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The Effect of Oxytocin on the Body Weight of Male Rabbits

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Abstract: This study was carried out to investigate the effect of two different doses of oxytocin on weight. A 15 adult male rabbits were weighed and provided with food twice daily, once in the afternoon and once in the evening, for three weeks to determine the amount of food consumed daily and the time spent feeding by each rabbit. After three weeks the rabbits were weighed and divided randomly into three groups: the control group, the low dose group, and the high dose group. The animals were injected daily for three weeks. During that time, the amount of food consumed and the time spent feeding in both periods were determined. After the end of the treatment period, the rabbits were weighed and then put down. The results of this study showed that before treatment the rabbits consumed more food in the evening period than they did in the afternoon period. The mean time spent feeding in the evening period was slightly higher than that spent in the afternoon period. However, this difference was not statistically significant. After treatment, there was still a significant difference between the means of the consumed food in the afternoon and the evening period for the control group. The mean amounts of food consumed in both periods by the treated groups were slightly reduced, but this reduction was not statistically significant. Furthermore, the mean time spent feeding in the evening period was slightly higher than that of the afternoon period for the three groups; however, these differences were not significant. The mean weight of the control group was slightly increased after treatment with the hormone, and the mean weights of the treated groups were slightly reduced after treatment. However, changes in body weight were not statistically significant.

Keywords: Oxytocin, Body Weight, Rabbits; Food Consumed.

INTRODUCTION

Oxytocin (OT) is a nonapeptide synthesized by the magnocellular neurons located in the supraoptic and paraventricular nuclei of the hypothalamus. It is secreted to the circulation by the posterior pituitary and nerve terminals in response to various stimuli (Gimpl & Fahrenholz, 2001; Luck & Jungclas, 1987). Oxytocin exerts a variety of actions, which is involved in a large number of physiological and pathological processes. These actions include the regulation of the hypothalamo-pituitary-adrenal axis in response to pregnancy (Apter-Levy *et al.,* 2013). However, its best-known and most well-established roles, stimulation of uterine contractions during parturition (Blanks & Thornton, 2003) and milk release during lactation (Breton *et al.,* 2001). It has gained more recent attention for its therapeutic potential in the treatment of obesity (Deblon et al., 2011; Kublaoui et al., 2008; Maejima et al., 2011; Morton et al., 2012; Zhang et al., 2011; Zhang & Cai, 2011). The vast majority of stud-

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ies investigating oxytocin and social behavior in humans, including its effect on reducing weight, have utilized nasal delivery (nasal spray) as the administration method (MacDonald & MacDonald, 2010). Oxytocin was first reported to inhibit food intake following systemic administration in rodents by Arletti and colleagues in 1989 and 1990 (Arletti et al., 1989; Arletti et al., 1990). Subsequent studies showed these effects could be reproduced following administration of much lower doses when given directly into the CNS (Deblon et al., 2011; Kublaoui et al., 2008; Lokrantz et al., 1997; Morton et al., 2012; Rinaman & Rothe, 2002; Zhang & Cai, 2011). These effects were completely blocked by pretreatment with an oxytocin antagonist (Arletti et al., 1989; Arletti et al., 1990; Olson et al., 1991).

This ability to reduce food intake appears to be through a specific effect of oxytocin to reduce meal size (Blouet et al., 2009; Lokrantz et al., 1997; Yamashita et al., 2013) and increase latency to the first meal (Arletti *et al.,* 1990). But to our knowledge, there are no studies dealing with the effect of oxytocin on the weight of rabbits. Therefore, this study was carried out to investigate the effects of two different doses of oxytocin on the weight of male rabbits.

MATERIALS AND METHODS

Animals: Fifteen, 4 month old adult male rabbits, known locally as Egyptian (Arabic) rabbits, were bought from a local market. The rabbits were kept in a temperature controlled room divided by concrete molds to separate and keep each rabbit alone.

The rabbits were provided with water and special rabbit chow imported from Private Group Company, Egypt. The animal spaces were cleaned daily with detergent and bleach.

Drug: Oxytocin was purchased from a local veterinary pharmacy. The hormone was manufactured by Bio-Pharmachemie (Vietnam) and imported and distributed by Selvium Pharmaceutical Co. Ltd. (Benghazi-Libya). Sterile physiological saline (0.09 % NaCl) was purchased from a local pharmacy.

Experimental Procedure: The rabbits were weighed (1.590±0.680), and each rabbit was provided with 70 g of rabbit chow twice daily, one at 2 p.m. and the second at 2 a.m. for three weeks to determine the amount of food consumed daily by each rabbit and to determine the time spent feeding by each rabbit. After each meal, the amount of food consumed by each rabbit was determined by subtracting the leftover in each bowl from the 70g provided. The time spent feeding was determined by watching the animal from the moment the food was provided (0 time) till it stopped eating, using a stopwatch. After three weeks, the rabbits were weighed again and divided randomly into three groups (five rabbits in each group): the control group (injected with 1 ml/kg of physiological saline), the low dose group (injected with 20 µg/kg; five IU/kg of oxytocin), and the high dose group (injected with 40 µg/kg; 10 IU/kg of oxytocin).

The animals were injected subcutaneously daily in the afternoon for three weeks. During that time, the rabbits were monitored for any signs of disease or any physical changes. In addition, the amount of food consumed and the time spent feeding in both periods were determined as above and recorded. After the end of the treatment period, the animals were weighed, and the weights were recorded.

Statistical Analysis: Statistical analysis was performed using a computer run package (Graph Pad Prism version 4 Graph Pad Software, San Diego, USA). One-way ANOVA followed by Tukey's HSD test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± standard error of the mean (SEM), N= 5. A P-value below 0.05 was considered to be statically significant.

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RESULTS

None of the rabbits in this study exhibited overt clinical signs of toxicity in response to treatment with oxytocin. The animals were given food during two periods, one at 2 p.m. and the other at 2 a.m. The amount of food consumed and the time spent eating was determined for each animal. Figure 1 represents the amount of food consumed (in grams) by the control group and the treated groups in the afternoon period and the evening period before injection of oxytocin (before treatment). The mean amount of food were consumed by the control group, the low dose group, and the high dose group in the afternoon period were 38.59 ± 0.6031, 37.68 ± 1.378, 37.91 ± 1.962, respectively. There were no significant differences between the means of the three groups. In the evening period, the amounts of food consumed were 45.49 ± 2.169, 45.86 ± 1.267, and 45.01 ± 1.437, respectively. There were no significant differences between these means. However, there was a significant difference between the mean of the afternoon period and that of the evening period for each group ($p < 0.05$). The rabbits consumed more food in the evening period.

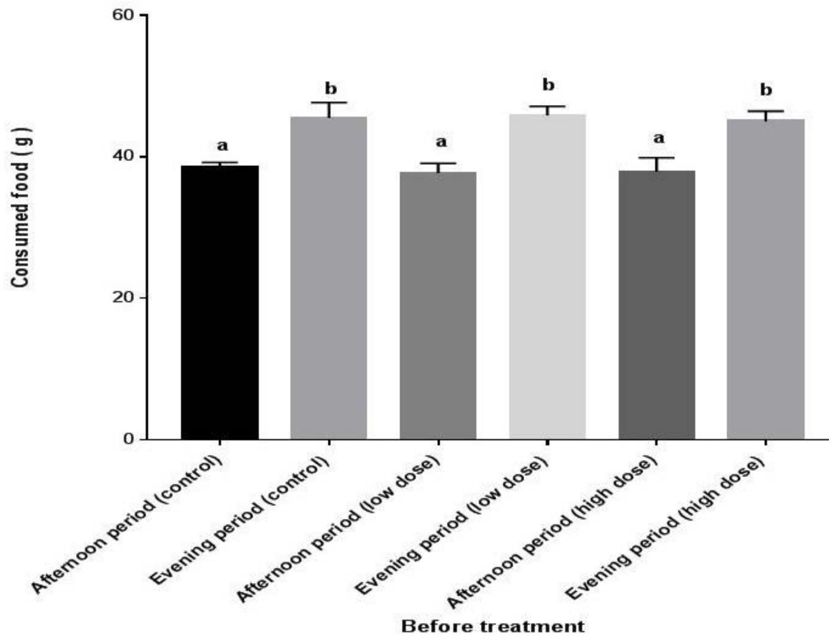

Figure 1. The mean amount of food consumed (in grams) by the control group and the treated groups in the afternoon period and the evening period before injection of oxytocin (before treatment). Results are Mean ± SEM. Different letters indicate significant differences between the means. Similar letters indicate no differences.

Figure 2 represents the mean amount of time (in minutes) spent feeding by the control group, the low dose-treated group, and the high dose-treated group in the two periods before treatment. It was found that in the afternoon period, the mean times spent feeding were 46.05 ± 2.529, 42.25 ± 0.653, and 39.03 ± 1.907, respectively, and for the evening period were 52.46 ± 3.394, 46.57 ± 1.421, and 43.98 ± 2.959, respectively. Even though the mean time spent feeding in the evening period was slightly higher than that for the afternoon perod for each group, this increase, however, was not statistically significant ($p > 0.05$). The amounts of consumed food in the afternoon period and the evening period were also measured after the end of the treatment period (after injection of oxytocin).

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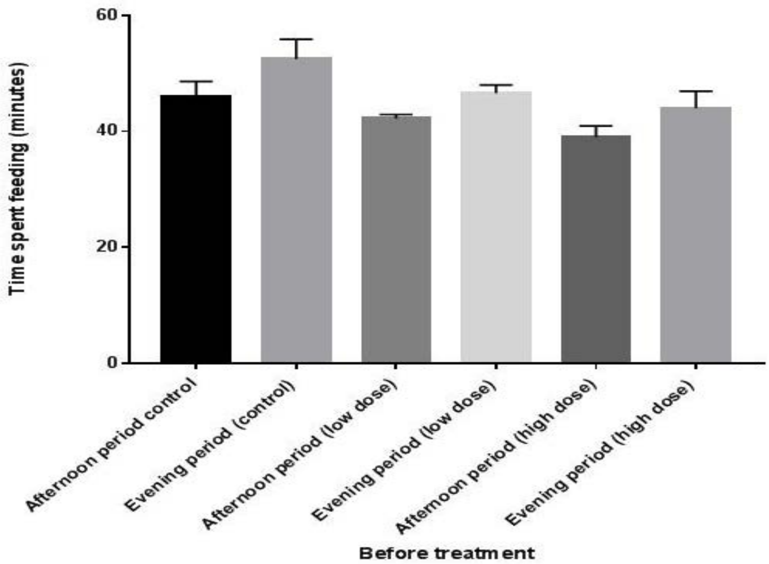

Figure 2. The mean amount of time (in minutes) spent feeding by the control group, the low dose-treated group, and the high dose-treated group in the two periods before treatment. Results are Mean ± SEM

Figure 3. represents the amounts of consumed food in the afternoon period were 33.72 ± 1.616, 32.74 ± 1.832, and 30.14 ± 2.347 for the control group, the low dose-treated group, and the high dose-treated group, respectively. For the evening period, the amounts of consumed food were 41.28 ± 1.895, 36.05 ± 2.58, and 30.30 ± 1.798, respectively. There was still a significant ($p < 0.05$) difference between the means of the consumed food in the afternoon and the evening period for the control group ($p = 0.044$). However, the amounts of food consumed by the treated groups in the afternoon period were slightly reduced in comparison with the amount consumed by the control group for the same period. Likewise, the amounts of food consumed in the evening period by the treated groups were reduced in comparison with that of the control group and in comparison with the same period for the treated groups before the injection of the hormone. However, these reductions were not statistically significant.

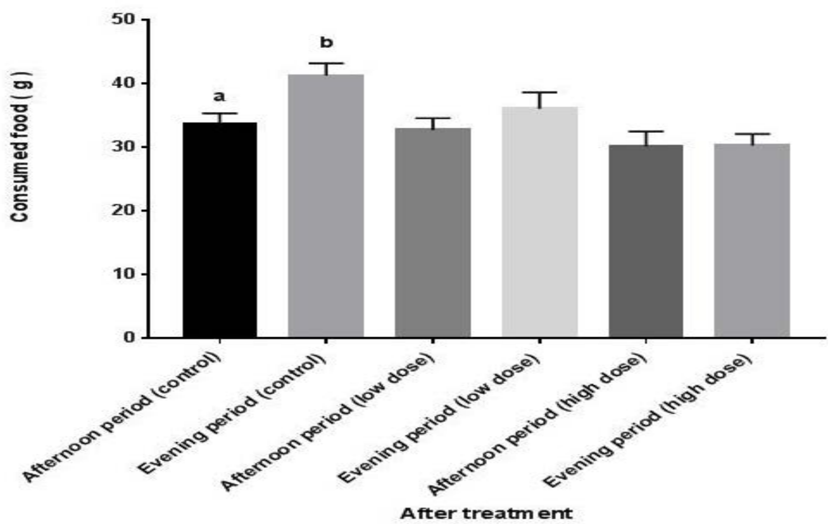

Figure 3. The mean amounts of food consumed in the afternoon period and the evening period of the control group, the low-dose-treated group, and the high-dose-treated group. Results are Mean ± SEM. Different letters indicate significant differences between the means.

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Figure 4 represents the mean time spent (in minutes) feeding in both periods for the three groups after treatment with the hormone. For the afternoon periods, the times spent feeding were 36.22 ± 0.971, 36.81 ± 2.539, and 33.38 ± 2.664 for the control group, the low-dose-treated group, and the high-dose-treated group, respectively. For the evening periods, the numbers were 41.4 ± 1.85, 39.66 ± 2.76, 37.45 ± 2.569, respectively. Even though the mean time spent feeding in the evening period was slightly higher than that in the afternoon period for the three groups; however, these differences were not significant. The weights of the rabbits were measured before and after treatment with the hormone.

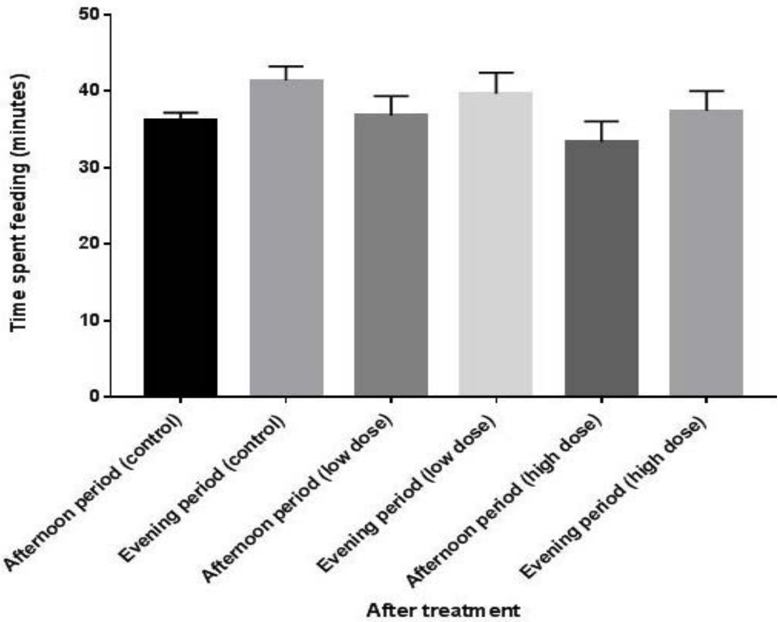

Figure 4. The mean amount of time spent feeding in both periods for the three groups after treatment with the hormone. Results are Mean ± SEM.

Figure 5 represents the mean weights of the control group, the low-dose-treated group, and the high-dose-treated group.

The mean weights (Kg) of the rabbits of the three groups before treatment were 1.361 ± 0.112, 1.601 ± 0.199, and 1.494 ± 0.142, respectively; and the mean weights after treatment were 1.651 ± 0.083, 1.317 ± 0.128, and 1.330 ± 0.118, respectively. From this figure it is clear that the weights of the rabbits in the control group had increased after the end of the treatment period; this increase, however, was not statistically significant. On the other hand, weights of the hormone-treated rabbits were slightly reduced after treatment with the hormone, but this reduction was also not statisti cally significant. Some of the internal organs (kidneys, liver, and heart) were also weighed, but there were no significant differences between the mean weights of the control organs and those of the treated ones.

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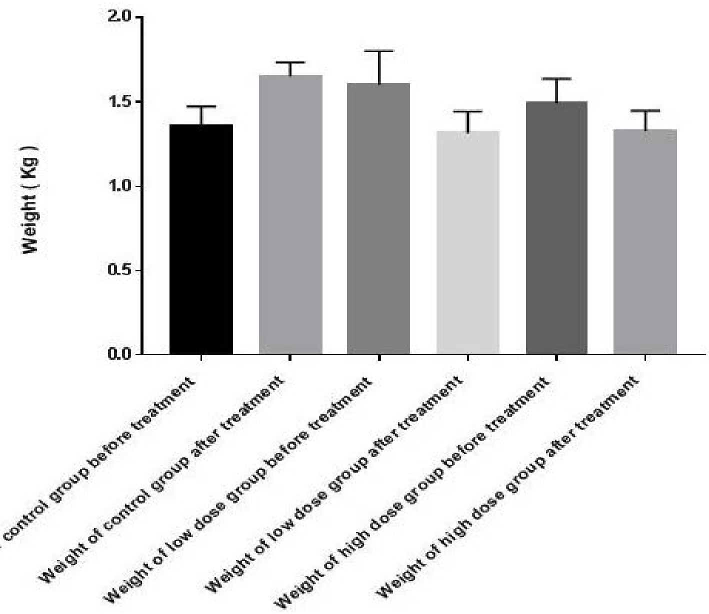

Figure 5. The mean weights of the control group, the low-dose-treated group, and the high-dose-treated group before and after treatment. Results are Mean ± SEM.

DISCUSSION

This study was carried out to investigate the effect of oxytocin on the weight of male rabbits. The reason for feeding in 2 periods was that one period of feeding was not good enough for the rabbits to reach satiety. However, from this, it was found that the animals consumed more food in the evening period than in the afternoon period. In this study, the weights of the treated rabbits decreased slightly, and this reduction, however, was not statistically significant. This is probably because the doses used in this study were lower than those used by most other researchers who have reported a reduction in weight with the injection of oxytocin (6000 μg/kg) (Arletti et al., 1989; Maejima et al., 2011). However, (Li *et al.,* 2007) were able to increase stomach and duodenum motility in rabbits by the intravenous injection of oxytocin (0.1 - 0.8 μg/kg). Furthermore, the differences could be due to the methods of injection. Injecting the hormone intra-cerebroventricularly and intra-peritoneally. In this study, the hormone was injected subcutaneously (Arletti *et al.,* 1989); however, injection of the hormone subcutaneously was found to stimulate lipid metabolism in mice and rats when fed standard or high-fat diet (Maejima *et al.,* 2011). The increase in motility of the stomach and duodenum is believed to speed up the emptying of the gut and, therefore, reduce the weight of the animal (Li *et al.,* 2007). The reduction in body weight could be the result of reduced food intake. Indeed, the results of this study show that the amounts of food consumed in both periods were reduced; and since there were no significant differences in the amounts of time spent feeding in both periods before and after treatment, we believe that the loss of weight is due to the loss of appetite. The results of this study are consistent with other studies that have used oxytocin to reduce weight.

Experiments in rodents and nonhuman primates demonstrate that chronic peripheral or central administration of oxytocin results in sustained weight loss attributed to reduced food intake, maintenance of energy expenditure despite weight loss, and increased lipolysis (Blevins et al., 2014; Deblon et al., 2011; Maejima et al., 2011; Morton et al., 2012). Interestingly, obese rodents seem to be more sensitive to oxytocin treatment than their lean

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counterparts (Morton *et al.,* 2012). Preclinical studies have demonstrated a preferential effect of oxytocin in reducing carbohydrate consumption (Amico et al., 2005; Blevins et al., 2014; Mullis et al., 2013). Although some rodent studies have not found that oxytocin affects intake of lipid emulsions (Miedlar et al., 2007; Sclafani et al., 2007), others have demonstrated that oxytocin suppresses consumption of high-fat diets (Deblon et al., 2011; Morton et al., 2012; Zhang & Cai, 2011). To date, three human studies all exclusively in men, which investigated the effects of intranasal administration of oxytocin on food intake in individuals without eating disorders, have been published (Lawson et al., 2015; Ott et al., 2013; Thienel et al., 2016).

In a randomized study with healthy men without diabetes mellitus, oxytocin reduced caloric intake by 122 kcal with a preferential effect on fat consumption (Lawson *et al.,* 2015). To date, only one study has examined whether oxytocin administration reduces body weight in humans who have overweight or obesity, but are otherwise healthy. The study demonstrated that oxytocin led to substantial weight loss over eight weeks (Zhang *et al.,* 2013). The reduction in weight observed in this study could also be due to the breakdown of fats. Preclinical studies indicate that oxytocin induces lipolysis (Blevins et al., 2014; Deblon et al., 2011) and fat oxidation (Blevins et al., 2016; Maejima et al., 2011), which in turn, independent of food intake, lead to reduced body fat and weight (Deblon *et al.,* 2011). Notably, oxytocin reduces visceral and liver fat (Maejima *et al.,* 2011), which are metabolically important fat depots associated with an increased risk of metabolic syndrome and cardiovascular disease (Pischon *et al.,* 2008).

CONCLUSION

These results clearly confirm and extend the recent data that show the anti-obesity effects of the peripheral administration of oxytocin. However, further studies are needed to establish whether this hormone has any side effects.

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تأثير الأوكسيتوسن على وزن جسم ذكور الأرانب

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المستخلص: أُجريت هذه الدراسة للتحقق من تأثير جرعتين مختلفتين من الأوكسيتوسين على الوزن. تم وزن 15 من ذكور الأرانب، وقُدم لكل أرنب 70 جراما من علف الأرانب مرتين في اليوم، مرة بعد الظهر، ومرة في المساء، لمدة 3 أسابيع، لتحديد كمية الطعام المستهلكة يوميا، ولتحديد الوقت المستغرق في الأكل من قبل كل أرنب. وبعد 3 أسابيع تم وزن الأرانب ووُزعت عشوائيا إلى 3 مجموعات: المجموعة الضابطة، ومجموعة الجرعة المنخفضة، ومجموعة الجرعة المرتفعة. حُقنت الحيوانات يوميا لمدة 3 أسابيع، خلال تلك الفترة حُددت كمية الطعام المستهلكة، والوقت المستغرق لاستهلاك هذه الكمية في كل من الفترتين، وبعد انتهاء مدة فترة المعاملة تم وزن الأرانب. بينت النتائج أن الأرانب قبل المعاملة بالهرمون استهلكت كمية من الطعام أكثر في الفترة المسائية، وكمية الوقت المستغرقة في استهلاك الطعام في الفترة المسائية كانت أطول بقليل من تلك المستغرقة في فترة ما بعد الظهر، لكن هذا الفرق في الوقت لم يكن معنويا. وبعد انتهاء فترة المعاملة بالهرمون كان هناك فرق معنوي في كمية الطعام المستهلكة بين الفترتين بالنسبة للمجموعة الضابطة، أما المجموعات المعاملة فقد انخفضت قليلا كمية الطعام المستهلكة في الفترتين، لكن هذا الانخفاض لم يكن معنوياً. وكان الوقت المستغرق لاستهلاك الطعام في الفترة المسائية أطول قليلا منه في فترة ما بعد الظهر بالنسبة للمجموعات الثلاثة، لكن هذه الفروقات لم تكن معنوية. متوسط وزن المجموعة الضابطة ارتفع قليلا بعد المعاملة بالهرمون، ومتوسطات أوزان المجموعتين المعاملتين كانتا منخفضتين قليلا بعد المعاملة، لكن هذه التغيرات في الأوزان لم تكن معنوية.

الكلمات المفتاحية: الأوكسيتوسن، وزن الجسم، الأرانب، استهلاك الغذاء.

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