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# Airway Hyper-reactivity to Histamine in Clinically Abnormal and Experimentally Sensitised Horses

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**Abstract:** Airway hyper-reactivity to histamine was studied in horses with respiratory disease (n = 7) and in normal horses (n = 4) selected on the basis of physical and laboratory examination. The animals were challenged with aerosolised histamine and the lung function was measured. The normal horses were sensitised to *Ascaris suum* and then challenged again with the same dose of histamine. The responses of same horse to the same dose of histamine before and after sensitisation were compared between themselves and with diseased horses which suggested that the abnormal horses were hyper-reactive to histamine when compared to normal horses in which the reactivity to histamine was significantly greater after sensitisation. The response of normal horses after sensitisation to *A. suum*, resembled more with abnormal group than with their own response before sensitisation. The study suggest the presence of hyper-reactivity in horses suffering from respiratory disease and also suggest that hyper-reactivity can be induced in horses with experimental sensitisation.

**Key words:** Hyper-reactivity, Equine COPD, bronchial asthma

## Introduction

One of the characteristic features of the bronchial asthma is an exaggerated airway reactivity to several specific and non specific stimuli. The finding is so consistent that it has been included as an integral part of the asthma syndrome. Hyper-reactivity of airways in the man and in laboraytory animals can be produced by sensitisation to foreign proteins and respiratory pathogens (Boushey *et al.*,1980; Shishikura *et al.*, 1990; Jorres *et al.*, 1996). In addition, hyper-reactivity to histamine, serotonin, cholinergic agonists, oleukotriens, PGF2α and certain irritant gases such as ozone and sulphur dioxide has been documented (Boushey *et al.*, 1980). The mechanisms associated with this exaggerated response are not well understood at present.

The syndrome of Chronic Obstructive Pulmonary Disease (COPD) in the horse shares several clinical, etiological and patho-physiological mechanisms with human asthma. Similarities between the two syndromes have been referred to in the literature for many years (Cook and Rossdale 1963; Lowell 1964; McPherson and Thomson 1983; Resarowksi *et al.*, 1996). Obel and Schmiterlow (1948) observed a more severe response to intravenous histamine in horses with respiratory disorders than in normal controls. A similar exaggerated response to aerosolized histamine has been reported for ponies with COPD (Derksen *et al.*, 1982). These studies suggest that analogous to airway hyper-reactivity in asthmatic man, the equine airways are also hyper-reactive in at least, some diseased individuals. As part of an evaluation of model of allergic bronchospasm in the horse, we examined whether this hyper-reactivity was induced.

## Materials and Methods

Eleven standardbred horses, of mixed weight (345 to 444kg), age 3 to 8 years) and of either sex were subjected to a detailed protocol to determine their respiratory health status. The criteria which were used to achieve this goal included a detailed clinical examination at rest and after exercise, resting blood gas values, bronchoscopy and bronchoalveolar lavage (BAL), quantitative cytology of the lavage fluid and control Pulmonary Function Tests (PFT).

Table 1: Baseline values (Mean ± S.E.M.) for lung mechanics in normal (n = 4) and abnormal (n = 7) horses

| | Normal | Abnormal |
|---|---|---|
| $C_{dyn}$ (1/cm$H_2O$) | 3.66 ± 0.94 | 1.17 ± 0.19* |
| Max. $P_{pl}$ (cm$H_2O$) | | |
| a) Total airways | 4.09 ± 0.55 | 7.786 ± 0.67* |
| b) Lower airways | 1.19 ± 0.10 | 4.13 ± 0.45* |
| Non-elastic work (kg cm/1) | | |
| a) Total airways | 2.21 ± 0.26 | 4.71 ± 0.93* |
| b) Lower airways | 0.31 ± 0.11 | 0.61 ± 0.23 |

*P < 0.05

Table 2: Ventilation volumes and airflow rates (means ± S.E.M.) in normal (A: n = 4); experimentally sensitized (B: n = 4) and abnormal (C: n = 7) horses

| | | Baseline | Minutes after histamine inhalation | |
|---|---|---|---|---|
| | | | 5 | 20 |
| $V_T$ (1) | | | | |
| | A | 3.8 ± 0.4 | 3.3 ± 0.6 | 3.9 ± 0.5 |
| | B | 4.7 ± 0.6 | 4.2 ± 0.65 | 5.1 ± 1.0 |
| | C | 4.3 ± 0.5 | 4.1 ± 0.6 | 4.4 ± 0.7 |
| $V_I$ (1/min) | | | | |
| | A | 88.1 ± 18.2 | 89.0 ± 42.0 | 50.9 ± 11.6 |
| | B | 47.3 ± 9.0 | 56.0 ± 1.1 | 66.9 ± 25.1 |
| | C | 52.7 ± 8.7 | 80.9 ± 17.4 | 54.1 ± 8.00 |
| f (B/min) | | | | |
| | A | 24.6 ± 6.3 | 25.7 ± 11.1 | 13.1 ± 1.9 |
| | B | 10.5 ± 2.2 | 13.5 ± 1.8 | 12.2 ± 2.0 |
| | C | 13.6 ± 2.8 | 22.0 ± 4.8 | 12.8 ± 1.0 |
| Average flow (1/sec). | | | | |
| a) Inspiration | | | | |
| | A | 3.2 ± 0.9 | 3.0 ± 1.7 | 1.98 ± 0.4 |
| | B | 1.8 ± 0.3 | 2.0 ± 0.5 | 2.5 ± 0.7 |
| | C | 2.2 ± 0.4 | 3.0 ± 0.5 | 2.0 ± 0.3 |
| b) Expiration | | | | |
| | A | 3.7 ± 0.8 | 3.4 ± 1.5 | 1.98 ± 0.6 |
| | B | 1.7 ± 0.4 | 2.1 ± 0.4 | 1.6 ± 0.3 |
| | C | 1.9 ± 0.3 | 3.4 ± 1.0 | 2.4 ± 0.4 |

On the basis of above tests, four horses were considered normal as they exhibited no clinical signs, their baseline lung function values (Table 1) were within the normal range (Sasse, 1971; McPherson *et al.*, 1978) and showed no abnormality on bronchoscopy. The differential cell count of the BAL fluid was normal. These horses were referred to as normal controls.

The control horses were challenged with an aerosol of histamine (1% w/v solution of histamine diphosphate nebulized for 5 minutes) to determine their response before sensitisation and then they were experimentally sensitised to *A. suum* extract injected three times at weekly intervals (2ml of 1/10 dilution of *A. suum* mixed with 8 ml of sterile saline). After sensitisation, these horses (referred to as sensitised group) were challenged again with the same dose of histamine and their responses were quantified.

The remaining seven horses were found to have respiratory problems and constitute the abnormal group. In the absence of previous medical history and the fact that these animals were observed only for a short period of time, no conclusive diagnosis with regard to the particular lung disease could be made. They showed a mixture of clinical signs, particularly after exercise. Poor exercise tolerance was observed in all these animals. The baseline values for pulmonary mechanics in these animals were significantly higher than the normal horses (Table 1). Bronchoscopy revealed hyperaemia and mucopurulent exudate. A higher proportion of neutrophils was detected on the differential cell count of the BAL fluid.

**Pulmonary function test (PFT):** All horses were conscious and tested while standing in a 1 x 2 m stock without sedation. The methods were the same as described previously (Willoughby and McDonell, 1979; Mirbahar *et al.*, 1985; 2000). Airflow, ventilation volumes and transpulmonary pressure ($P_{p1}$) were measured using a Fleisch pneumotachograph and esophageal balloon and recorded simultaneously on a photographic recorder (VR-6, Electronics for Medicine, White Plains, NY, USA.). The use of two differential pressure transducers permitted the determination of total (mouth to esophageal) and lower (tracheal to esophageal) airway pressure changes. Pulmonary resistance ($R_L$) was calculated by relating the changes in $P_{p1}$ to instantaneous airflow at 25, 50 and 75 % of inspiratory and expiratory tidal volumes ($V_T$), while dynamic compliance ($C_{dyn}$) was determined by relating the changes in volume to changes in $P_{p1}$ at the points of zero airflow.

**Nebulization method:** The concentration of histamine (1% w/v solution) and the time of exposure to the histamine aerosol (5 minutes) were selected from preliminary experiments. All horses appeared to tolerate this dose. Histamine diphosphate was dissolved in 0.9% sterile saline and nebulized through an ultrasonic nebulizer (675-Monaghan, Wilder Medical, Kitchner, Ontario, Canada). In the beginning of each experiment, each horse was challenged with an aerosol of 0.9% saline for 5 min and PFT were performed to obtain baseline values. Each animal was then challenged with histamine for 5 min. The lung function was measured at approximately 5 and 20 min. After the histamine challenge (The nebulization method has been described previously in detail (Mirbahar *et al.*, 1985;2000).

**Statistical analysis:** A two way analysis of variance was applied to compare pre-histamine challenge responses (saline) with responses at 5 and 20 min post-histamine challenge for each group. The differences between means were specified using Duncan Multiple Range Test. A paired t-test was used to compare pre and post-sensitization responses of the four horses at a given time period.

## Results

**Control Group:** Horses in this group did not show any signs of respiratory embarrassment and the local edematous reaction of the external nares was not observed after histamine inhalation. The quantification of pulmonary mechanics revealed a significant increase in the total and lower max. $\Delta P_{p1}$ while $C_{dyn}$ decreased ($p < 0.05$) (Fig. 1 and 2). Total and lower $W_b$ (Fig. 3) and upper and total $R_L$ did not change. Increases in lower inspiratory and expiratory $R_L$ were conspicuous (Fig. 4a and b). $V_T$, $V_I$, f and flow rates did not change (Table 2).

**Sensitised group:** The sensitized group of horses responded with varying degrees of respiratory distress compared with their consistently weak response before sensitization. On a subjective scale of weak, moderate and severe, the responses of two horses were categorised as severe, one as moderate and one as weak. In one of the severe responders, the effect was so severe that the initial experiment was terminated and the horse was treated with a $B_2$-bronchodilator (salbutamol). One week later, the experiment was repeated using the same concentration of histamine but reducing the level of exposure from 5 to 4 min.

Despite variability between horses, significant ($P < 0.05$) increases occurred in max. $\Delta P_{p1}$ and $W_b$, both for lower and total airways while $C_{dyn}$ decreased (Fig. 1,2 and 3). While $V_T$, $V_I$, f and flow rates did not change (Table 2), the increases in lower inspiratory $R_L$ at 75% $V_T$ and expiratory $R_L$ at 25, 50 and 75% of $V_T$ were significant (Fig. 4). Similarly the changes in total $R_L$ were either significant ($P < 0.05$) or prominent but statistically non-significant (Fig. 4). Upper inspiratory and expiratory $R_L$ did not change although a moderate local

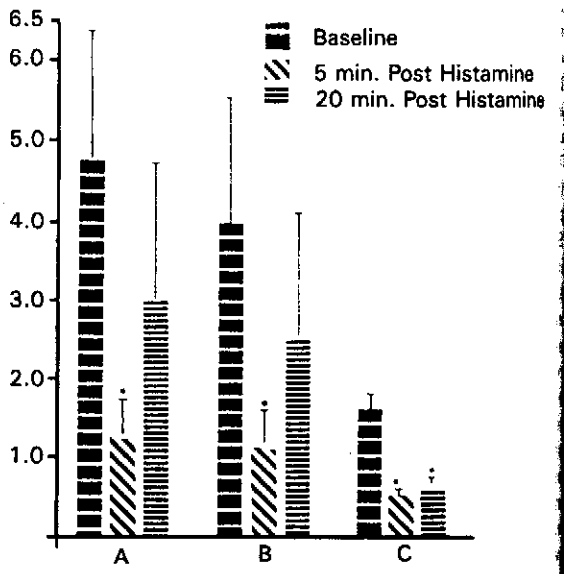


Fig. 1: Absolute values (mean ± S.E.M.) For dynamic compliance before (Baseline) and after the inhalation of 1% solution of histamine disphosphate in normal (A, n = 4), experimentally sensitized (B, n = 4) and abnormal (C, n = 7) horses
* Different from the baseline ($P < 0.05$)

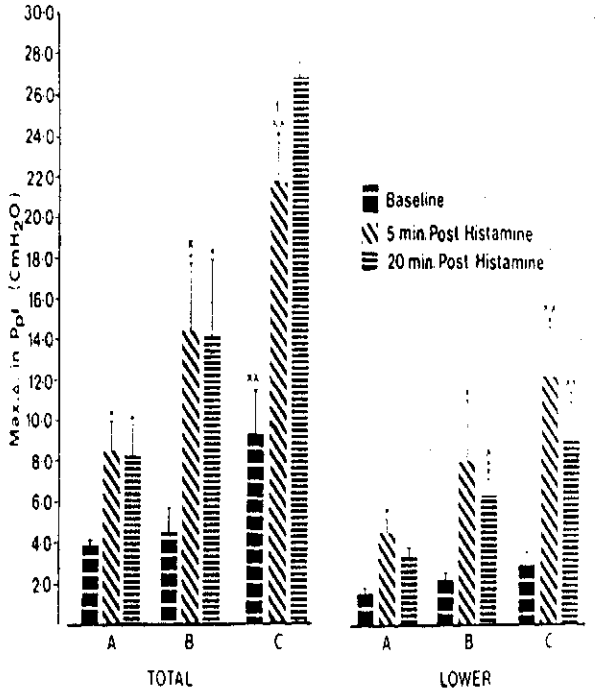


Fig. 2: Absolute values (mean ± S.E.M.) For maximum changes in pleural pressure for total and lower airways before (baseline) and after the inhalation of histamine in normal (A, n = 4) experimentally sensitized (B, n = 4) and abnormal (C, n = 7) horses
*Different ($p < 0.05$) from baseline
x and xx: Different ($p < 0.01$ and $0.1 > p > 0.05$ respectively) from normal group at a given time period
t and tt: Differ ($p < 0.01$ and $0.1 > p > 0.05$ respectively) from sensitized group at a given time period

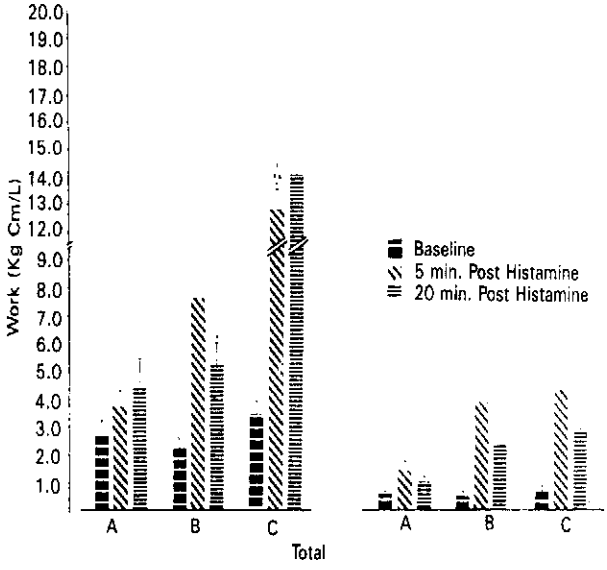


Fig. 3: Absolute values (mean ± S.E.M) for non-elastic work of breathing for total and lower airways before (baseline) and after the inhalation of histamine in normal (A, n = 4), experimentally experimentally sensitized B, n = 4), and abnormal (C, n = 7) horses
*Different ($p < 0.05$) from baseline
x and xx: Different ($p < 0.01$ and $0.1 > p > 0.05$ respectively) from normal group at a given time period
t and t: Different ($p < 0.05$) from sensitized gorup

edematous reaction of the external nares was observed in two severely responding horses.

**Abnormal group:** All seven horses responded with severe respiratory distress. In two horses it was necessary to reduce the exposure time to 4 min due to the severe effect observed during histamine inhalation. In general, the effect was rapid in onset, becoming noticeable approximately one minute after the beginning of histamine exposure. The clinical signs observed during and immediately after histamine aerosolization included an increase in f, moderate to severe sweating, occasional mild salivation and a progressive edema involving the external nares, upper and lower lips and chin. This edema disappeared within 24 hours.

The measurements of lung function at 5 and 20 min post-histamine challenge indicated significant increases ($P < 0.001$) in total and lower max. $\Delta P_{pl}$ and $W_b$ with a parallel significant ($P < 0.001$) decrease in $C_{dyn}$ (Fig. 1.2 and 3)_. The total inspiratory and expiratory $R_L$ increased, although this change reached a level of significance ($P < 0.001$) only at 20 min post-histamine challenge (Fig. 4a). By this time, the local nasal edematous reaction has also peaked. At 20 min. the increases in total $R_L$ were essentially due to upper $R_L$, which on an average increased by 152% ($P < 0.05$) on inspiration and 193% ($P < 0.05$) on expiration as against an average increase of 8 and 62% respectively at 5 min post-histamine challenge. A multifold increase in lower inspiratory $R_L$ was not statistically significant from the control values (Fig. 4b), however, lower expiratory $R_L$ increased significantly ($P < 0.05$) at 5 and 20 min. $V_T$, $V_I$ expiratory and inspiratory flow rates did not change. Although f appeared very high during and immediately after histamine challenge, the computed values at 5 min post-challenge showed no significant increases (Table 2).

**Between group comparisons:** The baseline (saline) values obtained before and after sensitization in normal horses showed a non-significant increase in total and lower $R_L$, the values being higher after sensitization (Fig. 4). Comparisons between pre and post-sensitization responses to histamine revealed significant ($P < 0.05$) increase in lower inspiratory and expiratory $R_L$ in sensitized horses. The differences in lower and total max. $\Delta P_{pl}$ and $W_b$ were conspicuous, but not significant. Differences were observed in baseline mechanical values between abnormal and normal horses, although these differences were not always significant (Fig. 1, 2 and 4). The pulmonary functional impairment produced by histamine was greater in the abnormal group, than in the normal group of horses prior to sensitization. The differences were statistically significant for $W_b$, max $\Delta P_{pl}$ and inspiratory and expiratory $R_L$. The abnormal and sensitized groups responded to histamine in a reasonably similar manner. Comparisons of post-histamine responses for these two groups revealed a significant

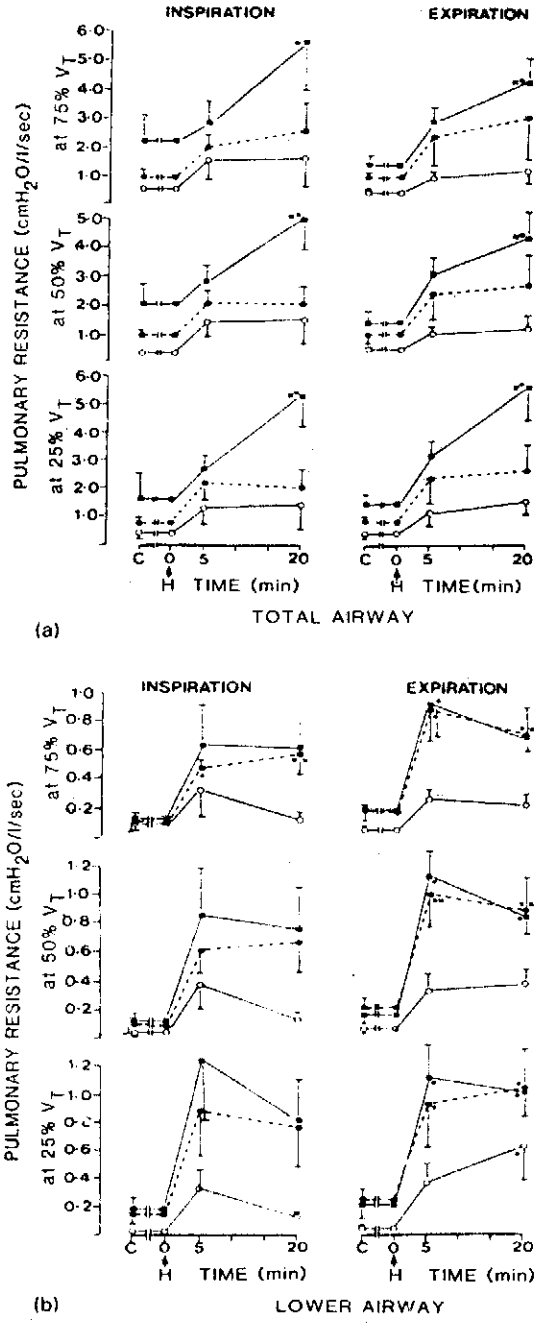


Fig. 4: Absolute values (mean ± S. E. M.) For total (Fig. 4a) and lower (Fig. 4b) pulmonary rersistance in normal (o------o; n = 4) (o----o; n = 4) sensitized (•------•; n = 7) horses before (c) and 5 and 20 min. after inhalation challenge with histamine. Baselin © values were obtained after saline inhalation. Histamine (H) was administered at time zero and lung function was recorded at 5 and 20 min after histamine inhalation
Different ($p < 0.05$) from baseline within a particular group
Different ($p < 0.05$) from normal group at a given time period

difference only in total $W_b$ ($P < 0.05$) at 5 min and in total max.$\Delta P_{pl}$ ($P < 0.05$) and $W_b$ at 20 min, values being higher in abnormal horses. The differences between lower $P_{pl}$, $W_b$ and $R_L$ were not noticeable.

## Discussion

From the statistical comparisons and clinical qualitative observations on the horses during experiment, it was apparent that compared to the normal group, the animals in the abnormal group, reacted severely to the same dose of histamine. Obel and Schmiterlow (1948) recorded a significantly greater change in $P_{pl}$ in "heavy" horses compared to normal controls after intravenous administration of histamine. Derksen *et al.* (1982) have also reported similar findings in ponies suffering from COPD. This suggests that analogous to asthmatic man, the hyper-reactivity of airways is also a feature of COPD in horses.

The hyper-reactivity to histamine observed in the same "normal" horses after sensitization was interesting. Although no solid conclusions could be drawn from the present study, it is tempting to think some plausible explanation for the exaggerated airway response in this group 1) Microbial infections are known to induce airway hyper-reactivity in man and in laboratory animals (Boushey *et al.*, 1980). This does not seem likely since animals used in this study were continuously monitored during the period of study. Body temperature, pulse and respiration rates were recorded daily. No health problem was encountered in these horses 2) Popa *et al.* (1973) failed to demonstrate airway hyper-reactivity in allergic guinea pigs until after they were challenged with appropriate antigen. This suggested that antigenic challenge rather than existing allergy is responsible for exaggerated airway response to histamine. This also seems unlikely because we later discovered that a significant population of horses possess natural allergy to *A. suum*. These horses, when discovered are already hyper-reactive to histamine and repeated bronchial provocation with *A. suum* did not change their sensitivity to histamine (Mirbahar *et al.*, 1988). Sensitization to foreign proteins is known to cause an increase in the airway sensitivity to specific and non-specific stimuli (Boushey *et al.*, 1980; Chand, 1980; Mirbahar and Eyre, 1982; Morcillo *et al.*, 1984; Shishikura *et al.*, 1990; Tarayre *et al.*, 1991; Jorres *et al.*, 1996). This seems a likely speculation for our findings, which is supported by the fact that the degree of the response to histamine in experimentally sensitized animals closely paralleled their response to inhalation challenge with *A. suum*. Animals responding severally to antigen also responded severely to histamine and vice versa suggesting a relationship between the immunological status of individual animal and airway hyper-reactivity. Similar findings have been reported by Hargreave *et al.* (1981) who has observed a positive correlation between the degree of airway hyper-reactivity and severity of asthma in man. The etiology and pathogenesis of asthma is not well defined. The experimental bronchospasm in horse, described in this and other papers (Mirbahar *et al.*, 1985; 1988; 2000) simulate all the characteristic features of naturally-occurring disease in the horse. Further investigations using these methods should help in the better knowledge of the disease and in the development of effective and appropriate treatment. The use of horse as an experimental model of a naturally-occurring disease in this species seems ideal since it bypasses the problems of known species differences encountered in the extrapolations made

from one species to another, particularly from laboratory animals to man. In addition, the syndrome of obstructive pulmonary disease in the horse appears to be the only naturally-occurring disease in animals which could be compared to bronchial asthma on clinical, etiological and patho-physiological basis (Cook and Rossdale, 1963; McPherson and Thomson, 1983). Anatomical similarities between human and equine lungs have long been recognised (McLaughlin *et al.*, 1961). Changes in pulmonary function observed in COPD horses compare well with those reported in asthmatic man. The association of airway hyper-reactivity with COPD in the horse suggests another important analogy between these two syndromes. Based on these considerations, it appears therefore, that if the inconveniences of the species associated with the size and management of the animals could be overcome, the horse may prove a valid model in the laboratory investigations of bronchial asthma.

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