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Carbon disulphide exposure affects the response of rat adrenal medulla to hypothermia and hypoglycaemia

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1 The effects of hypothermia and hypoglycaemia on adrenal catecholamines and dopamine-β-hydroxylase were compared in control and carbon disulphide (CS_2) exposed rats 24 h after the last of ten daily 4 h inhalation exposures to CS_2, 2 mg l^{-1} air. Animals were either kept in a cold room (0°C) for 210 min with or without immobilization or were injected with insulin 100 u kg^{-1}. Before these treatments CS_2 exposed rats had more dopamine and less adrenaline in their adrenals than controls, and CS_2 exposure also elevated the adrenal synthesis of catecholamines.

2 Cold with immobilization or insulin treatment depressed the adrenal adrenaline content and increased the plasma concentrations of noradrenaline and adrenaline. There were no consistent differences between control and CS_2 exposed rats.

3 The adrenal dopamine content increased during cold exposure with immobilization or after insulin treatment both in CS_2 exposed and control rats. The increase was smaller in CS_2 exposed rats but the final dopamine values were nearly identical in the two groups.

4 Exposure to cold (without immobilization) increased the adrenal dopamine content and the rate of catecholamine synthesis in control, but not in CS_2 exposed rats. The increase in controls was less than the difference between the pre-cold exposure values of control and CS_2 exposed rats.

5 It is concluded that the elevation of adrenal dopamine content and catecholamine synthesis in CS_2 exposed rats satisfy part of the demand placed on the adrenal medulla by hypothermia and hypoglycaemia. Consequently the changes induced by the latter treatments were smaller in CS_2 exposed than in non-exposed rats. Moreover, when CS_2 exposed rats were subjected to cold stress without immobilization their catecholamine synthesis was higher than the level measured in control rats after cold exposure.

Introduction

Activation of the sympatho-adrenal system by procedures, which are commonly called 'stress', first leads to catecholamine discharge (Lewis, 1975) and after repeated stress to the induction of tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DβH) and phenylethanolamine-N-methyltransferase (PNMT) (Ungar & Phillips, 1983). When depletion is induced by cold (Kvetnansky *et al.*, 1971), immobilization (Kvetnansky, 1973) or insulin-induced hypoglycaemia (Silbergeld *et al.*, 1971), TH and DβH are increased substantially, but PNMT only slightly. Exposure to carbon disulphide (CS_2), which inhibits DβH, (Caroldi *et al.*, 1984b) shares some of the short and long term stress-induced effects on adrenal catecholamines. CS_2 depresses the concentration of hydroxylated amines more in the brain than in the adrenals (Magos & Jarvis, 1970) and after repeated exposure induces adrenal DβH (Caroldi *et al.*, 1984a). There are no data on the effect of CS_2 on adrenal PNMT, but since the elevated dopamine stores are associated wih an increased conversion of dopamine to noradrenaline (NA) this indicates that TH must be induced with DβH (Caroldi *et al.*, 1984a). Moreover, it has been shown that the brains of rats repeatedly exposed to CS_2 have elevated *in vitro* TH activity (Heubusch & DiStefano, 1978).

Based on the effects of CS_2 on adrenal catecholamine metabolism, it seemed reasonable to study the effect of repeated exposure to CS_2 on the concentration of adrenal catecholamines and the synthesis of dopamine in rats exposed to cold or treated with insulin. Experiments were carried out after ten daily 4 h exposures to CS_2 2 mg l^{-1} air. Such an exposure results in approximately 17 μg ml^{-1} free plus reversibly bound CS_2 in blood (Lam & DiStefano, 1982). The elimination half-life of free and reversibly bound CS_2 from the whole body is 35 min (Magos *et al.*, 1974) and in agreement with this fast elimination the conversion

of dopamine to NA returns to normal 2–4 h after a single exposure to CS_2 (Caroldi *et al.*, 1984b). However, as at the end of exposure the conversion of dopamine to NA is influenced by the presence of an inhibitor (Caroldi *et al.*, 1984a) and in the adrenals of repeatedly exposed rats the content of DβH remains high 24 h after exposure to CS_2 (Caroldi *et al.*, 1984a), rats were submitted to cold, cold plus immobilization or insulin treatment 24 h after the last of ten exposures to CS_2.

Methods

Animals and carbon disulphide exposure

Porton-Wistar male rats weighing 120–160 g were exposed to CS_2 2 mg l^{-1} in dynamic inhalation chambers for 4 h a day (between 10 h 00 min and 14 h 00 min) for 10 consecutive days, and the CS_2 concentration was continuously monitored in the chambers by infrared spectrometry (Magos *et al.*, 1970). Control rats were exposed to similar conditions of air flow but without CS_2. During exposure-free periods, rats were kept in groups of six in stainless steel wired cages (46 × 32 − 24 cm) under laboratory conditions. Tap water and food (Labsure, animal diet 41 BM) were supplied *ad libitum*. All further treatments were carried out 24 h after the last exposure.

Dopamine turnover rate in adrenal glands

CS_2 exposed and control rats were killed by decapitation either without further treatment or at different times after the i.p. injection of α-methyltyrosine (αMT) 400 mg kg^{-1}. The adrenals were quickly removed, weighed, frozen in liquid nitrogen and stored at −30°C until the catecholamine assay. The rate of dopamine synthesis was calculated from the rate constant of dopamine decline and the dopamine content measured before the administration of αMT (Brodie *et al.*, 1966). This method compares favourably with those using radioactive tracer techniques (Moore & Dominic, 1971).

Insulin treatment

Rats were anaesthetized with an i.p. injection of sodium pentobarbitone 60 mg kg^{-1}. The tail artery was cannulated with PE-50 tubing containing heparin 30 iu ml^{-1} saline. Animals were kept in individual restraining cages throughout the experiment. When animals recovered from anaesthesia, 2 ml blood samples were drawn into a heparinized tube through the cannula. Approximately 0.1 ml of blood was weighed, mixed with 1 ml of 0.33 M perchloric acid and stored at 4°C for glucose determination (Test-combination, Glucose, Boehringer Mannheim). The remaining blood was centrifuged for 10 min at 4°C in a bench centrifuge and 0.8 ml of plasma was taken and stored at −70°C for the assay of catecholamines. After separation, red blood cells were immediately resuspended in 0.8 ml of 1% w/v NaCl solution and reinjected into the tail artery to reduce the fall in blood volume. Insulin (100 u kg^{-1}) was injected into the tail artery and after 60 and 120 min further blood samples were taken and treated as above. Two hours after the injection of insulin all rats were killed by decapitation, their adrenals removed and stored at −30°C for catecholamine assay.

Cold exposure

The tail arteries of CS_2 exposed and control rats were cannulated and blood samples taken as described above. To prevent dislodgement of the cannulae, animals were kept immobilized in restraining cages. These cages consisted of two perspex endplates with perforations for stainless steel rods which hold the animal in a prone position. One of the endplates had an aperture for the cannulated tail. After sampling the rats were kept in a cold room (0°C) in restraining cages for 3½ h. Such an exposure decreases the core temperature by 1°C (Shum *et al.*, 1969). At the end of this period a second blood sample was taken and the animals killed, their adrenals removed and stored at −30°C for catecholamine assay. Non-cannulated CS_2 exposed and control rats were kept in groups of two at 0°C for the same period of time without immobilzation, in stainless steel wired cages for the determination of the rate of dopamine turnover.

Catecholamine and metabolite assay

Adrenals were homogenized by a Ystral laboratory disperser in 1 ml of 0.1 M ice-cold phosphate buffer, pH 7.4, containing 0.1% Triton X-100 and then shaken in an ice bath for 30 min. Tissue extracts were prepared from 0.5 ml homogenates according to the method of Atack & Magnusson (1978). Extracts were centrifuged at 40,000 *g* for 1 h at 4°C, the supernatant filtered and 25 or 30 µl aliquots injected into h.p.l.c. system for the estimation of dopamine, noradrenaline (NA), adrenaline (Ad) and 3,4-dihydroxyphenylacetic acid (DOPAC; the deaminated metabolic product of dopamine) levels. The protocol for the extraction of plasma catecholamines was a modification of the method of Hallman *et al.* (1978). Approximately 25 mg of activated and acid washed alumina was added to 0.8 ml of plasma with dihydroxybenzylamine as an internal standard, followed by 1 ml of 1.5 M. Tris buffer (pH 8.6 containing 0.025 g Na_2 EDTA ml^{-1}). Samples were shaken vigorously for 10 min, the alumina allowed to settle, washed three times with

water and then transferred to a micro-tube for centrifugation. Catecholamines were eluted from the alumina by the addition of 300 µl of 0.1 M acetic acid and 200 µl aliquots were taken for h.p.l.c. analysis. The h.p.l.c. column was a pre-packed, reversed phase Hichrom Spherisorb 5 ODS2 (25 cm × 5 mm i.d.) and the solvent used to obtain a good separation of peaks was 90% 0.1 M KH_2PO_4, 10% methanol, 0.1 mM Na_2EDTA, 0.7 mM octane sulphonic acid adjusted to pH 3.5 with orthophosphoric acid. The flow rate was adjusted to 1.5 ml min^{-1}. Catecholamines and their metabolites were measured by an electrochemical detector with an applied potential of 0.720 V. The analysis was based on the comparison of peak heights given by adrenal or plasma samples with those of standards. Catecholamine values in plasma were corrected according to the recovery of dihydroxybenzylamine after alumina extraction.

Table 1 Catecholamine and 3,4-dihydroxyphenyl acetic acid (DOPAC) concentrations in rat adrenals after repeated exposure to carbon disulphide

	Controls	*CS_2*
Dopamine	2.39 ± 0.10	3.79 ± 0.24*
DOPAC	1.07 ± 0.05	1.41 ± 0.06*
Noradrenaline	155 ± 6	143 ± 10
Adrenaline	506 ± 13	445 ± 12*

Carbon disulphide (CS_2) 2 mg l^{-1} air was administered for 4 h a day for 10 consecutive days. The values shown (in nmol kg^{-1} body wt per pair of adrenals) are the means ± s.e.mean of 13 rats.
*Significantly different from control, $P < 0.01$; Student's t test.

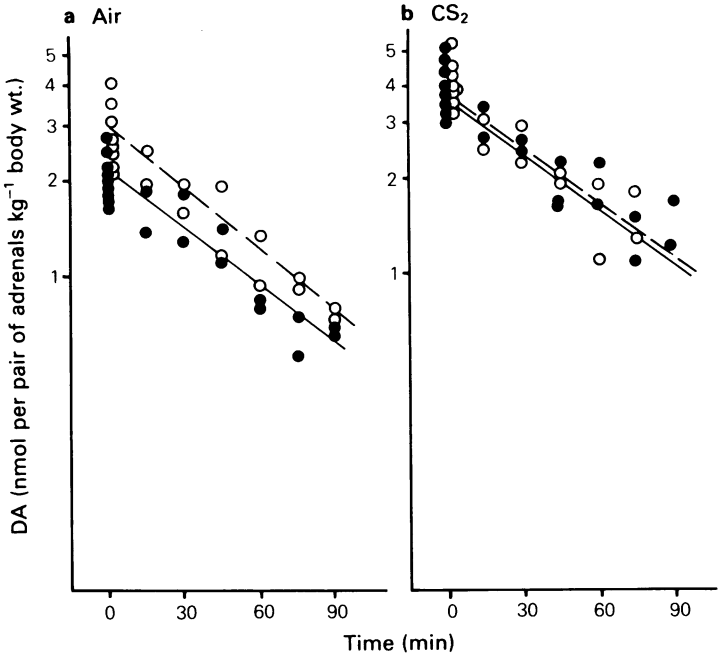

Figure 1 The effects of carbon disulphide (CS_2) exposure on adrenal dopamine (DA) depletion caused by the injection of α-methyltyrosine (αMT) without cold exposure (solid circles) or after exposure to 0°C for 210 min (open circles). Rats were exposed in inhalation chambers 4 h a day for ten consecutive days either to CS_2-free air (a) or to CS_2 2 mg l^{-1} air (b). Treatment with αMT or cold started 24 h after the last exposure.
Regression lines and correlation coefficients were: (a) air exposed, without cold: $\log y = 0.351 - 0.007x$, $r = 0.936$; after cold: $\log y = 0.440 - 0.007x$, $r = 0.962$; (b) CS_2 exposed, without cold: $\log y = 0.567 - 0.006x$, $r = 0.903$; after cold: $\log y = 0.599 - 0.006x$, $r = 0.919$. Analysis of variance between regression lines gave no significant differences between slopes in any variation. For elevations (heights) there were significant differences between air and CS_2 exposed rats ($P < 0.05$) but cold increased the adrenal dopamine content significantly only in the air exposed group.

Chemicals

Carbon disulphide (CS_2), analytical grade, was purchased from BDH Chemicals Ltd, Dagenham. α-Methyl-DL-*p*-tyrosine (αMT) (Sigma Chemical Co., Poole) was dissolved in 1% saline (50 mg ml^{-1}) immediately before i.p. injection and to avoid local irritation pH was adjusted to 5.0 with NaOH. Neutral insulin BP (80 u ml^{-1}) was purchased from Boots Company PLC, Nottingham. Other chemicals were of the highest purity grade commercially available.

Results

Rats exposed up to ten days to CS_2 showed neither changes in body weight nor in the weights of their adrenals and appeared otherwise normal. Twenty four hours after the last of ten exposures to CS_2, the dopamine and 3,4-dihydroxyphenyl acetic acid (DOPAC) content of the adrenals were, respectively, 59% and 32% higher than in controls, while the β-hydroxylated amine content remained normal or only slightly reduced (Table 1). These changes in the adrenal glands of rats repeatedly exposed to CS_2 closely resembled our previously published data (Caroldi *et al.*, 1984a).

Figure 1 shows that the higher dopamine content measured in CS_2 exposed rats reflected a faster rate of dopamine synthesis and conversion (dopamine content × dopamine turnover) as turnover rate constants were not different in the adrenals of CS_2 exposed (0.014 ± 0.001 min^{-1}; ± s.e.mean) from those in control rats (0.016 ± 0.001 min^{-1}). Turnover rates were not changed by keeping CS_2 exposed or control animals in a cold room at 0°C for 210 min. However, in control animals the rate of dopamine synthesis increased by approximately 25%, as indicated by the change in dopamine content, during exposure to cold. In the adrenals of rats exposed to CS_2 the rate of dopamine synthesis did not rise after exposure to cold (Figure 1).

In the adrenals of control rats, both exposure to cold with immobilization (210 min at 0°C) and insulin treatment (100 u kg^{-1} 120 min before decapitation) reduced the adrenaline (Ad) content by approximately 20% whereas the NA content was unaffected and the concentrations of dopamine and DOPAC more than doubled (Table 2). Multiple exposure to CS_2 produced similar changes.

When CS_2 exposed rats were subjected to cold or treated with insulin a further decline in the levels of Ad was seen and the contents of dopamine and DOPAC increased 50% less than in controls. This difference in the degree of these changes cancelled the initial differences which were present between CS_2 exposed and control rats.

Both hypothermia and insulin increased significantly the concentration of catecholamines in arterial plasma. As expected NA was increased more after cold and Ad more after insulin treatment. No differences in the concentrations of plasma Ad and NA were detected between CS_2 rats and controls either before or after 210 min of cold exposure. In agreement with

Table 2 The effects of cold exposure with immobilization or insulin treatment on adrenal concentrations of catecholamines and 3,4-dihydroxyphenyl acetic acid (DOPAC) in control and CS_2 exposed rats

			Concentration (nmol per pair of adrenals kg^{-1} body wt.)				*Difference between means (no stress = 100%)*			
CS_2	*Stress*	n	*NA*	*Ad*	*Dopamine*	*DOPAC*	*NA*	*Ad*	*Dopamine*	*DOPAC*
–	–	5	140 ± 12	488 ± 30	2.55 ± 0.22	0.93 ± 0.02				
–	cold	5	133 ± 10	396 ± 20†	5.12 ± 0.36†	1.97 ± 0.15†	−6.3	−18.9	+100.8	+112
+	–	5	154 ± 16	435 ± 21	3.98 ± 0.25‡	1.48 ± 0.03‡				
+	cold	5	161 ± 11	399 ± 23	4.88 ± 0.24†	1.88 ± 0.22	+4.5	−8.2	+22.6	+27
–	–	4	134 ± 11	474 ± 22	3.32 ± 0.57	1.15 ± 0.15				
–	insulin	12	157 ± 7	398 ± 26	8.49 ± 0.24†	3.20 ± 0.15†	+17.2	−16.0	+155.7	+178.3
+	–	4	128 ± 8	412 ± 33	4.99 ± 0.51	1.50 ± 0.15				
+	insulin	12	138 ± 9	342 ± 15	8.18 ± 0.50†	2.90 ± 0.20†	+7.8	−17.0	+63.9	+93.3

Rats were exposed to carbon disulphide (CS_2) 2 mg l^{-1} air for 4 h a day on ten consecutive days. Controls were kept in inhalation chambers for the same time. Before cold exposure (0°C for 210 min) or insulin treatment (100 u kg^{-1}) the tail artery was cannulated and rats were kept in restraining cages; They were killed by decapitation after cold exposure or 2 h after insulin. When variance was not homogeneous, Mann-Whitney U test and in other cases a two-tailed Student's *t* test was used to calculate statistical significance.

Values show mean ± s.e.mean of *n* rats.

†Significantly different from the corresponding mean of non-stressed rats.

‡Significantly different from the corresponding mean of rats not exposed to CS_2.

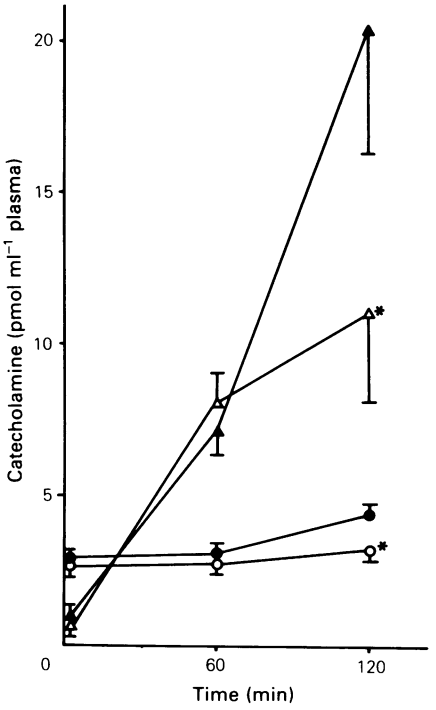

Figure 2 The effects of carbon disulphide and cold with immobilization on the concentrations of noradrenaline (circles) and adrenaline (triangles) in arterial plasma. Cold exposure (0° for 210 min) of immobilized rats started 24 h after the last of 10 exposures to ambient air (solid symbols) or CS_2 (open symbols) as described in Figure 1. Each point represents the mean and vertical lines s.e.means of five rats. Abscissa scale gives time in cold room.

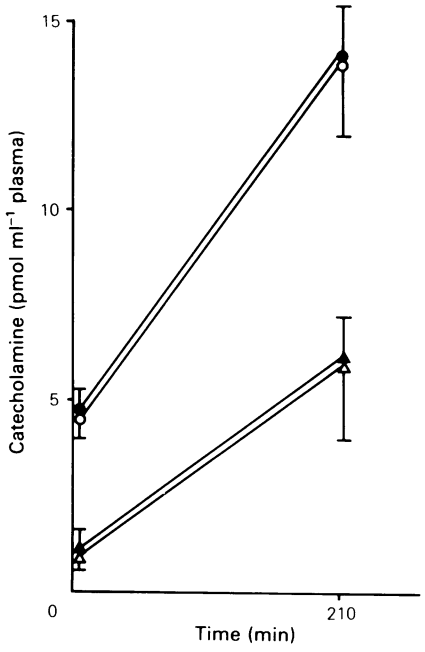

Figure 3 The effects of carbon disulphide and insulin on the concentrations of noradrenaline (circles) and adrenaline (triangles) in arterial plasma. Insulin 100 u kg^{-1} was injected 24 h after the last of ten exposures to ambient air (solid symbols) or CS_2 (open symbols). Each point represents the mean and vertical lines s.e.means of 6 rats. *Significant differences between air and CS_2 exposed rats, $P < 0.05$; directional Student's t test.

previous data (Cunningham, 1975) CS_2 exposure slightly reduced plasma glucose concentration, (6.05 ± 0.15 vs 6.66 ± 0.13 mmol l^{-1}; mean ± s.e.mean of 12 rats). However this difference was not detectable after insulin injection, when plasma glucose concentration dropped in both groups by 40% within 60 min. During the first hour after insulin injection the level of Ad in plasma increased to the same extent both in CS_2 and control rats and NA remained unchanged. However, 2 h after insulin, concentrations of Ad or NA were less in CS_2 exposed than in control rats.

Discussion

The experiments described in this paper aimed to investigate the effect of CS_2 exposure on changes induced by cold or insulin treatment on the adrenal and plasma concentrations of hydroxylated amines, the adrenal concentrations of dopamine and DOPAC and the decreased adrenal dopamine content induced by α-methyltyrosine. The monoexponential decline of dopamine after the inhibition of tyrosine hydroxylase by αMT depends principally on the conversion of dopamine to NA and it is used as an index of adrenomedullary hormone synthesis (Brodie *et al.*, 1966).

The adrenal content of NA and Ad indicated no apparent effect by CS_2 on changes induced by cold or insulin (see Table 2). Both cold and insulin treatment caused some decrease in the adrenal content of adrenaline in immobilized rats. Neither the pre- nor post-treatment values showed any significant differences between the CS_2 exposed or control rats although the initial adrenaline content was consistently lower in the CS_2 exposed group. A decrease in the adrenal

content of hydroxylated amines indicates the effects of cold or insulin on catecholamine discharge. Another indicator, despite their extremely short half-life in blood (Vane, 1969), is the concentration of NA and Ad in arterial plasma.

Figures 2 and 3 show that the arterial plasma concentrations of NA and Ad were increased by cold or by insulin treatment in immobilized rats. Insulin increased plasma Ad more than NA, whereas cold had the opposite effect. As the source of NA released into plasma is not the adrenal glands, but the sympathic nervous system (Leduc, 1961), the large increase of plasma NA induced by cold is not discordant with the lack of change in adrenal NA. Carbon disulphide did not influence the plasma concentrations of NA and Ad at all in cold exposed rats but 2 h after the administration of insulin, CS_2 exposed rats had significantly less NA and Ad in their plasma than control rats. In the case of NA the difference was small, but even though there were large variations in individual values at 2 h, the difference between mean Ad concentrations was still large enough to be significant.

A more consistent difference was observed in the adrenal dopamine content. Table 2 shows that in CS_2 exposed rats, cold with immobilization or insulin increased the adrenal dopamine content from its initial level by 23% and 64%, respectively. However, with the lower initial dopamine levels in control rats the corresponding increases were 101% and 156%. DOPAC content increased with dopamine and showed the same difference in percentage increase between CS_2 exposed and control rats.

As changes in the adrenal dopamine content reflect changes in adrenal NA and Ad synthesis (Almgren *et al.*, 1979), data described in Table 2 indicate that CS_2 influenced the extent of change in catecholamine synthesis while hypothermia and hypoglycaemia affected the absolute level of catecholamine synthesis. Insulin or cold with immobilization increased the adrenal dopamine content of control rats above the increase caused by CS_2 exposure. Consequently in CS_2 exposed rats, only insulin or cold with immobilization, but not cold without immobilization, increased dopamine content. Furthermore, Figure 1 shows that neither cold nor exposure to CS_2 affected the decreased dopamine content seen after αMT treatment. Slopes and consequently turnover rates were practically identical in the four groups. However, identical turnover and different dopamine contents means different synthesis rates. After cold exposure the synthesis rate of dopamine in pairs of adrenals corrected to 1 kg body weight was 0.040 nmol min^{-1} in controls and 0.053 nmol min^{-1} in CS_2 exposed rats. Extrapolation of the same slope to adrenal dopamine contents measured in insulin-treated rats gives 0.12 nmol min^{-1} synthesis rates for both control and CS_2 exposed rats.

Thus, these exeriments were not able to show any definite influence of CS_2 exposure on changes in the adrenal and plasma concentrations of hydroxylated catecholamines. However, the changes in adrenal dopamine and in catecholamine synthesis suggest that CS_2 exposed rats compared with control rats, when subjected to cold with immobilization or insulin, require less adaptive changes in their adrenal catecholamine metabolism. Furthermore, they require no change at all in this metabolism when subjected to cold without immobilization, probably because they already have elevated catecholamine synthesis.

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