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METABOLISM OF ETHANOL AND ACETALDEHYDE IN PARENCHYMAL AND NON-PARENCHYMAL RAT LIVER CELLS

MATS SJÖBLOM and JØRG MØRLAND
Institute of Medical Biology, University of Tromsø, N-9001, Tromsø, Norway

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Abstract—Suspensions of isolated parenchymal (P) and non-parenchymal (NP) cells were prepared by collagenase perfusion followed by centrifugation of the primary cell suspension. Suspensions of P cells were able to metabolize ethanol (8–16 nmoles/min/10^6 viable cells) while NP cells did not metabolize ethanol at all. Acetaldehyde was metabolized in P-cell suspensions at rates ranging from 14 to 20 nmoles/min/10^6 viable cells. Some acetaldehyde metabolism also occurred in NP-cell suspensions (0.18–0.33 nmoles/min/10^6 viable cells). In accordance with these studies on ethanol and acetaldehyde metabolism we found alcohol dehydrogenase activity only in homogenates of P cells, and aldehyde dehydrogenase activity in homogenates of P cells was 20 times higher per cell than in homogenates of NP cells. It was concluded that the P cells of rat liver are responsible for ethanol metabolism and probably also responsible for most, if not all metabolism of acetaldehyde arising from ethanol oxidation. Biochemical effects which are consequences of ethanol metabolism are probably not found in NP cells.

It is usually assumed that ethanol metabolism takes place in the parenchymal (P) cells of the liver. Studies on isolated rat parenchymal liver cells in suspension have supported this view [1–4]. Whether non-parenchymal (NP) liver cells are able to metabolize ethanol has not been studied according to our knowledge. It appeared of interest to investigate this possibility since a series of cellular effects of ethanol in the liver has been considered to be the consequence of ethanol metabolism and not ethanol itself [5–8]. Furthermore, the main metabolite of ethanol, acetaldehyde, may have some importance in the toxic actions of ethanol, and it appeared of interest to study how the various types of liver cells were able to handle this metabolite. During ontogenetic development, the activity of ethanol and acetaldehyde metabolizing enzymes in the liver changes dramatically [9–13], as does the proportion of NP cells to P cells [14]. This, too, called for a further study of ethanol and acetaldehyde metabolism in the various cell types. In other studies we have obtained indirect evidence that NP rat liver cells do not metabolize ethanol [15, 16]. In the present report we studied the ability of isolated NP and P rat liver cells to metabolize ethanol and acetaldehyde by direct methods, as well as their content of enzymes responsible for ethanol and acetaldehyde metabolism.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–300 g) from Møllegaards Avlslaboratorium, Skensved, Denmark, were kept on a 12-hr light–12-hr dark cycle. The animals had free access to rat chow from Felleskjøpet AS, Oslo, Norway, and tap water until the moment of sacrifice.

Preparation of cells. Primary-cell suspensions containing both P and NP cells were prepared after perfusion of the liver with a collagenase solution according to Berry and Friend [17] as modified by Seglen [18] and described previously [19, 20]. The liver was disrupted at 0–5° after the perfusion. P cells were prepared by centrifugation of the primary cell suspension at 0–5° for 90 sec at 45 *g*. The pellet containing P cells was washed and recentrifuged twice at 35 *g* for 30 sec in the incubation medium (see below) at 20°. NP cells were usually prepared following the slightly modified procedure from Nilsson and Berg [21]. The 45 *g* supernatant obtained at 0–5° was centrifuged for 90 sec at 90 g at 0–5°. The supernatant was then centrifuged at 600 *g* for 3 min at 20°. The resulting pellet, containing NP cells, was washed and recentrifuged twice at 600 *g* for 3 min at 20° in the incubation medium (see below). P cells and NP cells prepared this way were used in both metabolism and enzyme experiments.

In one experiment NP cells were prepared by the method of Mills and Zucker-Franklin [22] as described by Berg and Boman [23], where the primary-cell suspension was incubated with 0.28% pronase for 60 min at 37° to destroy the P cells. The suspension was then centrifuged twice at 600 *g* for 3 min and the pellet treated as described above. These NP cells were used for enzyme activity studies only.

In some metabolism experiments suspensions of dead NP and P cells were used. Such preparations were obtained by freezing and thawing the various cell suspensions.

All preparations of cells were studied under a microscope and counted in a Bürker chamber. Viability was tested by Trypan-blue exclusion.

Metabolism experiments. Experiments on ethanol and acetaldehyde metabolism were carried out in 7.5-ml polyethylene tubes with four or five parallels of a 1-ml cell suspension oscillating 167 times/min under air at 37°. The tubes were sealed with polyethylene stoppers. Both P and NP cells were incubated in a medium consisting of 140 mM NaCl, 5.4 mM KCl, 0.2 mM $MgSO_4$, 2.0 mM $CaCl_2$,

0.34 mM Na_2HPO_4, 0.35 mM KH_2PO_4, 5.5 mM glucose and 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.5. In addition 20 mg bovine serum albumin, 30 μg penicillin and 250 μg streptomycin sulfate were added per millilitre of medium. The cell concentrations used were 8.4×10^6 P cells/ml and 84×10^6 NP cells/ml in the ethanol metabolism studies, and 1.3×10^6 P cells/ml and 13×10^6 NP cells/ml in the acetaldehyde metabolism studies. Only NP-cell suspensions completely uncontaminated with P cells were used in the metabolism experiments.

The ethanol elimination rates in the two types of cell suspension were studied by the addition of various amounts of ethanol to final concentrations of 11–87 mM. The reaction was stopped after 0, 15, 30, 60 and 90 min by the addition of 2 ml ice-cold 0.3 M perchloric acid. After centrifugation for 10 min at 1500 *g* at 4°, the clear supernatant was used for ethanol determinations with a F42 Perkin–Elmer head-space gas chromatograph. Propanol was used as the internal standard.

Acetaldehyde was added to 200 μM, together with 1 mM 4-methylpyrazole (final concentration) to prevent reduction to acetaldehyde to ethanol by alcohol dehydrogenase. The acetaldehyde oxidation was stopped by the addition of 5 ml ice-cold 0.6 M perchloric acid containing 50 mM thiourea after 0, 3, 6 and 9 min (P cells), or 0, 15, 30 and 60 min (NP cells). The samples were centrifuged immediately at 4° for 10 min at 1500 *g*. The clear supernatant was used for determinations of acetaldehyde in a F42 Perkin–Elmer head-space gas chromatograph as described by Eriksson *et al.* [24].

Enzyme experiments. The activities of alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1, ADH) and aldehyde dehydrogenase (aldehyde: NAD oxidoreductase, EC 1.2.1.3, ALDH) were studied at different stages of the preparation of cells as follows: in a biopsy from the unperfused liver consisting of a small lobulus removed after ligation, in another small lobulus of the remaining liver after perfusion with collagenase, in a sample of the primary-cell suspension, and in samples from each step in the preparation of P and NP cells. In some of these experiments, however, the final NP-cell suspensions were contaminated with P cells (approximately 3 per cent), probably due to a small difference during the initial steps of the cell preparation procedure (centrifuging the 45 *g* supernatant at 60 *g* instead of 90 *g*). Results from such suspensions were included to demonstrate the importance of having pure NP-cell suspensions. In experiments where ADH and ALDH were studied in the cell suspensions the cells were not incubated. After preparation of the cell suspensions, 5 ml was centrifuged for 1 min at 1600 *g* and enzyme activities were measured in both the supernatants representing the media and the pellets representing the cells. Protein was determined in these fractions as well as in liver samples to calculate specific activities and the total recovery of enzyme activities. Liver samples, medium and cells were frozen and stored at −80° until analysis.

The cells and livers were homogenized in an isotonic sucrose medium [25] with a Potter–Elvehjem homogenizer. The homogenates were made 10 per cent (w/v) and used fresh for enzyme activity measurements. The cell incubation media were used undiluted. ADH was measured at 30° spectrophotometrically according to Büttner [26] by following the oxidation of NADH at 340 nm during the reduction of acetaldehyde to ethanol. There are at least two different forms of ALDH in rat liver [27, 28]. ALDH I is located in mitochondria and has a high affinity (low K_m) for acetaldehyde, while ALDH II is found both in mitochondria and microsomes and has a much lower affinity (high K_m) for acetaldehyde. Both ALDH I and II were measured spectrophotometrically at 30° by following the reduction of NAD at 340 nm during the oxidation of acetaldehyde according to Tottmar *et al.* [28]. Protein was measured according to Lowry *et al.* [29] with bovine serum albumin as reference.

Chemicals. Collagenase (Type I), albumin (Fraction V defatted), HEPES, NAD, NADH, TRIS and rotenone were obtained from Sigma Chemicals Co., St. Louis, U.S.A. Crystalline penicillin was purchased from A/S Apotekernes Laboratorium, Oslo, Norway, and streptomycin sulfate from Novo Industri A/S, Copenhagen, Denmark. Pronase (B-grade) was obtained from Calbiochem., San Diego, U.S.A. 4-Methylpyrazole was purchased from Labkemi AB, Gothenburg, Sweden. Pyrazole was obtained from Fluka AG, Buchs, Switzerland. Other chemicals used were p.a.

RESULTS

Ethanol metabolism

Ethanol metabolism in P cells was studied at different ethanol concentrations. There were no differences in the rates of metabolism between cells from the same preparation incubated at different ethanol concentrations ranging from about 10 to 90 mM (Fig. 1). In all cell suspensions studied, the rate of ethanol oxidation was constant for 90 min (data not shown), and varied from about 8 to 16 nmoles/min/10^6 living cells between the various suspensions (Fig. 1). Ethanol metabolism might also be expressed as 4–8 nmoles/min/mg protein or as 0.8–1.6 μmoles/min/g packed cells. Ethanol disappearance was only found when incubated in tubes containing living cells. Known amounts of ethanol added to cell suspensions immediately before sampling were completely recovered during subsequent analysis.

NP cells were incubated with 22 or 87 mM ethanol only—on several occasions. At neither concentration was there any disappearance of ethanol and it was concluded that NP cells do not possess the ability to metabolize ethanol. The cell concentration used was 10 times that for the P cells and almost equal when referred to the protein content.

The initial viability of P and NP cells used in these experiments with living cells was between 80 and 90 per cent in all experiments, as was the viability after incubation for 90 min.

Acetaldehyde metabolism

Acetaldehyde metabolism was studied for 60 min in three experiments in P and NP cells in the presence of 200 μM substrate. Both cell types metabolized

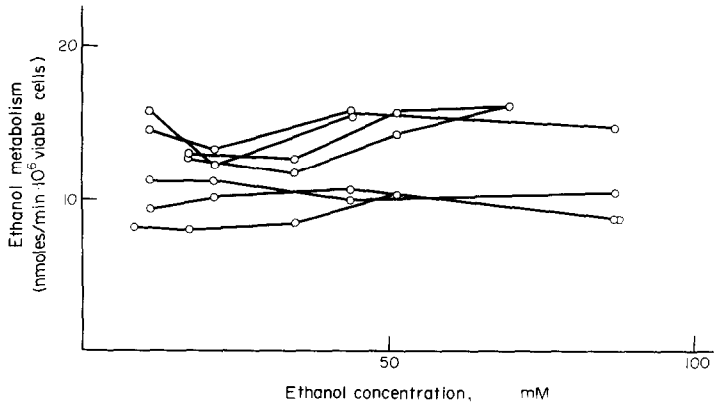

Fig. 1. Ethanol metabolism in suspensions of isolated parenchymal rat liver cells. 8.4×10^6 cells/ml were incubated in the presence of various ethanol concentrations (see abscissa) for 90 min. Each point represents the rate of ethanol elimination in one experiment determined from ethanol concentrations measured after 15, 30, 60 and 90 min. Points connected with a line represent experiments on cells derived from the same primary-cell suspension.

acetaldehyde at this concentration, and the rate of acetaldehyde oxidation was constant for the period studied. P cells were about 10 times more efficient than NP cells when referred to amount of cellular protein and about 100 times more efficient with respect to metabolism per cell (Table 1). The metabolism in P cells varied from 14.4 to 20.6 nmoles/min/10^6 living cells equivalent to 7–10 nmoles/min/mg protein or 1.4–2.0 μmoles/min/g packed cells. In NP cells the rate of acetaldehyde oxidation varied from 0.18 to 0.33 nmoles/min/10^6 living cells. Acetaldehyde disappearance in tubes containing only dead cells was less than 5 per cent when compared with tubes containing living cells. The values given above are corrected with respect to this disappearance. No loss of acetaldehyde was found when incubated only with the medium. The recovery of the method was above 90 per cent.

The viability of P and NP cells was between 80 and 90 per cent during the whole incubation period in all experiments on acetaldehyde metabolism with living cells.

Enzyme activities

The viability in the final suspensions were 72.9 per cent (P cells) and 97.5 per cent (NP cells) before homogenisation for enzyme determination was performed (mean of six experiments). No change in the specific activity of ADH was found when samples taken at different stages of the preparation procedure were studied, except in the case of NP-cell suspensions (Table 2). In the pure NP-cell suspensions no ADH activity was detected (Table 2). The specific activity in the NP-cell suspension which was contaminated with about 3 per cent P cells, was roughly as would be expected when NP cells were completely devoid of ADH activity. The protein concentrations of the pure cell suspensions were 2.1 mg/10^6 cells (P) and 0.25 mg/10^6 cells (NP). The mean ADH activity in P-cell suspensions could therefore also be expressed as 84.3 nmoles/min $\times$ 10^6 cells.

The specific activities of ALDH I and II were unchanged in the following preparations; intact liver, perfused liver, primary-cell suspension and parenchymal-cell suspension, while they were lower in NP-cell suspensions (Table 3). It should be noticed that even in pure NP-cell suspensions, there were substantial amounts of ALDH I and II activities. The activities of ALDH were 20 times lower in NP cells than in P cells when referred to activity per cell (Table 3). However, since a P cell contains about eight times more protein than a NP cell, the ratio between the specific activities was approximately 2.5.

When the enzyme activities of either ADH, ALDH I (low K_m) or ALDH II (high K_m) in the total of P cells and NP cells were combined they constituted about 75–85 per cent of the corresponding activities found in cells in the primary suspensions. The same values were found when similar calculations were carried out for cell protein. The amount of the total ADH activity of the primary-cell suspension which was found in the medium varied considerably between experiments, from 2 to 74 per cent. The high medium values were, however, not correlated to great leakage of ADH activity during the subsequent preparation steps or to low specific activities in the P cells prepared from these suspensions. Substantially no ALDH activity was

Table 1. Acetaldehyde metabolism in parenchymal (P) and non-parenchymal (NP) rat liver cells*

Primary-cell suspension	P cells (nmoles/min/10^6 viable cells)	NP cells
No. 1	14.4	0.18
No. 2	20.6	0.22
No. 3	17.9	0.33

* 1.3×10^6 P cells/ml or 13×10^6 NP cells/ml were incubated with 200 μM acetaldehyde as substrate in the presence of 1 mM 4-methylpyrazole. Each value represents the rate of acetaldehyde oxidation in each experiment determined from acetaldehyde concentrations measured at 0, 3, 6 and 9 min (P cells) or 0, 15, 30 and 60 min (NP cells). Results derived from the same primary suspension are presented on the same line.

Table 2. Alcohol dehydrogenase (ADH) activity during the cell preparation procedure*

Preparation	No. of exp.	ADH (nmoles/min/mg protein)	Parenchymal-cell protein† (% of total proteins)
Intact liver	2	48.1 (45.9–50.2)	
Perfused liver	2	45.6 (42.8–48.3)	
Primary-cell suspension	2	48.2 (45.4–50.9)	97.8 (97.6–97.9)
Parenchymal-cell suspension	6	41.7 ± 2.7	99.7 ± 0.4
Non-parenchymal-cell suspension—pure	4	0 ± 0	0 ± 0
Non-parenchymal-cell suspension—contaminated‡	2	9.6 (4.4–14.8)	37.8 (34.5–41.0)

* Each value represents means ± S.E.M. (or range in parentheses).
† The percentage of protein originating from parenchymal cells was calculated from the proportion of parenchymal cells present in the respective preparations.
‡ Containing about 3 per cent parenchymal cells.

found in the medium of the primary-cell suspension and less than 5 per cent in the media of subsequent cellular preparation.

In one additional experiment the NP cells were prepared by the pronase method. No activity of ADH was found, and ALDH I and II activities were 0.29 and 1.80 nmoles/min/10^6 viable cells respectively. The latter value was in agreement with that obtained with cells prepared by centrifugation, while the former was markedly lower although at the same order of magnitude as the lowest values recorded in cells prepared by centrifugation.

DISCUSSION

Ethanol metabolism

In our study isolated parenchymal rat liver cells metabolized ethanol at rates ranging from 0.8 to 1.6 μmoles/min/g packed cells. These rates are close to those reported for similar preparations [3, 4, 30–37] and small discrepancies may be attributed to differences with respect to feeding state of the liver donor [30, 33, 36], the presence of albumin during cell preparation [33] and the choice of buffer for cell incubation [4, 36]. The rates of ethanol metabolism measured in isolated cells are generally lower than corresponding rates found in the isolated perfused rat liver, ranging from 1 to 2 μmoles/min/g liver [38–41], and markedly lower than those found *in vivo*, ranging from 2.3 to 5 μmoles/min/g liver [31, 42]. The discrepancy could have been due to a loss of ADH in isolated cells, although this has been suggested not to be the cause [32, 35]. Our ADH measurements in isolated P cells supported the latter suggestion since the specific activity of ADH was the same in homogenates of P cells as in homogenates of whole liver. Since the rate of ethanol metabolism in intact P cells (8–16 nmoles/min/10^6 cells) was lower than the corresponding capacity of these cells calculated from their ADH-activity (about 85 nmoles/min/10^6 cells) other factors than ADH appeared to be rate limiting in isolated cells as has been suggested previously [3, 4, 30–33, 35, 36]. The difference between ADH-activity measured and ability to metabolize ethanol in our cell preparation could also partly be due to the present method of measuring ADH-activity with acetaldehyde as substrate giving four to five times higher V_{max} than when ethanol is used as substrate [31, 32].

Anyhow, these observations also called for a closer study of whether the metabolism occurring in suspensions of P cells was representing metabolism in some kind of diluted homogenate only, being independent of intact, living cells. Experiments with dead cells showing no ethanol metabolism clearly demonstrated that ethanol metabolism depended on living cells.

Our preparation of hepatocytes did not reveal increased rate of ethanol metabolism at high ethanol concentrations which has been reported by some other workers [3, 34, 43–45]. Differences in the degree of depletion of substrates in isolated cells as well as the composition of the gas phase may explain this, as discussed by Khanna *et al.* [46]. In any case, because of the low rates of ethanol metabolism in isolated cells, it is doubtful whether results in this preparation will have any relevance to the situation prevailing *in vivo*.

Suspensions of NP cells did not metabolize ethanol at all. ADH-activity could not be detected in homogenates of NP cells either prepared by the centrifugation or the enzymatic method (pronase). It could be argued that ADH had been washed out completely from NP cells during preparation. This would imply rather marked effects on cellular integrity, which were unlikely since the NP-cell fractions usually contained a higher proportion of viable cells than the corresponding P-cell fractions. Our results thus indicate that hepatic NP cells are not able to metabolize ethanol at all. Similar results have been obtained in another laboratory (G. Bengtsson, personal communication).

Acetaldehyde metabolism

In our study P cells metabolized acetaldehyde at rates ranging from about 1.4 to 2.0 μmoles/min/g packed cells at a substrate concentration of 200 μM when acetaldehyde reduction was blocked with 1 mM 4-methylpyrazole. These rates compared well to those observed in isolated cells [47], the perfused rat

Table 3. Aldehyde dehydrogenase (ALDH) activities during the cell preparation procedure*

Preparation	No. of exp.	ALDH I (low K_m)		ALDH II (high K_M)	
		(nmoles/min/mg protein)	(nmoles/min/10^6 cells)	(nmoles/min/mg protein)	(nmoles/min/10^6 cells)
Intact liver	2	10.1 (8.9–11.3)		20.2 (17.1–23.2)	
Perfused liver	2	10.0 (8.8–11.2)		21.1 (17.3–24.8)	
Primary-cell suspension	2	10.8 (9.5–12.1)		19.9 (18.1–21.7)	
Parenchymal-cell suspension	6	11.1 ± 1.0	24.8 ± 2.4	18.6 ± 0.8	40.0 ± 2.1
Non-parenchymal-cell suspension—pure	4	5.46 ± 1.42	1.44 ± 0.35	7.14 ± 1.78	1.72 ± 0.43
Non-parenchymal-cell suspension—contaminated†	2	4.08 (3.58–4.57)		9.52 (7.53–11.5)	

* Each value represents mean ± S.E.M. (or range in parentheses).
† Containing about 3 per cent parenchymal cells.

liver [38, 48], and in rat liver homogenates [49, 50] at similar substrate concentrations. Suspensions of P cells thus oxidized acetaldehyde at this concentration at a rate somewhat faster than its rate of formation (Fig. 1), probably partly explaining the lack of accumulation of acetaldehyde when ethanol was added to suspensions of P cells [16]. Other explanations obviously exist also, since increasing the rate of ethanol metabolism in cell suspensions does not increase the acetaldehyde level [32]. The rates of acetaldehyde metabolism in suspensions of our P cells were very close to the rates of acetaldehyde oxidation by ALDH I (low K_m) measured in homogenates of such cells. Accordingly a mitochondrial enzyme with low K_m is considered to be the enzyme responsible for acetaldehyde oxidation [3, 27, 28, 47, 51–53], but comparisons between metabolism and enzyme activity must of course be interpreted with caution. Since acetaldehyde was not metabolized in suspensions of dead cells, it could be ruled out that liver cell suspensions were acting merely as diluted homogenates.

Suspensions of NP cells metabolized acetaldehyde at a rate approximately 100 times slower than P cells when metabolism per cell was calculated (Table 1). The activities of ALDH I and II were about 20 times lower in NP cells than in P cells (Table 3). Seen together this indicated that NP cells exhibited some acetaldehyde metabolism. According to our knowledge this has not been studied previously.

Importance of various liver cell types with respect to ethanol metabolism in the whole rat

It has been shown that about 90 per cent of the ethanol metabolism which takes place in an intact animal occurs in the liver [6, 8, 54]. From the present results it appears that P cells only are responsible for this removal of ethanol. However, some fraction of NP cells might have been lost during preparation, so ethanol metabolism in some sub-type of NP cells cannot be completely ruled out.

About 95 per cent of the metabolism of acetaldehyde generated from ethanol takes place in the liver [8, 42, 55]. From the present results it appears that P cells are responsible for nearly all of this metabolism since: (a) acetaldehyde is generated in P cells only, (b) the capacity for acetaldehyde metabolism per cell is about 100 times greater in suspensions of P cells than in suspensions of NP cells, (c) the activities of ALDH I and II per cell are about 20 times higher in P cells than in NP cells, (d) there are about twice as many P cells as NP cells per liver [56, 57].

Consequences of ethanol metabolism in various liver cells

A series of biochemical effects of an acute dose of ethanol have been ascribed to the metabolic alterations caused by alcohol metabolism by ADH [5–8]. Such effects would be expected not to occur in NP cells in the light of the present study. Recent experiments in similar preparations of NP cells have shown that the lactate/pyruvate ratio did not change after the addition of ethanol [15, 16], indicating no redox change in such cells. Some of the metabolic consequences of ethanol might, however, be exported

from P cells to NP cells in the intact organ. It has been shown that a small fraction of the acetaldehyde generated leaves the liver during ethanol oxidation [8, 42, 55] and some acetaldehyde might reach the NP cells. One may speculate whether the levels of ALDH in NP cells are sufficient to protect these cells against possible toxic effects of acetaldehyde. The concentration of ALDH per mg protein was only 2.5 times lower in NP cells than in P cells, which may yield enough protection against the probably lower concentrations of acetaldehyde reaching the NP cells. Other factors as the subcellular localization of ALDH in NP cells would probably also be of great importance in this respect. It has been suggested that several toxic effects of ethanol in the liver are linked to ethanol metabolism. With the present report in mind it is interesting to notice that chronic consumption of ethanol reduces the ratio of P/NP cells in the liver [58–61], indicating a more pronounced cytotoxic effect of ethanol on P cells. Other differences between P and NP cells in the response to ethanol [15, 16] could have a bearing on this as well.

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