitrophenol (PNP); 25 mM *o*-aminophenol (OAP)], microsomal protein (1 mg/ml), uridine diphosphoglucuronic acid (3.3 mM), and either PNP (0.13 mM) or OAP (0.27 mM) in a total volume of 1.5 ml. Activity of the enzyme using PNP as acceptor substrate was assayed by a modification of the method of Temple *et al.* (15) in which 5 N KOH was used in the final step. The activity of the enzyme using OAP as acceptor substrate was assayed by the method of Dutton and Storey (16).

N-Acetyltransferase activity with either *p*-aminobenzoic acid (PABA) or sulfadiazine as acceptor substrate was estimated as described by Weber (17). The incubation mixture contained acetyl-coenzyme A (600 nmol), phosphate buffer (0.33 mM, pH 6.8), soluble protein (0.5–6-mg total depending on species studied), and either PABA (100 nmol) or sulfadiazine (100 nmol).

Gluthathione S-aryltransferase activity was determined in a Gilford 2400 spectrophotometer with 1,2-dichloro-4-nitrobenzene as substrate according to the method of Grover and Sims (18).

NADPH-cytochrome *c* reductase activity was determined by the method of Williams and Kamin (19) as described by Gigon *et al.* (20), and cytochrome P-450 was estimated by its dithionite difference spectrum as described by Omura and Sato (21) using a Shimadzu MPS-50L recording spectrophotometer.

Electron Microscopy. Samples were prepared for electron microscopy as described previously (22).

Results

Biochemistry of Microsomal Fractions. *Liver.* Quantitative data on drug oxidation and conjugation by hepatic enzymes from five animal species are presented in table 1. There was no obvious or consistent correlation between levels of cytochrome P-450 and drug oxidation among the different species, although the relatively high levels of NADPH-cytochrome *c* reductase activity in the hamster appeared to parallel the generally high levels of drug oxidation in this species. Rabbit was the only species to demonstrate a reproducible, measurable activity of N-acetyltransferase toward sulfadiazine as substrate. No single species was seen to be either totally superior or totally inferior in its hepatic drug-metabolizing ability.

Lung. Table 2 shows the pulmonary drug-metabolizing enzyme activity in the five species studied. It can be seen that all species have substantially less pulmonary enzyme activity than they do hepatic activity. In addition to a nearly total inability of the pulmonary system to N-acetylate sulfadiazine, lung from most species demonstrated very low aniline and biphenyl hydroxylase activities. A lone exception to this trend is the high biphenyl hydroxylase activity seen here and reported previously for the rabbit lung (5, 6, 23). Mouse lung contains considerably more glutathione S-aryltransferase activity than do other species

TABLE 1

Species differences in hepatic drug metabolism

Data are reported as mean ± SD of 4–8 separate determinations.

Parameter	Species				
	Rat	Rabbit	Mouse	Hamster	Guinea pig
Protein[a]					
Microsomal	27.0 ± 3.6	24.1 ± 3.9	34.3 ± 4.4	28.0 ± 3.1	35.9 ± 10.3
Supernatant	53.1 ± 9.3	67.4 ± 6.8	77.6 ± 15.9	63.8 ± 11.6	70.7 ± 13.7
Cytochrome P-450[b]	0.098 ± 0.025	0.177 ± 0.038	0.108 ± 0.022	0.140 ± 0.023	0.125 ± 0.038
NADPH-cytochrome *c* reductase[c]	187.0 ± 51.0	152.0 ± 16.0	109.0 ± 33.0	277.0 ± 48.0	225.0 ± 67.0
Hydroxylases[c]					
Aniline	0.8 ± 0.4	0.6 ± 0.4	1.5 ± 0.3	2.7 ± 0.9	0.8 ± 0.4
Biphenyl	1.6 ± 0.4	1.7 ± 0.5	2.8 ± 0.6	3.4 ± 1.3	4.1 ± 3.1
Aminopyrine demethylase[c]	10.8 ± 2.8	9.8 ± 3.5	11.0 ± 1.9	18.8 ± 3.4	9.2 ± 2.6
Glutathione S-aryltransferase[c]	76.4 ± 28.9	21.9 ± 4.3	74.4 ± 31.1	35.0 ± 11.1	57.0 ± 13.0
N-Acetyltransferase[c]					
PABA	1.8 ± 0.6	1.8 ± 0.4	6.5 ± 2.1	13.1 ± 5.3	7.1 ± 1.9
Sulfadiazine	0.2 ± 0.5	0.3 ± 0.2	<0.1	<0.1	<0.1
UDP-glucuronyltransferase[c]					
PNP	4.4 ± 3.5	6.6 ± 2.8	2.2 ± 1.6	5.0 ± 3.0	2.7 ± 2.4
OAP	0.9 ± 0.3	2.3 ± 1.1	1.0 ± 0.4	1.6 ± 0.9	2.5 ± 1.1

[a] mg of protein per g tissue.
[b] $\Delta A_{490-450}$ per mg of protein per ml.
[c] nmol of product per mg of protein per min.

TABLE 2

Species difference in pulmonary drug metabolizing enzyme activity

Data are reported as mean ± SD of 4–8 separate determinations.

Parameter	Species				
	Rat	Rabbit	Mouse	Hamster	Guinea pig
Protein[a]					
Microsomal	9.4 ± 2.9	7.1 ± 2.3	9.3 ± 1.8	8.2 ± 2.6	10.2 ± 2.2
Supernatant	55.6 ± 14.7	47.1 ± 4.8	53.1 ± 11.8	54.5 ± 8.0	55.6 ± 14.1
Cytochrome P-450[b]	0.007 ± 0.002	0.024 ± 0.011	0.016 ± 0.002	0.010 ± 0.005	0.013 ± 0.005
NADPH-cytochrome *c* reductase[c]	55.0 ± 31.0	94.0 ± 35.0	133.0 ± 44.0	97.0 ± 27.0	93.0 ± 8.0
Hydroxylases[c]					
Aniline	<0.1	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.4	0.2 ± 0.1
Biphenyl	0.2 ± 0.1	1.8 ± 0.5	0.7 ± 0.2	1.2 ± 1.0	0.8 ± 0.6
Aminopyrine demethylase[c]	0.3 ± 0.1	1.5 ± 0.8	0.4 ± 0.2	0.3 ± 0.2	0.6 ± 0.3
Glutathione S-aryltransferase[c]	2.1 ± 0.3	5.3 ± 1.5	21.3 ± 4.2	2.8 ± 0.4	4.7 ± 2.9
N-Acetyltransferase[c]					
PABA	0.6 ± 0.5	2.0 ± 0.5	1.6 ± 0.8	2.0 ± 0.9	2.4 ± 0.8
Sulfadiazine	0.2 ± 0.4	<0.1	<0.1	<0.1	<0.1
UDP-glucuronyltransferase[c]					
PNP	0.8 ± 0.8	0.4 ± 0.8	1.5 ± 0.7	0.9 ± 1.3	<0.1
OAP	0.2 ± 0.1	0.3 ± 0.3	0.6 ± 0.4	1.0 ± 0.5	0.9 ± 0.6

[a] mg of protein per g of tissue.
[b] $\Delta A_{490-450}$ per mg of protein per ml.
[c] nmol of product per mg of protein per min.

and this species reproducibly demonstrated UDP-glucuronyltransferase activity with PNP.

Kidney. Renal activities of drug-metabolizing enzymes for the five species are shown in table 3. Drug-metabolizing ability of the kidney is considerably less than for the liver but generally somewhat greater than for lung.

Morphology of Microsomal Fractions. *Liver.* Previous investigations have concerned themselves with the fine structure of hepatic microsomal fractions from rats and other species (24, 25). In the present study, no consistent or significant ultrastructural differences were noted among hepatic microsomes from the five species examined. A typical field consisted primarily of rough- and smooth-surfaced vesicles and glycogen rosettes together with occasional structures resembling lysosomes and fragments of Golgi apparatus.

Lung. It has been previously reported (23) that microsomal vesicles prepared from the lungs of rabbits appear in electron micrographs as aggregates or clusters of vesicles in a microfibrillar matrix. We have confirmed this observation and extended it to include all five species investigated. Rough and smooth microsomal vesicles were seen to be aggregated with large numbers of electron-dense particles resembling polysomes in a rather amorphous or fibrillar matrix. These aggregates were seen in lung microsomes from all five species.

Kidney. Renal microsomes consisted predominantly of rough and smooth vesicles, free polysomes, and a minor amount of amorphous material. There was a slight tendency toward vesicular aggregation which was less pronounced and much less consistent than with pulmonary microsomes.

Discussion

The literature contains widely discrepant reports concerning the relative MFO activity in hepatic and extrahepatic tissue of different laboratory species (7, 26, 27). On the one hand, very low levels of enzyme activity have been reported in the lungs and kidneys of rats, guinea pigs, and mice (7, 28), while on the other hand, our laboratory and others have reported enzyme activities in rabbit lung microsomes equal to or slightly higher than the activities in rabbit liver (5, 6, 29). The present investigation was initiated in order to shed further light on this apparent species-related difference.

In order to facilitate direct comparison of hepatic and extrahepatic enzyme activities, the data presented in tables 1–3 have been recalculated

TABLE 3

Species differences in renal drug metabolizing enzyme activity

Data are reported as mean ± SD of 4–8 separate determinations.

Parameter	Species				
	Rat	Rabbit	Mouse	Hamster	Guinea pig
Protein[a]					
Microsomal	17.9 ± 5.0	13.6 ± 2.6	18.6 ± 2.9	16.2 ± 2.8	13.4 ± 2.3
Supernatant	43.9 ± 3.3	44.8 ± 4.2	44.2 ± 10.7	40.5 ± 5.5	45.7 ± 9.3
Cytochrome P-450[b]	0.013 ± 0.003	0.023 ± 0.015	0.036 ± 0.021	0.018 ± 0.001	0.029 ± 0.017
NADPH-cytochrome *c* reductase[c]	52.0 ± 10.0	34.0 ± 3.0	77.0 ± 19.0	62.0 ± 9.0	57.0 ± 4.0
Hydroxylases[c]					
Aniline	<0.1	<0.1	0.2 ± 0.1	0.4 ± 0.2	<0.1
Biphenyl	<0.1	0.2 ± 0.1	<0.1	0.6 ± 0.3	<0.1
Aminopyrine demethylase[c]	0.6 ± 0.4	<0.1	0.6 ± 0.4	0.7 ± 0.2	0.3 ± 0.2
Glutathione S-aryltransferase[c]	3.8 ± 1.4	7.4 ± 2.8	36.0 ± 14.0	3.6 ± 1.7	7.5 ± 2.2
N-Acetyltransferase[c]					
PABA	1.8 ± 0.4	0.7 ± 0.2	6.1 ± 1.9	6.2 ± 1.3	9.4 ± 2.2
Sulfadiazine	<0.1	<0.1	0	0	<0.1
UDP-glucuronyltransferase[c]					
PNP	3.3 ± 1.8	2.9 ± 1.3	0.7 ± 0.5	1.8 ± 1.2	1.0 ± 0.6
OAP	0.8 ± 1.1	<0.1	0.2 ± 0.1	0.3 ± 0.1	1.1 ± 0.5

[a] mg of protein per g of tissue.

[b] $\Delta A_{490-450}$ per mg of protein per ml.

[c] nmol of product per mg of protein per min.

and are expressed (table 4) as lung to liver or kidney to liver ratios. Of the animal species examined in this investigation, it appears that only the rabbit and perhaps to a lesser degree the hamster have significant and consistent levels of pulmonary MFO activity (table 4). The qualitative relation between the activity of the MFO system in lung of the various species studied (table 2) confirms work of others (6, 7, 29) and extends our comparative knowledge to two other species. With the exception of UDP-glucuronyltransferase activity, the rabbit lung is consistently the most active pulmonary system over a wide range of parameters. The rat and the guinea pig, and with one exception the mouse, had consistently low pulmonary MFO activity. Low pulmonary cytochrome P-450 concentration has previously been reported (23, 29) for rabbit, and the same trend appears for other species (table 2) and for NADPH-cytochrome *c* reductase activity (23, 29, 30). Results of pulmonary UDP-glucuronyltransferase activity with PNP as acceptor substrate (table 2) are quantitatively different than those of (9) for guinea pig and rat but correlate well with those of (29) for the rabbit, where values of glucuronyltransferase activity are near zero in the lung.

With regard to renal microsomal MFO activity, the mouse appears to be the only species tested that exhibited appreciable activity when compared to the hepatic system (table 4), although enzyme activity from some other species approached that of the mouse. Little work with the renal MFO system of the mouse has been previously published, but work with other species have demonstrated that protein yield and cytochrome P-450 levels were similar to ours (23, 29, 31), and that the mouse kidney has the same qualitative relation of protein content as is found with liver and lung.

It seems as if the two extrahepatic organs that were studied are approximately equal in overall MFO activity. In 40% of the parameters, the lung had the greater value, whereas in 33% of the instances the kidney had the greater value. The parameters in which each organ excelled and their absolute values, of course, are different for each organ, but the dominance of one extrahepatic system by the other is not demonstrated.

The N-acetyltransferase activity toward sulfadiazine was at or below the limits of detection in hepatic and extrahepatic tissues of all species examined (tables 1 and 4). By contrast, the same activity measured with PABA as substrate was easily measurable in most tissues. However, no consistent organ or species trends were readily discernible from the data (tables 1–4). These data are consistent with published results for rabbit pulmonary activity of PABA and for glutathione S-aryltransferase (5, 32).

The explanation for the low enzyme activities in extrahepatic organs may be either lower amounts of enzymes or less active enzymes. Several other explanations, however, are also possible. It may be that the extrahepatic organs elaborate an inhibitor of enzyme activity or the metabolic profile (*i.e.*, the

TABLE 4

Ratio of hepatic to extrahepatic drug metabolism in several animal species

Parameter	Rat		Rabbit		Mouse		Hamster		Guinea Pig	
	Lung/ liver	Kidney/ liver	Lung/ liver	Kidney/ liver	Lung/ liver	Kidney/ liver	Lung/ liver	Kidney/ liver	Lung/ liver	Kidney/ liver
Cytochrome P-450	0.07	0.13	0.14	0.13	0.15	0.33	0.07	0.13	0.10	0.23
NADPH-cytochrome *c* reductase	0.29	0.28	0.62	0.22	1.22	0.71	0.35	0.22	0.41	0.25
Hydroxylases										
Aniline	<0.05	<0.05	0.33	<0.05	0.20	0.13	0.19	0.15	0.25	<0.05
Biphenyl	0.13	<0.05	1.06	0.12	0.25	0.05	0.35	0.18	0.20	<0.05
Aminopyrine demethylase	0.30	0.06	0.15	<0.05	<0.05	<0.05	0.20	<0.05	0.07	<0.05
Glutathione S-aryltransferase	<0.05	<0.05	0.24	0.24	0.29	0.48	0.08	0.10	0.08	0.13
N-Acetyltransferase										
PABA	0.33	1.00	1.11	0.39	0.25	0.94	0.15	0.47	0.34	1.32
Sulfadiazine	1.00	<0.05	0.01	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
UDP-glucuronyltransferase										
PNP	0.18	0.75	0.06	0.44	0.68	0.32	0.18	0.36	<0.05	0.37
OAP	0.22	0.89	0.13	<0.05	0.60	0.20	0.63	0.19	0.36	0.44

type of metabolites and their relative amounts) is different from the liver. In addition, the product we assayed may be further metabolized to other metabolites. For example, rat liver microsomes hydroxylate aniline almost exclusively in the 4-position. The colorimetric assay we utilized in estimating aniline hydroxylase activity detects 4-hydroxyaniline specifically, and if renal or pulmonary microsomes hydroxylated aniline in the 2- or 3-positions, these products would not be detected with our methodology. Previous work from our laboratory has dealt extensively with the question of whether an inhibitor was produced in pulmonary incubations and no evidence was found to support this hypothesis (33). The question of an altered metabolic profile in extrahepatic organs is beyond the scope of the present work. In an attempt to appraise whether the reaction products that we assayed in our experiments were being produced but further metabolized, we incubated pulmonary and renal microsomes from rabbit, rat, and mouse in the presence of at least two representative concentrations of the following products: formaldehyde, *p*-aminophenol, (PAP) and *p*-nitrophenyl-β-D-glucuronide. No substrate was present, but all other incubation conditions were held constant. In part of the samples the protein was denatured prior to incubation and the rest were denatured in the usual manner at the end of the incubation period. With *p*-aminophenol, PNP-glucuronide, and formaldehyde, the amount of product recovered from the zero-time blanks was equal to that in the incubated samples, suggesting that for these three pathways in the tissues mentioned, there was no further metabolism of the products we assayed. A single preliminary experiment suggested, however, that 4-hydroxybiphenyl might be further metabolized by extrahepatic tissues under certain conditions. Until further experiments are conducted to determine whether the metabolic profile is the same in the three organs, it may be assumed that the reason for low microsomal enzyme activity in extrahepatic organs may be due to a decrease in either the amount or activity of enzymatic protein from microsomes of these organs.

It is obvious from the data presented that no single animal species is universally desirable for studies of extrahepatic and hepatic drug metabolism. For example, the mouse is relatively deficient in hepatic cytochrome P-450 and NADPH-cytochrome *c* reductase but is nearly unexcelled in its extrahepatic complement of these components. In addition, no single species can be said to be most active with respect to any single organ. The rabbit liver, for example, provides the highest activity in the glucuronyltransferase pathway toward two different substrates but has quite low relative ability to demethylate or hydroxylate substrates, whereas the rabbit pulmonary system is unexcelled in all activities.

When the data are carefully examined, however, some trends do appear. When all of the pathways or components are examined for each of the three organs in each species, the rat, perhaps the most commonly utilized species in drug metabolism research, appears to be relatively deficient in overall drug-metabolizing activity. The rat activity was the highest obtained in less than 25% of all analyses. This is contrasted with the hamster, which had the highest values in 50% of the activities analyzed. Organ-specific differences are even more striking. The rat values exceed those for all other species in only 27% of the pathways studied with hepatic enzymes, whereas 67% of the values obtained with the hamster liver were unexceeded by other species.

As can be expected, species differ not only in organ activity but also in the pathway studied. Mouse tissue was unexcelled in its activity of soluble fraction enzymes greater than 90% of the time but had the poorest hepatic and renal glucuronyltransferase activity of all species studied.

The hazard in making interlaboratory comparisons of hepatic enzyme activity from a single species is apparent from the wide range of absolute specific activities reported in the literature for a given MFO pathway or component. The picture becomes even more complicated when one then adds studies of extrahepatic drug metabolism in more than one species. It is hoped that the present contribution will aid in making comparisons more valid, if only by providing qualitative ratios of activity for the contribution from hepatic and extrahepatic sources in species.

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