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INFLUENCE OF PHENOBARBITAL ON THE DISTRIBUTION AND ELIMINATION OF DESMETHYLIMIPRAMINE IN THE RAT

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Abstract—Male rats received an i.p. injection of 25 mg/kg desmethylimipramine (DMI); the concentrations of the drug and its metabolite didesmethylimipramine (DDMI) were measured after 1–13 hr in liver, lung, kidney, brain and plasma by thin-layer chromatography. Levels of DMI declined steadily with a half-life of approximately 6–7 hr except in lung, where the concentration remained almost unchanged. Oral pretreatment with phenobarbital (PB) for 5 days enhanced elimination slightly. Brain–plasma concentration ratios and partly also kidney–plasma and liver–plasma ratios were decreased in PB-pretreated rats. This also happened when PB administration was stopped 48 hr before DMI injection. DDMI could not be determined reliably in plasma. PB pretreatment led to significantly lower lung–liver, kidney–liver and brain–liver concentration ratios at nearly all times. A decreased brain–plasma ratio for DMI was also observed as a consequence of PB pretreatment in rats sacrificed 5 hr after oral administration of 30 mg/kg DMI.

The concomitant administration of barbiturates to patients receiving tricyclic antidepressants has been observed to result in a lowering of antidepressant plasma levels [1–4]. Similarly, volunteers given nortriptyline reached lower steady-state levels when they ingested additional drugs including barbiturates [5]; the same observation was made in treated epileptics [6]. Though these phenomena are likely to be due to enzyme induction resulting in enhanced metabolic elimination of the antidepressants, no animal experiments are available to support this assumption. Rather, the hydroxylation of desmethylimipramine (DMI) and nortriptyline in the tricyclic system, which can be regarded as major routes of metabolism *in vivo* [7, 8], were reduced in liver microsomes from phenobarbital(PB)-pretreated rats [9]. In preliminary experiments, the DMI elimination from rats *in vivo* was unaltered by previous PB administration [10].

The present studies were therefore concerned with the time courses of DMI and its metabolite didesmethylimipramine (DDMI) in rats without additional treatment, under continuous PB administration, and 48 hr after termination of a PB pretreatment. The experiments revealed an enhancement of DMI elimination, and in addition pronounced alterations of its distribution among tissues when rats had received PB.

MATERIALS AND METHODS

Animal treatment. Male Wistar rats weighing 250–310 g were used. They had free access to tap water and a standard laboratory chow (Altromin R, Altromin GmbH, Lage, Germany) except before i.p. or oral administration of DMI when they were fasted overnight. DMI·HCl (Ciba–Geigy, Basel, Switzerland) was dissolved in 0.6% NaCl at a concentration of 14.2 mg/ml for i.p. injection. Of this solution, 2 ml/kg corresponding to 25 mg/kg free base was injected at 8 a.m. After 1, 5, 9 or 13 hr, the animals were anesthetized with ether and exsanguinated from the inferior caval vein into a heparinized syringe. Plasma, liver, lungs, kidneys and brain were stored at −20°.

For PB pretreatment, 50 mg/kg PB was administered by gavage as an aqueous solution of the sodium salt; the animals subsequently received 1 g/liter PB in their drinking water for 5 days. In one group, the PB treatment was continued right up to the time of sacrifice, while in another one tap water was substituted for the PB solution 48 hr before the DMI injection.

Measurement of drug levels. (a) Determination of DMI and DDMI in liver, lung and kidney. Liver tissue (5 g) was homogenized with 20 ml of 10% NaCl and extracted with 25 ml of *n*-heptane after addition of 0.2 ml of 10% sodium deoxycholate solution and 3.5 ml of 25% ammonia. After centrifugation, a measured aliquot of the heptane was evaporated. Both lungs and kidneys were extracted similarly, using 10 ml of 10% NaCl, 0.1 ml of 10% sodium deoxycholate, 1.2 ml of ammonia and 15 ml of heptane. The residues of the extracts were subjected to thin-layer chromatography (TLC) on 20 × 20 cm plates manually coated with 0.4 mm of silica gel containing a fluorescent indicator (Kieselgel GF_{254}, Merck, Darmstadt, Germany). The extracts were dissolved in chloroform and applied as bands of 4 cm; the plates were pre-run in chloroform–isopropanol (10:1) to the upper edge followed after drying for 3 min by isopropanol–chloroform–acetone–25% ammonia (10:8:0.5:1.5). Bands containing DMI (R_f 0.60) or DDMI (R_f 0.72) were visualized under ultraviolet light (254 nm) and removed and extracted as described previously [11].

Abbrevations—DMI: desmethylimipramine, DDMI: didesmethylimipramine, PB: phenobarbital.

The isolated substances were dissolved in 3 ml of 0.1 N H_2SO_4 and shaken with 0.5 ml of 1,2-dichloroethane. The absorption difference $A_{275}-A_{300}$ of the aqueous phase was used for quantitation, a value of 1.000 from a light path of 1 cm corresponding to 204 μg of DMI or 189 μg of DDMI in 3 ml.

(b) Determination of DMI and DDMI in plasma and brain. Plasma was extracted with toluene, and DMI was determined by *in situ* photometry on TLC plates[12]. Single brains were extracted as described for lung and kidney. The bulk of the lipid material was removed from the extracts by distribution between heptane and 0.01 N H_2SO_4[12]. The purified extracts were chromatographed in the same way as plasma extracts; standards run between the samples contained 200–1000 ng of DMI and 40–240 ng of DDMI.

Average recoveries in the described procedures were 72–81 per cent for DMI and 68–81 per cent for DDMI, depending on the tissue investigated. All values were corrected for recovery.

PB plasma levels were determined by quantitative TLC[13].

Total plasma protein was measured by a modification of the biuret procedure.

RESULTS

Kinetics after i.p. administration to unpretreated rats. Maximal DMI concentrations were observed after 1 hr, the highest level being present in lung followed by liver, kidney and brain (Fig. 1). In liver and kidney, DMI levels declined nearly exponentially, with a half-life of around 6–7 hr. Similar eliminations were observed in brain and plasma in the first 8 hr. In lung, however, the DMI concentration remained nearly constant. As a consequence, the lung–plasma concentration ratio almost doubled with time (Fig. 2). The brain–plasma ratio declined by 30 per cent while the ratios liver–plasma and kidney–plasma did not change appreciably.

Only in liver did the DDMI concentration exhibit a decrease with time (Fig. 3). In lung, it increased more than 2-fold, while in kidney and brain it remained at a constant level. Thus, in all organs it reached 40–50 per cent of the DMI concentration after 13 hr. Levels in plasma were too low for reliable determinations to be made.

Kinetics after i.p. administration to rats pretreated with PB. (a) Studies with PB treatment up to the time of sacrifice. Phenobarbital plasma levels declined steadily from 45 μg/ml one hr after DMI injection to 12 μg/ml 12 hr later. Except in plasma, DMI declined more steeply in all tissues; in brain, its concentration was decreased from the beginning as compared with controls (Fig. 1). This resulted in a decreased brain–plasma concentration ratio at nearly all times, while liver–plasma and kidney–plasma ratios were diminished only after 13 hr (Fig. 2). The increase of the lung–plasma ratio with time was less pronounced than in unpretreated animals.

DDMI was virtually unchanged in liver, whereas significantly lower levels were found in brain at all times and in lung and kidney at most of the times investigated. When DDMI concentrations in extra-

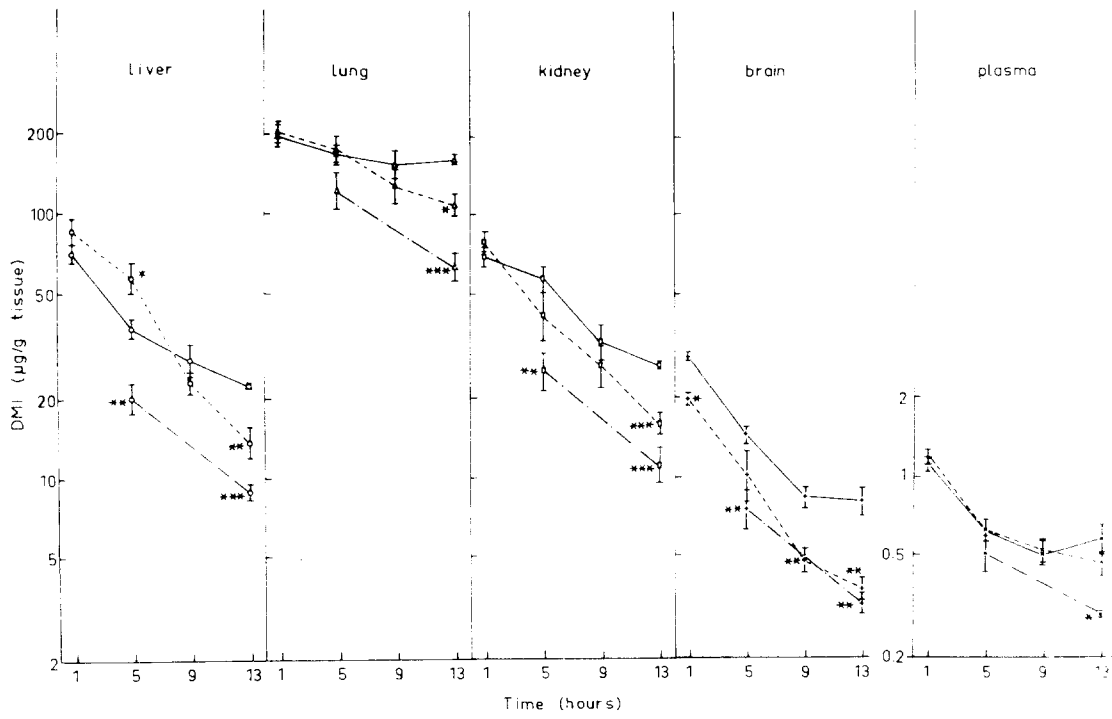

Fig. 1. DMI concentrations in tissues of male rats after i.p. injection of 25 mg/kg DMI. Rats were unpretreated (○——○) or they had received PB orally to the time of sacrifice (△- - - - -△) or up to 48 hr before DMI injection (□– – –□). Vertical bars indicate S.E.M., $N = 4$–7. Differences between pretreated and control groups were significant at levels of $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

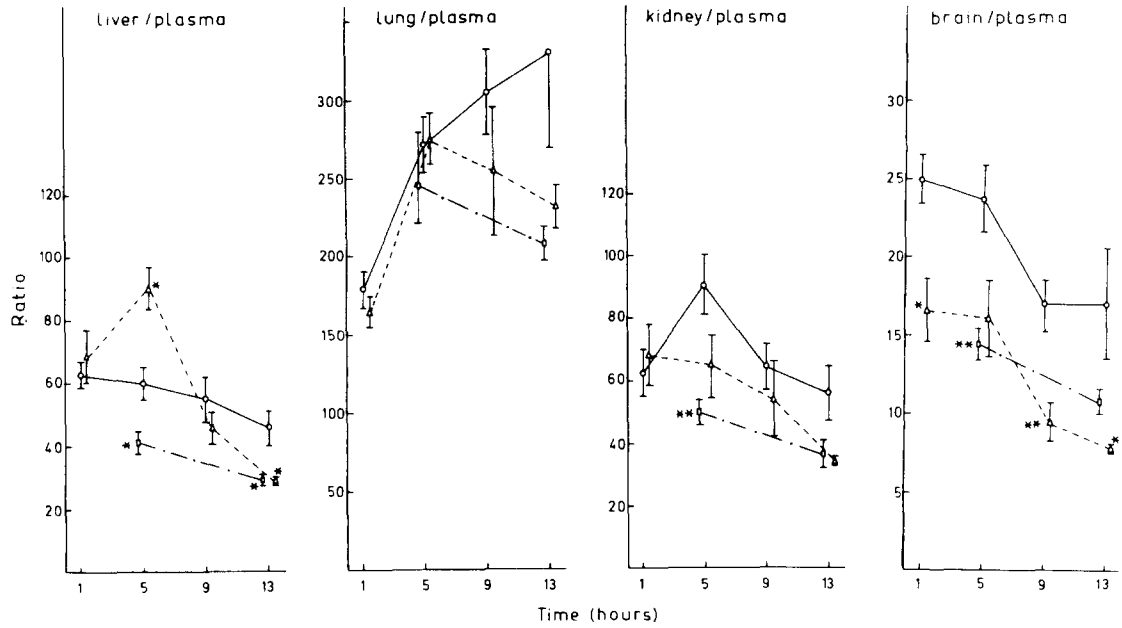

Fig. 2. Organ/plasma concentration ratios for DMI in male rats after i.p. injection of 25 mg/kg DMI. Symbols are the same as in Fig. 1.

hepatic organs were related to those in the liver, decreased ratios were observed almost regularly in PB-treated rats, and the increases of the ratios with time were either abolished (kidney, brain) or substantially diminished (lung) (Fig. 4).

(b) Studies with PB removal 48 hr before DMI injection. Measurements were confined to two times. At both of them, PB plasma levels were around 1 μg/ml (range 0.5–1.9 μg/ml). DMI was decreased as compared to controls in most tissues, and its decline appeared to be steeper, especially in lung, brain and plasma (Fig. 1). Organ–plasma ratios were decreased to an extent similar to cases in which PB administration was not discontinued (Fig. 2). Also

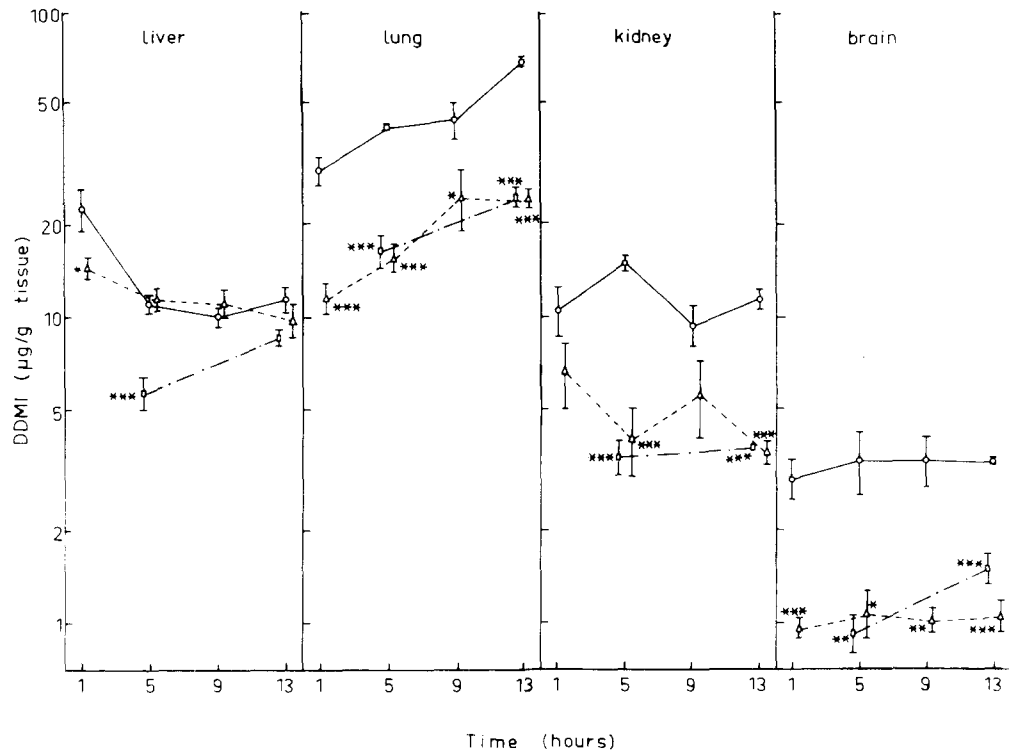

Fig. 3. DDMI concentrations in organs of male rats after i.p. injection of 25 mg/kg DMI. Symbols are the same as in Fig. 1.

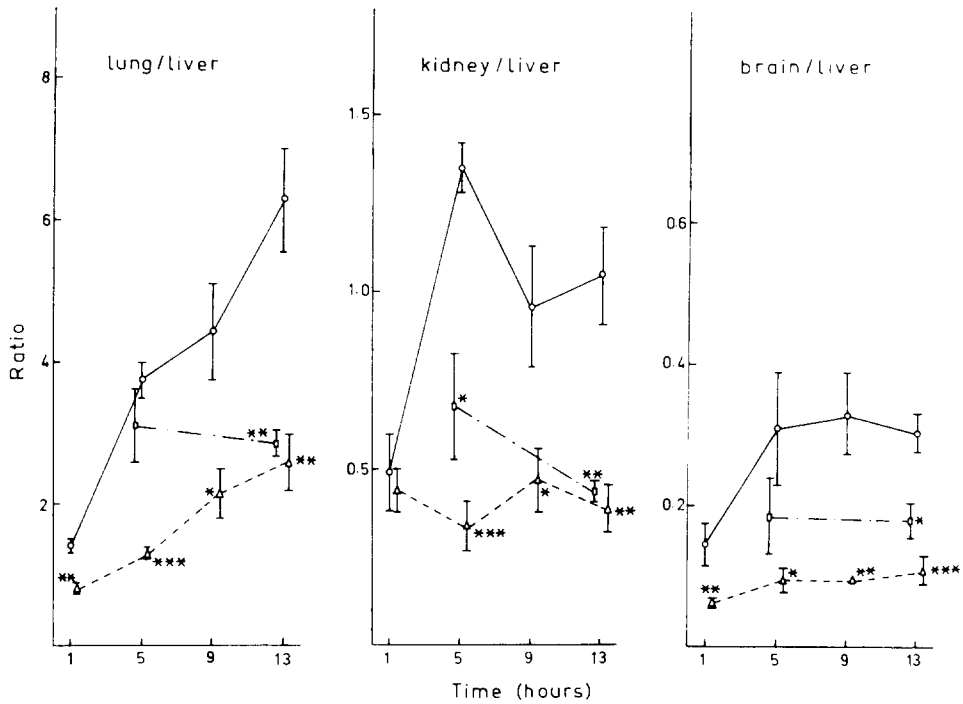

Fig. 4. Extrahepatic organ–liver concentration ratios for DDMI in male rats after i.p. injection of 25 mg/kg DMI. Symbols are the same as in Fig. 1.

DDMI kinetics were changed in much the same way except for a significantly lowered concentration in liver 5 hr after injection (Figs. 3 and 4).

Total plasma protein was determined in some of the rats, and averaged out at 59.9 mg/ml (range 51.1–64.7) in 7 unpretreated animals and 57.5 mg/ml (range 51.4–63.1) in 10 rats given PB up to 48 hr before DMI injection. There was a dependence of plasma protein values on the time interval between DMI injection and sacrifice which probably was due to the formation and reabsorption of a peritoneal exudate. Rats killed after 1 or 5 hr had a mean concentration of 56.0 mg/ml (range 51.1–60.5), whereas those sacrificed after 9 or 13 hr had one of 60.6 mg/ml (range 57.1–64.7, $P < 0.01$).

Brain and plasma concentrations after oral DMI administration. Lower levels were produced by gavage of 30 mg/kg DMI than by i.p. injection of 25 mg/kg. Brain–plasma ratios, however, were similar, and PB treatment without a PB-free interval again led to a significant reduction (Table 1).

DISCUSSION

The preferential accumulation of DMI in parenchymatous organs agrees with previous findings [14–16]. The elimination from lung was unusually slow resulting in a steep increase of lung–plasma ratios with time. Also DDMI increased in lung relative to other organs. In a recent report [17], the authors also described a longer persistence of DMI in lung as compared to liver following a high dose. This behavior corresponds to the kinetics of imipramine [18] and methadone [19] in the perfused rabbit lung. With both drugs, a "non-effluxable" pool exhibiting a half-life of more than 5 hr was observed and it was suggested that a connection existed between this kinetic behavior and the formation of "foamy cells" [16]. The present and previous data [17] on DMI confirm the existence of a slowly emptying pool of a basic amphiphilic drug in the lung *in vivo* and they show that it exists also in the rat, though perfusion experiments with imipramine in the rat lung did not reveal the presence of a non-effluxable pool [20].

The failure of DMI plasma levels to decline expo-

Table 1. Influence of continuous PB pretreatment on brain and plasma levels of DMI 5 hr after oral administration of 30 mg/kg to male rats

Pretreatment	DMI concentration (μg/g) Brain	Plasma	Brain–plasma ratio	N
—	5.8 ± 2.7	0.32 ± 0.09	18 ± 5	4
PB	3.1 ± 1.5	0.40 ± 0.03	8 ± 4*	4

Data represent means ± S.D.
* $P < 0.05$.

nentially was probably a consequence of changes in the protein concentration. These also led to a decrease of liver–plasma, kidney–plasma and brain–plasma ratios during the last 8 hr of the experiment. In preliminary studies* it could be shown that the unbound DMI fraction was higher in the plasma of rats killed 5 hr after injection than of those killed after 13 hr, while the relationship of the protein concentrations was reversed.

The observation that DDMI was formed in considerable quantities is in accordance with data obtained in the isolated perfused liver [8]. Ratios of DMI–DDMI decreased with time e.g. in lung, kidney and brain of unpretreated rats from 7 to 2–2.5 within 12 hr. This is in agreement with the slower elimination of the primary amine from whole rat bodies [14].

Like other tricyclic psychoactive drugs, DMI is subject to a high hepatic extraction. This results from an estimate of its total clearance in humans [21] as well as from its kinetics in the isolated perfused rat liver [10, 22] and in the rat *in vivo* [10]. Evaluation of the *in vivo* data [10] taking into account a red cell–plasma ratio for DMI of 1.47 [16] leads to a total blood clearance of 44 ml hr^{-1} (kg body wt)$^{-1}$. This value is in the order of magnitude of liver blood flow in the rat [23–27]. Therefore, an increase in hepatic drug-metabolizing activity alone will not necessarily lead to an enhancement of drug elimination [28]. An increased clearance will, however, be brought about by an increase in blood flow to the liver [28, 29], as has been shown to occur as a consequence of PB treatment of rats [25, 27]. Therefore the slight enhancement of DMI elimination observed in rats under continuous PB administration does not permit one to conclude that the capacity of the liver to metabolize DMI was augmented. Concerning total metabolism, the present results are not in conflict with experiments on rat liver microsomes which revealed that PB pretreatment led to impaired hydroxylation and enhanced demethylation of DMI [9]. However, DDMI tissue levels were either reduced or unchanged in the PB-treated groups.

An increase in the metabolic activity of the liver will reduce the availability of substances undergoing hepatic extraction when they enter the body *via* the hepato-portal system [28]. Such an effect seems to exist in rats given PB until 48 hr before DMI injection. DMI tissue levels were reduced at both times though a distinctly steeper decline than in the controls was seen only in the lung.

A conspicuous alteration brought about by PB was the reduction of brain–plasma ratios for DMI and the irregular reduction of kidney–plasma and liver–plasma ratios. Additional experiments with oral DMI administration confirmed that the effect on brain–plasma ratios was not confined to studies using the i.p. route. Also the distribution of DDMI was altered, liver concentrations being relatively higher in pretreated rats.

The concentration ratios cannot be taken as reflecting true equilibria. Plasma DMI levels had to be re-established during each circulation as a result of high hepatic extraction. The values obtained represented the net result of the relative rates of drug distribution into the tissues and redistribution back into the plasma. Apparently in the brain this latter process was relatively faster than the former one when rats had been pretreated with PB; other tissues also showed a tendency towards this behavior. Since DDMI plasma levels were not obtained, it is not clear whether PB alters the distribution and redistribution rates between plasma and liver or between plasma and the extrahepatic organs.

The observed alterations in tissue concentration ratios are similar to those brought about by PB with regard to the distribution of a phenothiazine drug metabolite in the rat [30]. The reason for these effects might be found in changes in binding. Apparently these changes are not related to the presence of appreciable PB levels, since they were also seen 2 days after termination of PB administration, when plasma levels had declined to very low values. Therefore, metabolic alterations must have occurred; these are currently under investigation. Preliminary results point to enhanced DMI binding to plasma proteins after PB treatment.†

Considering the problem of antidepressant-barbiturate interaction in psychiatric therapy, it appears that in addition to a reduced systemic availability of orally administered tricyclic antidepressants and possibly enhanced elimination, the effectiveness may be further impaired by a decreased distribution to the central nervous system.

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* Brinkschulte and Breyer-Pfaff, unpublished results.

† Brinkschulte, Jahns and Breyer-Pfaff, unpublished results.

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