(U) Plasma Volume Expansion in Rats: Effects on Thermoregulation and Exercise

R. Francesconi, M. Bosselaers, C. Matthew and R. Hubbard

Administration of polyethylene glycol (PEG, intraperitoneal, 3ml, 30% solution) to adult, male rats (300g) resulted in an approximate 20% increment in plasma volume (PV) 24 h after PEG injection. When these animals were exercised (9.14 m/min, level treadmill) in a warm 30°C, 30-40% rh) environment, their mean endurance was increased from 67.9 min (saline-treated controls, CONT) to 93.6 min (P<0.01). Total water loss was increased from 12.2 g (CONT) to 17.2 g (PEG) (P<0.01). Atropine administration (ATR, 200 ug/kg, tail vein) significantly (P<0.05) reduced both the endurance and the salivary water loss of CONT and PEG-treated rats while increasing the heating rate (P<0.01) of both groups. PEG treatment reduced (P<0.01) the hematocrit and circulating protein levels both prior and subsequent to exercise in the warm environment. Clinical chemical indices of heat/exercise injury were generally unaffected by pharmacological intervention while clinical chemical responses to exercise were related to the endurance time of each group. We concluded that expansion of PV by PEG provided significant beneficial effects on performance and thermoregulation during exercise in a warm environment.
PLASMA VOLUME EXPANSION IN RATS: EFFECTS ON THERMOREGULATION AND EXERCISE

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Abstract

Administration of polyethylene glycol (PEG, intraperitoneal, 3ml, 30% solution) to adult, male rats (300g) resulted in an approximate 20% increment in plasma volume (PV) 24 h after PEG injection. When these animals were exercised (9.14 m/min, level treadmill) in a warm (30°C, 30-40% rh) environment, their mean endurance was increased from 67.9 min (saline-treated controls, CONT) to 93.6 min (P<0.01). Total water loss was increased from 12.2 g (CONT) to 17.2 g (PEG) (P<0.01). Atropine administration (ATR, 200 ug/kg, tail-vein) significantly (P<0.05) reduced both the endurance and the salivary water loss of CONT and PEG-treated rats while increasing the heating rate (P<0.01) of both groups. PEG treatment reduced (P<0.01) the hematocrit and circulating protein levels both prior and subsequent to exercise in the warm environment. Clinical chemical indices of heat/exercise injury were generally unaffected by pharmacological intervention while clinical chemical responses to exercise were related to the endurance time of each group. We concluded that expansion of PV by PEG provided significant beneficial effects on performance and thermoregulation during exercise in a warm environment.

KEY WORDS: Polyethylene glycol, atropine, salivation, physical performance, indices of heat injury
INTRODUCTION

For a number of years we have been interested in the identification and investigation of pharmacological, physiological, or training interventions which may be effective in reducing the physiological cost of work in the heat, increasing heat dissipation during work in the heat, or increasing heat/exercise endurance. Similarly, we have undertaken research designed to identify and quantitate the debilitating effects of factors which predispose animals or humans to heat injury. To this end we have reported the decremental effects on exercise in the heat of a low potassium diet (16), alcohol consumption (10), preinduced hyperthermia (11), phenothiazine administration (9), and acute pyridostigmine administration (5). Alternatively, we have documented the beneficial effects on exercise in the heat of preinduced hypothermia elicited by acute administration of tryptophan (7) or a glucose analogue (8) followed by acute cold exposure. In continuing this line of research, the current investigation was designed to evaluate the effects of marked hyperhydration on endurance and thermoregulation during exercise in a warm environment.

In an early paper Stricker (25) used the subcutaneous administration of hyperosmotic polyethylene glycol (PEG, 10% or 30% solution) and inferior vena caval ligation to "elicit more drinking with fluid retention than any other experimental procedure known." However, he further reported that 0.15 M NaCl was consumed in greater quantities than water over the ensuing 24 h period. Initially, the extravascular administration of PEG to rats is followed by a period in which water is removed from the intravascular space under the strong osmotic influence of the exogenously administered PEG (26). This period persists for at least 8 h during which plasma volume deficits persisted as
evidenced in hematocrit levels from 55-60%, urine outputs were low, and fluid consumption was high (24). However, during a 24 h period following the subcutaneous administration of 5 ml of 30% PEG, Stricker (26) has reported that total fluid consumption may be as high as 55 ml and urinary output was approximately 15 ml while in a group of control animals fluid consumption was approximately 25 ml and urine output was about 22 ml. Further Stricker and MacArthur (28) have reported that when PEG was administered intraperitoneally (IP), then PEG is found in the plasma between 12-18 h, and plasma volume first equilibrates, then begins to expand under the influence of the increased drinking with fluid retention, the osmotic effect of the PEG within the intravascular space, and the greatly increased concentrations of plasma hormones subserving water reabsorption and electrolyte retention (27). Physiological differences in the effects of intraperitoneally and subcutaneously administered PEG may be due to the presence of major lymphatics underlying the diaphragm and draining the peritoneal cavity which transport the PEG to the vascular system.

Stricker and MacArthur (28) reported that 24 h after IP administration of PEG, mean hematocrit levels were reduced from 45 ± 0.8% to approximately 41% while in wild rabbits receiving 4 g/kg body weight of 30% PEG, hematocrits were reduced from 43% to 38% after 48 h and to 32% after 72 h (1). Thus, although not extensively investigated, there appear clear indications that following the acute hypovolemia of PEG administration, there ensues an interval (24 -72 h) wherein expansion of plasma volume elicits an apparent intravascular hyperhydrated state. The current study was designed to assess the effects of such plasma volume expansion on physical performance and thermoregulation in a warm environment.
Additionally, rats secrete copious amounts of saliva for evaporative heat loss during exposure to a warm environment (12,13). However, under an exercise contingency this excessive water loss is of questionable physiological benefit due to the inability of the exercising rats to spread this saliva behaviorally for evaporation and consequent heat dissipation (5,22). We have reported (17,21) that, in rats, atropine, a potent and widely used anticholinergic, is effective in inhibiting saliva secretion and evaporative water loss when the animals were passively exposed to a hot environment. However, we wished to evaluate these effects in an appropriate animal model of human heat/exercise illness (15,18), and especially during the euhydrated and hyperhydrated condition.

**Methods**

Adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were obtained at approximately 250-275g, and maintained at our facility for 5-7 d or until experimental weight of approximately 300 g was attained. The animals were housed singly in wire-bottomed cages and food (Agway 3000) and water were available ad lib. Fluorescent lighting (on, 0600-1800 h) was automatically controlled in a windowless room maintained at 21 ± 1°C, and animals were frequently weighed as they approached experimental weight. Since no effects of training or previous heat exposure were of interest, naive rats were used in all experiments; the slow treadmill speed (9.14 m/min) assured that the vast majority of the animals would run under these conditions without prior training.

At approximately 48 h before an experimental trial, a Silastic catheter was permanently implanted into the external jugular vein while the animal was anesthetized (sodium pentobarbital, 40 mg/kg body weight) using aseptic
techniques. This minor surgical intervention had no effects on the subsequent ability to exercise. At 24 h before an experimental trial, a small sample of blood (200 ul) was removed from the catheter to determine the hematocrit ratio before experimental manipulation. Rats were then randomly divided into 4 groups as follows: 1. a control group (CONT, n=10) which received 3.0 ml of sterile physiological saline by IP injection 24h prior to run, and 0.2 ml saline IV 30 min before the run; an atropine-treated group (ATR, n=9) which also received 3.0 ml physiological saline IP and 24 h later (i.e. 30 min before an experimental run) was also administered 200 ug/kg atropine sulfate (0.2 ml) by tail vein injection; 3. a third group received 3.0 ml of 30% polyethylene glycol solution (PEG, n=10) 24 h prior to an experimental trial and 0.2 ml saline IV 30 min before running; and 4. the final group was injected with 3.0 ml of 30% PEG 24 h before an experimental run and 200 ug/kg atropine (0.2 ml) 30 min before the run (PEG-ATR, n=10).

All experiments were conducted in a large stainless steel chamber set at 30 ± 0.5°C; the treadmill speed was set at 9.14 m/min (0° angle of incline) and a shock avoidance contingency was employed. Animals ran under these conditions until hyperthermic exhaustion ensued (Tre=42°C, animal unable to right itself) or 99 min, whichever occurred first. During the entire exercise interval Tre (6 cm) and Tsk (tailskin, midlength) were automatically sampled and recorded (HP85 desktop computer, HP3456A digital volt meter, and HP3495A scanner) at one minute intervals.

Small blood samples (0.8 ml) were taken in heparinized syringes approximately 15 min before initiating the run and hematocrit was immediately determined by microcentrifugation. The plasma fraction from the microhematocrit tube was immediately analyzed for protein content by refractometry. The
remainder of the blood sample was centrifuged (4°C, 10,000g), and a fresh plasma sample was stored in ice for osmolality determination (freezing point depression, uOsmette, Precision Systems). The remainder of the plasma was deep frozen (-20°C) and stored for subsequent analysis of creatine phosphokinase, lactic acid dehydrogenase, lactate, urea nitrogen, and creatinine. All of these assays were performed using a Gilford semi-automated spectrophotometer (Stasar IV) and Gilford Diagnostic reagent kits according to methods outlined in the respective technical bulletins. Sodium and potassium levels were measured by flame photometry (Radiometer, FLM 3). A second blood sample was taken immediately upon termination of the treadmill run; this sample was processed, stored, and analyzed exactly as the first.

Statistically significant effects were established by analysis of variance followed by the application of Tukey's test for critical differences of the means (19,20). Since only 9 animals were available in the ATR group, a single calculated value was used for this group (19). The null hypothesis was rejected at p<0.05.

Results

It was initially necessary to estimate the change in plasma volume elicited by the experimental regimen (i.e. 3.0 ml of 30% PEG 24 h prior to trial). To this end preliminary experiments were performed on blood samples taken immediately prior to PEG administration and 24 h later. Both blood samples were analyzed for hematocrit and hemoglobin (cyanometemoglobin method) and percent changes in plasma volume were calculated by the method of Dill and Costill (2). These preliminary results (n=4) demonstrated that the experimental intervention reduced hematocrit levels from (X ± SEM) 42.1 ± 0.63 to 36.9 ± 0.51 and hemoglobin from 14.2 ± 0.3 g/100ml to 12.65 ± 0.2 g/100ml eliciting a mean
percentage increment in plasma volume of 21.5 ± 1.4% (range = 18.7 - 25.1%). We concluded that this dosage, route of administration, and time lapse (24 h) provided an optimal range of plasma volume expansion for the current studies.

Figs. 1 and 2 demonstrate the effects of the four treatment regimens on thermoregulatory responses (Tre and Tsk) to exercise in a warm environment. Through the first 30 min of the treadmill exercise, Fig. 1 illustrates no effects of the treatments on Tre while at 40 min, mean Tre for the ATR group is significantly (P<0.01) elevated when compared to the PEG group. This difference (P<0.01) persists and is exaggerated after 50 and 60 min with the CONT and PEG-ATR groups falling between (without significant difference from) the extremely hyperthermic ATR group and the much cooler PEG groups. Tsk (Fig. 2) was elevated by the constant work rate and the warm ambient temperature, but was apparently unaffected by any of the pharmacological interventions. Standard errors of the means were ordinarily less than 0.5°C for both Tre and Tsk.

Data depicted in Table 1 confirm what is apparent in Fig. 1 -- that the rapidly developing hyperthermia of the ATR group resulted in a significantly (P<0.05) reduced endurance capacity compared to that of the saline-treated CONT group. Additionally, the expansion of the intravascular volume of the PEG-treated group led to a significant (P<0.01) increase in physical performance (5 of 10 animals in this group could have continued beyond the 99 min criterion). Interestingly, the combined PEG-ATR treatment elicited a mean endurance that was significantly (P<0.01) greater than that of the ATR group, significantly (P<0.05) less than that of the PEG-treated animals, and not significantly different from the endurance of the CONT group. The significant decrease in total water loss in the ATR-group (P<0.01 from CONT) and the elevation in the PEG group (P<0.01) obviously had no beneficial effects for the ATR-treated or
decremental effects for the PEG-treated rats. Mean weight loss/min in the PEG-treated rats was not different from controls despite the significantly increased endurance time. As suggested in Fig. 1, increments (\(\Delta\) Tre/min) in Tre in the ATR-treated group were significantly higher (P<0.01) than controls while PEG-treated rats manifested significant (P<0.05) decrements in this variable.

Table 2 reports the effects of the treatment regimens and exercise in a warm environment on several indices of hydration status. Most importantly, hematocrit levels in both PEG-treated groups were significantly (P<0.01) less than respective control levels before and after exercise. Prior to exercise sodium (Na\(^+\)) levels were significantly (P<0.01) reduced in the PEG-treated group, but significance was not attained in the PEG-ATR group. Exercise in the warm environment effected significant (P<0.05, minimal) increments in plasma Na\(^+\) in all groups. While plasma osmolality was increased (P<0.01) in all groups by exercise, the significantly (P<0.01) reduced level in the post-exercise sample of the ATR-treated group may be related to the decremented endurance; however, the similar significant (P<0.01) reduction in the PEG-ATR group cannot be explained on this basis. Total protein levels of both PEG-treated groups reflected the hemodilutional effects of this treatment prior and subsequent to exercise in the warm environment. It is speculative that the post-exercise reduction in total protein in the ATR-treated group may be related to the lowered osmolality of the respective sample and a physiological effect of the atropine.

Table 3 summarizes the effects of the treatments on several indices of exercise duration and intensity. The results generally indicate that the exercise interval was sufficient to induce increments (P<0.05) in lactic acid levels. Considerable variability in the responses of the enzymes (CPK and LDH)
precluded significant differences among the CONT, ATR, and PEG-ATR groups; however, post-exercise levels of both enzymes in the longest running group (i.e. PEG) were significantly (P<0.01, PRE vs POST) increased by the exercise interval. Table 4 illustrates the effects of the treatments and exercise in a warm environment on several indices of heat/exercise injury. Urea nitrogen and creatinine were consistently and significantly (P<0.01) elevated by the exercise/heat regimen. Potassium levels were generally unaffected by the pharmacological treatments or the exercise regimen except in the PEG group wherein the exercise was accompanied by a reduction in concentration sufficient to elicit a significant (P<0.05) difference from the respective control value.

**DISCUSSION**

As noted earlier, administration of hyperosmotic PEG solutions has been used extensively to effect acute decrements in plasma volume in rats (24-26). Similarly, Horowitz and Nadel (14) administered 20% PEG to elicit 25% reductions in plasma volume in mongrel dogs while total body water was minimally affected. Generally, these and other reports have concluded that such reductions in plasma volume were accompanied by decrements in heat tolerance (14), acutely increased hematocrit (1,23,28), and similarly elevated circulating Na⁺ concentrations (1,23). Despite these physiological responses indicative of acute (6-12 h) intravascular hypohydration, and therefore, reduced capacity for exercise in heat, there was also evidence that the sequel to the initial decrement in plasma volume was a significant shift in fluid volume occurring from 24-72 h subsequent to PEG administration which might expand plasma volume. Thus, Stricker and MacArthur (28) reported a mean decrement in hematocrit of 4.2% 24 h after IP administration of PEG, and Denton (1) noted hematocrits falling from 43% to 38% and 32%, 48 and 72 h, respectively, after PEG injection.
to rabbits. To execute the current experiments, we had targeted mean plasma volume elevations of approximately 20%, and our preliminary experiments and calculations indicated that 24 h after an intraperitoneal injection of 3 ml of 30% PEG, such an increment was established.

This level of intravascular volume expansion provided marked physiological benefit to the PEG-treated rats in terms of increased endurance and decreased heating rate despite significantly prolonged and therefore increased salivary water loss during the treadmill interval. While we had previously demonstrated that increasing hematocrit ratios to 52% (4) or infusing 2 ml NaCl or sodium bicarbonate (3) had no significant effects on subsequent exercise endurance in the heat, we had not manipulated plasma volume markedly in this rat model of human heat/exercise injury. However, preinduced hypothermia (7,8) was shown to be very effective in increasing exercise endurance in a hot environment.

The current experiments indicated that the expanded plasma volume of PEG treatment provided some thermoregulatory benefit reflected in the reduced rate of heat gain secondary to the large water loss of the PEG-treated group. Although rats ordinarily require grooming behavior to optimize thermoregulatory benefit from salivation and usually receive little benefit from salivation while running at 26°C, it is probable that in the current experiments at 30°C the copious volumes of saliva lost during treadmill exercise provided a measure of passive spreading and evaporative cooling from the neck and mouth region and the ventral surface of the body. Possibly of equal importance, however, was the improved cardiovascular stability provided by the expanded plasma volume. We had previously reported (5) that in euhydrated rats increased cholinergic salivation during exercise in the heat did not elicit increased endurance, and we attribute these differences to the hyperhydrational and euhydrational status of the experimental animals in the two experiments.
As noted earlier, we had previously demonstrated the efficacy of atropine and other anti-cholinergics in reducing both salivation and grooming behavior in passively heat-stressed rats (17, 21). However, it was uncertain whether the inhibition of salivation during an exercise contingency would be beneficial or detrimental to the physical performance and thermoregulation of the running rat. Benefit might be derived by preventing a reduction in the plasma volume loss which accompanies salivation especially if this salivary water loss could not be fully translated to evaporative heat loss because of the inability to spread saliva behaviorally. Alternatively, a detriment could arise from a severe restriction in salivary water loss which at 30°C prevented or greatly reduced any evaporative cooling effect passively derived from dripping saliva wetting the neck region and ventral surface of the body. The results of the current study seem abundantly clear in answering this question.

Compared to saline-treated controls, atropinized rats displayed a mean decrement in endurance of 17.6 min; strikingly, atropinized rats which had been previously administered PEG manifested a 17.3 min reduction in endurance when compared to rats treated with PEG alone. While actively exercising on the treadmill, ATR-treated rats lost .061 g/min less body weight than saline-treated controls; atropinized and PEG-treated rats also lost .061 g/min less than those treated with PEG only. Thus, these results indicated that atropine alone reduces both endurance and salivary water loss when compared to saline treatment. When PEG was administered, total body water was probably increased by stimulated drinking and fluid retention; this was followed by plasma volume expansion as evidenced in significantly reduced hematocrit and hemoglobin levels, endurance was prolonged, and water loss was greatly elevated in a time-dependent fashion when compared with saline-treated controls. Atropine
administration to rats previously treated with PEG reduced performance and weight loss to approximately control levels. Thus, atropine had consistently detrimental effects on saline-treated rats and reduced the advantages of PEG administration to approximately control levels. The adverse effects of atropinization appear to be thermoregulatory in nature, and probably also dependent upon the ambient temperature at which the exercise is carried out.

Clinical indices of heat/exercise injury generally mirrored the dilutional effects of the PEG administration and the longer run time elicited by this treatment. The reduced plasma osmolality and circulating protein in the post-exercise samples of the atropinized group may reflect the decreased endurance of this group with attendant insufficient equilibrium time for protein to be returned to the circulatory system during the exercise interval. However, direct effects of atropine cannot be ruled out. The significant reduction in plasma potassium levels in the PEG-treated rats has been observed previously (6) in rats exercising lightly with the achievement of steady-state rectal temperature. When exercise is accompanied by marked hyperthermia, we ordinarily observed significant elevations of circulating potassium levels although these earlier experiments (5,9-11) were conducted at 35°C ambient temperature.

We concluded from these experiments that plasma volume expansion secondary to PEG administration had significant beneficial effects on exercise performance and thermoregulation. During exercise, total water loss among PEG-treated animals was significantly greater than that of saline-treated controls. Atropine reduced physical performance and salivary water loss in both saline- and PEG-treated rats. Hydrational markers and clinical chemical indices of heat/exercise injury suggested that the beneficial effects of polyethylene glycol and detrimental effects of atropine were attributable primarily to increased intravascular volume and decreased salivation, respectively.
The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

In conducting the research described in this report, investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

The authors express their gratitude to Mrs. D. Danielski and Mrs. S.E.P. Henry for the expert word processing support.
Figure Legend

Fig. 1. Effects of polyethylene glycol, atropine, and a polyethylene glycol-atropine combination on the rectal temperature responses to mild exercise (9.14 m/min, 0° angle) in a warm environment (30°C). Mean values are depicted for n=10/group. Exercise was terminated at 99 min or when animals could not continue to maintain the pace.

Fig. 2. Effects of polyethylene glycol, atropine, and a polyethylene glycol-atropine combination on the skin temperature response to exercise in a warm environment. All conditions are as noted under Fig. 1.
References


TABLE 1. EFFECTS OF POLYETHYLENE GLYCOL, ATROPINE, AND A POLYETHYLENE GLYCOL-ATROPINE COMBINATION ON PERFORMANCE AND THERMOREGULATION DURING EXERCISE IN A WARM ENVIRONMENT. MEAN VALUES ± SEM ARE REPORTED FOR n = 10 IN EACH GROUP.

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<td></td>
<td>67.9 ± 5.1</td>
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<td>12.2 ± .8</td>
<td>6.3** ± .4</td>
<td>17.2** ± 1.3</td>
<td>10.4 ± 1.3</td>
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* = SIGNIFICANTLY DIFFERENT FROM CONTROL, P < 0.05
** = SIGNIFICANTLY DIFFERENT FROM CONTROL, P < 0.01
TABLE 2. EFFECTS OF POLYETHYLENE GLYCOL, ATROPINE, AND A POLYETHYLENE GLYCOL-ATROPINE COMBINATION ON INDICES OF HYPOHYDRATION PRIOR AND SUBSEQUENT TO EXERCISE IN A WARM ENVIRONMENT. MEAN VALUES ± SEM ARE REPORTED FOR n = 10 IN EACH GROUP.

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<td>OSMOLALITY (MOSM/KG)</td>
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* = SIGNIFICANTLY DIFFERENT FROM RESPECTIVE CONTROL, P < 0.05
** = SIGNIFICANTLY DIFFERENT FROM RESPECTIVE CONTROL, P < 0.01
TABLE 3. EFFECTS OF POLYETHYLENE GLYCOL, ATROPINE, AND A POLYETHYLENE GLYCOL-ATROPINE COMBINATION ON CIRCULATING INDICES OF EXERCISE/HEAT STRESS PRIOR AND SUBSEQUENT TO EXERCISE IN A WARM ENVIRONMENT. MEAN VALUES ± SEM ARE REPORTED FOR n = 10 IN EACH GROUP.

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* = SIGNIFICANTLY DIFFERENT FROM RESPECTIVE CONTROL, P < 0.05
** = SIGNIFICANTLY DIFFERENT FROM RESPECTIVE CONTROL, P < 0.01
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<td>POTASSIUM</td>
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<td>UREA</td>
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<td>NITROGEN</td>
<td></td>
<td></td>
<td>17.3 ± 0.6</td>
<td>25.7 ± 1.4</td>
</tr>
<tr>
<td>(MG/100ML)</td>
<td>20.5 ± 1.3</td>
<td>24 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CREATININE</td>
<td>.5 ± .03</td>
<td>.8 ± .1</td>
<td>.5 ± .03</td>
<td>.8 ± .06</td>
</tr>
<tr>
<td>(MG/100ML)</td>
<td>.5 ± .04</td>
<td>.7 ± .02</td>
<td>.5 ± .03</td>
<td>.7 ± .04</td>
</tr>
</tbody>
</table>

* = SIGNIFICANTLY DIFFERENT FROM RESPECTIVE CONTROL, P < 0.05
END
DATE
FILMED
6-1988
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