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Cerebral Embolization Leads to Memory Impairment of Several Learning Tasks in Rats

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KIYOTA, Y , M MIYAMOTO, A NAGAOKA AND Y NAGAWA *Cerebral embolization leads to memory impairment of several learning tasks in rats* PHARMACOL BIOCHEM BEHAV **24**(3) 687–692, 1986 —The effects of cerebral embolization, produced by injecting microspheres into the left internal carotid artery, on passive and active avoidance tasks and water filled multiple T-maze task, were studied in male Wistar rats The rats with cerebral embolization were markedly impaired acquisition and retention of the one-trial passive avoidance response The impairment depended on the number of microspheres injected and continued for 2 weeks The cerebral embolized rats were also impaired acquisition of two-way active avoidance response in a shuttle box These impairments are not due to decrease in shock sensitivity, because there was no significant change in the flinch-jump threshold The embolized rats also exhibited a significant disturbance in performance of water filled multiple T-maze learning These results suggest that rats with cerebral embolization are impaired in three different types of learning tasks, and may be useful as an animal model for the vascular type of dementia

Cerebral embolization Passive and active avoidance tasks Water maze learning Memory impairment Animal model for dementia

ANIMALS with cerebral embolization produced by different manipulations (intra-arterial injection of arachidonate [5, 8, 9, 22], adenosine diphosphate [6], homologous blood clots [15,20] or air [7,10]) have been used as experimental models for brain ischemia Solid microspheres [4, 13, 21, 23] were also used to produce a permanent occlusion of microvessels It has been demonstrated by many investigations that cerebral embolization greatly affects brain energy metabolism an increase in lactate level, decrease in ATP level and decrease in oxygen or glucose utilization [13, 14, 17, 18] However, the effects of cerebral embolization on behavior, especially on learning behavior [12,16], have not been studied extensively, although cerebral embolization is thought to disturb learning and memory

The present study was conducted to investigate the effects of cerebral embolization, produced by injecting microspheres into the left internal carotid artery, on passive or active avoidance task and water filled multiple T-maze task in rats

METHOD

Subjects

The subjects were male JCL/Wistar rats that weighed 250–300 g and were 8–10 weeks old at the beginning of the experiments The animals were housed in groups of 8–10 in large wire-mesh cages under controlled conditions of temperature (25±1°C), humidity (55±5%) and light (7 00–19 00) Food and water were freely available in the home cage

Embolization

The cerebral embolization was performed according to the method described by Kogure *et al* [13] with minor modifications The rats were anesthetized with ethyl ether and the left carotid bifurcation was exposed The left external carotid artery and the pterygo-palatine artery, a branch of the internal carotid artery, were ligated The origin of the external carotid artery was occluded with a vascular clamp An incision was made in the artery, and a PE-50 polyethylene tubing filled with saline was inserted through the incision into the common carotid artery Carbon microspheres (35±5 μm in diameter) suspended in 50 μl saline containing 20% dextran were injected into the internal carotid artery through the tubing, the origin of the external carotid artery was then ligated Sham-operated rats were injected with the same volume of 20% dextran solution instead of the microsphere suspension

Flinch-Jump Threshold

Each rat was placed in a test chamber (30×30×30 cm) and was given a 1-min habituation period before the measurement Eight different AC currents of shock (0 1–1 0 mA) were applied in ascending order for 1 0 sec at 30 sec intervals through the grid floor A "flinch" was defined as a startle or crouching response and "jump" as removal of two or more paws from the grid floor The shock intensity at which rats exhibited each response was measured for the threshold

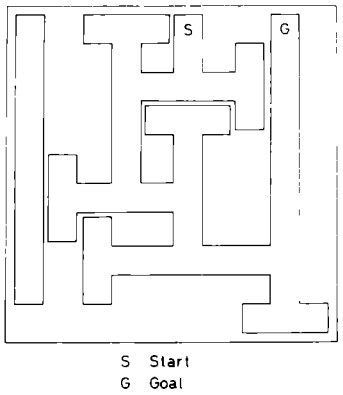

FIG 1 Maze pattern of water filled multiple T-maze Six choice points are present in the correct pathway 'S and "G' represent the start and goal points of the maze, respectively

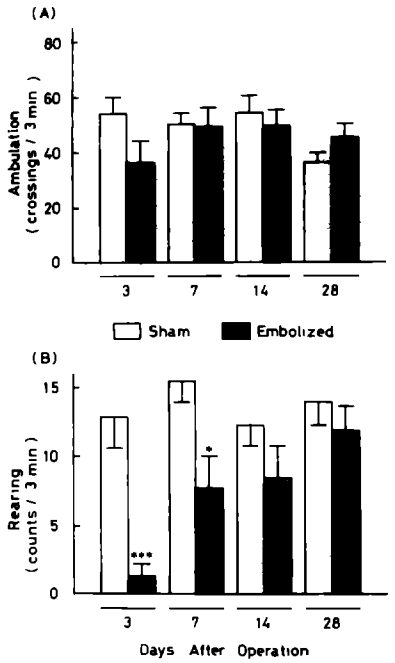

FIG 2 Ambulation (A) and rearing (B) behavior in the open-field test in rats with cerebral embolization produced by injecting 2000 microspheres into the left internal carotid artery Different groups of rats (9–12) were used for each test after the operation $*p<0.05$, $***p<0.001$, compared with sham-operated rats

TABLE 1

EFFECT OF THE CEREBRAL EMBOLIZATION ON FLINCH-JUMP THRESHOLD IN RATS

	Flinch (mA)	Jump (mA)
3 Days		
Sham	0 23 ± 0 02	0 45 ± 0 03
Embolized	0 33 ± 0 04*	0 61 ± 0 13*
1 Week		
Sham	0 21 ± 0 01	0 38 ± 0 06
Embolized	0 18 ± 0 04	0 44 ± 0 12
2 Weeks		
Sham	0 20 ± 0 04	0 38 ± 0 06
Embolized	0 17 ± 0 02	0 35 ± 0 09

Flinch-jump threshold was measured at various times after the cerebral embolization The embolization was produced by injecting 2000 microspheres into the left internal carotid artery

Each value is the mean ± S E (mA) of 10 or 11 rats $*p<0.001$ compared with sham-operated rats

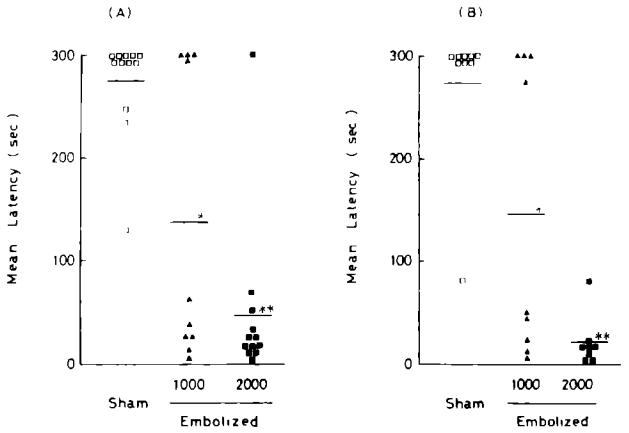

FIG 3 Effects of cerebral embolization on passive avoidance response in rats The embolization was produced by injecting 1000 or 2000 microspheres into the left internal carotid artery In the experiment A, the acquisition trial was done 7 days after the operation and the retention test was carried out 24 hr later In the experiment B the acquisition trial was done 24 hr before the embolization and the retention test was carried out 7 days after the operation Each point represents the individual avoidance latency and each bar shows the mean latency in the retention test $*p<0.05$, $**p<0.01$, compared with sham-operated rats

Open-Field Behavior

The general behavior of the rats was observed in the open-field as described by Hall [11] The testing apparatus consists of a circular floor 60 cm in diameter enclosed by a 50 cm high wall The floor is divided into 19 sectors, each of which has approximately same area marked with a black line The open-field is illuminated by 100 W bulb 80 cm above the center of the floor A rat was placed in the center of the floor, and two parameters were measured for 3 minutes "ambulation," expressed as the total number of sectors crossed by the rat, and "rearing," expressed as the frequency with which it stood on its hind limbs The open-field behavior was observed 3 to 28 days after the operation Different groups of rats were used in each test

Passive Avoidance Task

Rats were tested in a step-through type passive avoidance task [1] The experimental apparatus consists of two compartments, illuminated one (25×10×25 cm) and dark one (30×30×30 cm) equipped with a grid floor, and two compartments are separated by a guillotine door (8×8 cm) In the acquisition trial, a rat was placed in the illuminated compartment and allowed to enter the dark compartment, as soon as it did so, the door was closed and unescapable foot-

shock (2 5 mA, 3 sec) was delivered through the grid floor In the retention test, the rat was again placed in the illuminated compartment and the latency to enter the dark compartment was measured If the rat avoided longer than 300 sec, a ceiling score of 300 sec was assigned In the experiment to study the effects of cerebral embolization on the acquisition of the passive avoidance response, the acquisition trial was carried out 7 days after the embolization and the retention test was performed 24 hr after the acquisition trial The effects of cerebral embolization on the retention of passive avoidance response acquired 24 hr before the embolization, were evaluated by a retention test performed 7 days after the operation

Active Avoidance Task

Rats were trained in a shuttle box type of active avoidance apparatus [3] The experimental apparatus equipped with a grid floor, consists of two compartments (20×23×20 cm) separated by a hurdle (6 cm high) A buzzer is put on a ceiling of a shuttle box for the source of the conditioned stimulus The conditioned stimulus (2 8 kHz, 70 dB) was presented for a maximum 5 sec, and if the rat did not cross to the opposite side of the shuttle box, an unconditioned stimulus of footshock (1 0 mA) was delivered through the grid floor for a maximum 5 sec Training was started 7 days after the operation Each rat was given 20 trials daily for 6 days, a variable interval ranging from 30 to 50 sec between the trials was used Two measures of behavior were recorded "avoidance response," crossing to the opposite side during the conditioned stimulus and "intertrial response," spontaneous crossing between the trials

Water Filled Multiple T-Maze Task

Rats were trained in a water maze task [2] The experimental apparatus consists of a water tank (125×125×35 cm) comprising a multiple T-maze with 6 choice points and a straightaway (Fig 1) The maze pathway is 13 cm wide, and the tank was filled with water (21±1°C and 20 cm deep) A platform (22 cm high), with an inclined landing approach, is equipped at the goal where the rat is able to escape from the water Training was started 7 days after the operation On the first day, the rat was put in the straightaway for three preliminary swimming training trials the rat was put in one end of the straightaway and latency to reach the other end, equipped with the platform, was recorded The next day, the rat was put at the start point (S) of the maze, and latency to reach the goal (G) and the number of errors in choice was recorded Each rat was given 3 trials a day for 3 days (Days 1–3)

Statistics

Statistical comparison between different groups was made using Student's *t*-test and Mann-Whitney U-test (two-tailed), and analysis of variance (ANOVA) was used for data in active avoidance and the water maze tasks

RESULTS

Neurological Symptoms

The embolized rats exhibited ptosis and paralysis of contralateral paws Some occasionally showed circling or rolling behavior These neurological deficits were marked for three days after the embolization, then gradually improved and completely disappeared 7 days after the embolization Neurological deficits increased in proportion to the number of microspheres injected Incidence of death was 0, 8, 40 and 70% in the rats injected with 1000, 2000, 3000 and 4000 microspheres, respectively Based on this finding, we chose 2000 microspheres for standard experiments, because this number produced prominent neurological deficits but the incidence of death was relatively low

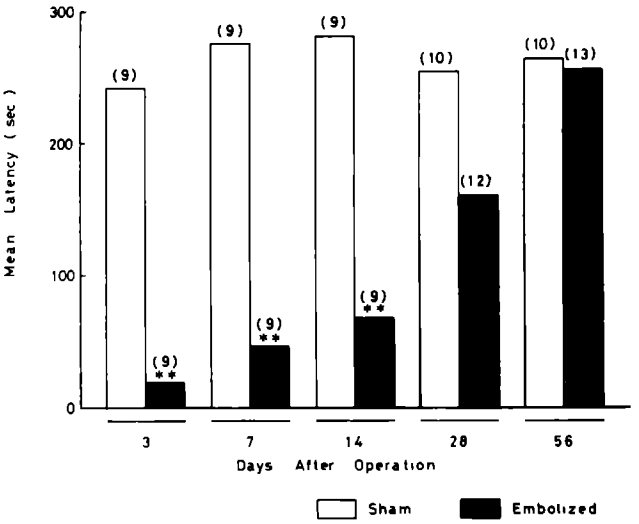

FIG 4 Acquisition of passive avoidance response at various times after cerebral embolization in rats The embolization was produced by injecting 2000 microspheres into the left internal carotid artery Different groups of rats were used in each test The retention test was performed 24 hr after the acquisition trial Number of rats is shown in parentheses $^{**}p<0.01$, compared with sham-operated rats

Nociceptive Thresholds in the Embolized Rats

The thresholds for the flinch and jump responses were significantly higher in the embolized rats than in sham-operated rats 3 days after the operation However, both thresholds recovered to the control level 7 days after the embolization (Table 1)

General Behavior

In all tests, ambulatory activities in embolized rats did not differ significantly from those in sham-operated rats (Fig 2A) However, the embolized rats showed less rearing behavior than the sham-operated rats, and the difference was significant 3 and 7 days after the operation (Fig 2B)

Effects of Cerebral Embolization on Acquisition of Passive Avoidance Response (Pre-Training Embolization)

To study the effect of cerebral embolization on acquisition of passive avoidance response, the acquisition trial was carried out 7 days after embolization produced by the injection of 1000 or 2000 microspheres In the retention test done 24 hr after the acquisition trial, most of the sham-operated rats exhibited long response latencies to enter the dark compartment In contrast, the embolized rats exhibited short response latencies and the change was more marked in rats injected with 2000 microspheres mean response latency was

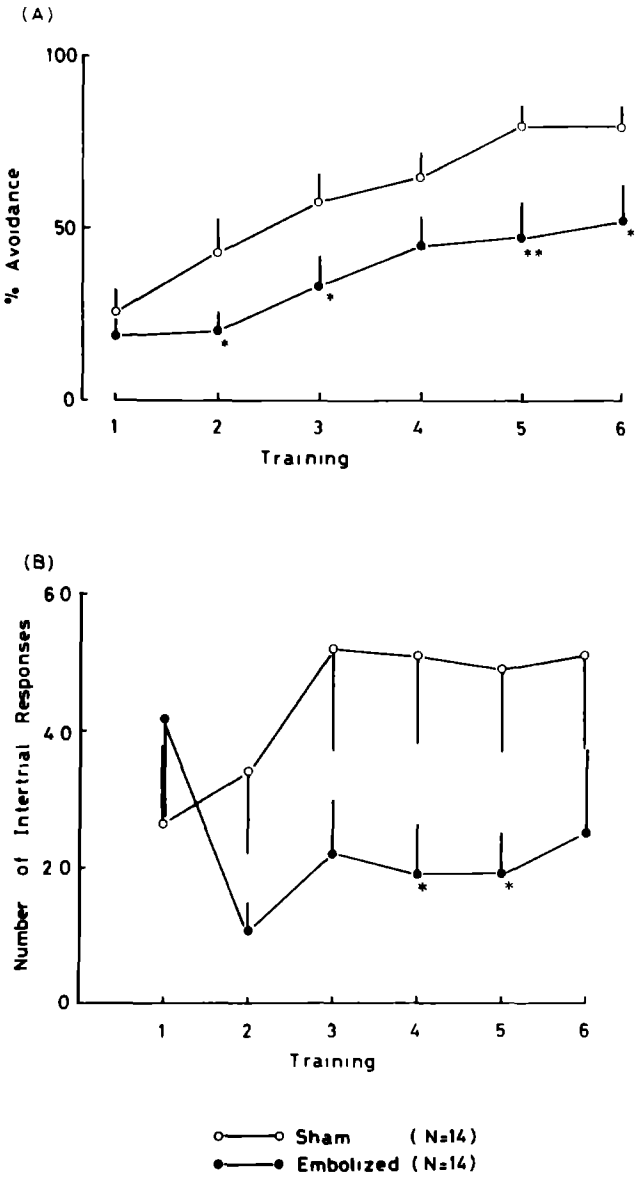

FIG 5 Effects of cerebral embolization on active avoidance response in rats Training was started at 7 days after the operation The embolization was produced by injecting 2000 microspheres into the left internal carotid artery Each value represents the percentage of avoidance responses (A) or the number of intertrial responses (B), which is shown as mean±S E $*p<0.05$, $**p<0.01$, compared with sham-operated rats

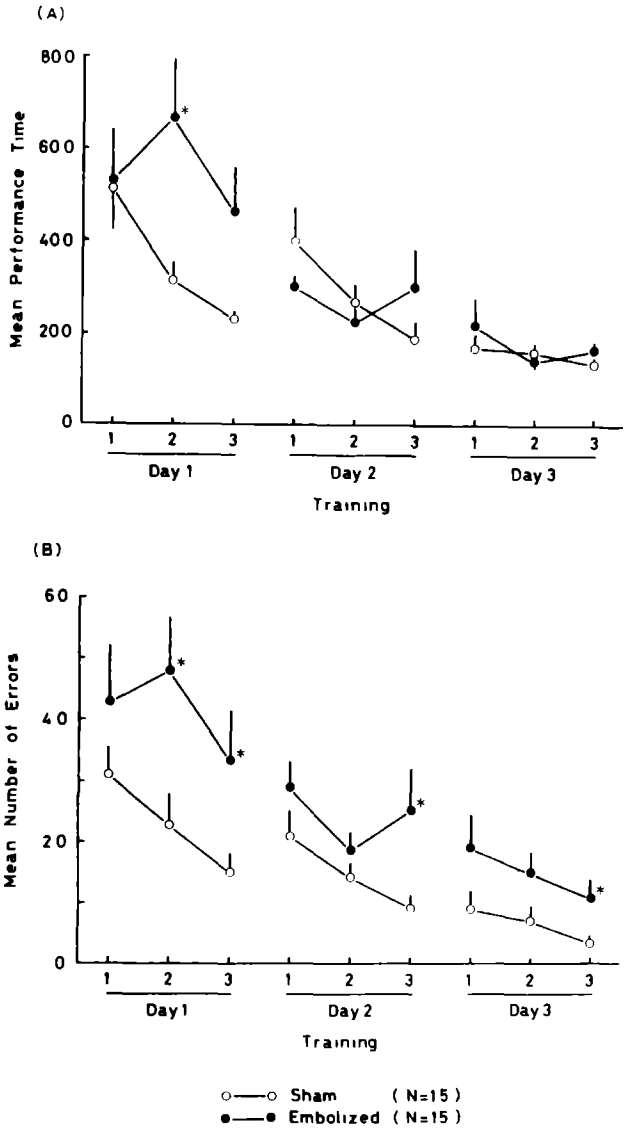

FIG 6 Effects of cerebral embolization on water maze learning in rats Training was started at 8 days after the cerebral embolization The embolization was produced by injecting 2000 microspheres into the left internal carotid artery Time to reach the goal (A) and the number of errors (B) are expressed as mean±S E $*p<0.05$, compared with sham-operated rats

136 7 sec (U=25 5, $p<0.05$) or 46 4 sec (U=7 5, $p<0.01$) in rats injected with 1000 or 2000 microspheres, respectively (Fig 3A)

Next, we determined whether the impaired acquisition of passive avoidance response in the embolized rats recovered time-dependently The acquisition trials were carried out at various times from 3 to 56 days after the embolization made by injecting 2000 microspheres, different groups of rats were used in each test Sham-operated rats showed long avoidance latencies in each test, performed 24 hr after the acquisition trial In contrast, the response latencies in the embolized rats were significantly short 3, 7 and 14 days after the operation However, the shortened response latencies were gradually recovered to the level of the sham-operated rats, and normal 56 days after the embolization (Fig 4)

Effects of Cerebral Embolization on Retention of Passive Avoidance Response (Post-Training Embolization)

Rats given an acquisition trial of passive avoidance task in the intact state, were embolized using 1000 or 2000 microspheres 24 hr after the acquisition trial Eight of 9 sham-operated rats showed a ceiling score of 300 sec in the reten-

tion test, performed 7 days after the operation In contrast, the response latencies were very short in the embolized rats Avoidance latencies were inversely related to the number of microspheres injected mean response latency was 145 2 sec ($U=16$, $p<0$ 05) or 21 4 sec ($U=1$, $p<0$ 01) in the rats embolized by 1000 or 2000 microspheres, respectively (Fig 3B)

Effects of Cerebral Embolization on Acquisition of Active Avoidance Response (Pre-Training Embolization)

The percentage of avoidance responses gradually increased with training in both the embolized and sham-operated rats However, the percentage of avoidance responses in the embolized rats was lower than that in the sham-operated rats in every session, $F(1,26)=6$ 2, $p<0$ 05 In the final session, the sham-operated rats showed an avoidance response in 80% of the trials whereas the embolized rats exhibited a response in only 50% of the trials (Fig 5A) The number of intertrial responses in the embolized rats was significantly less than that in sham-operated rats in the fourth and fifth session (Fig 5B) However, no significant difference was observed between the two groups in ANOVA, $F(1,26)=3$ 0, $p>0$ 05

Water Maze Performance in the Cerebral Embolized Rats

The embolized rats swam as well as the sham-operated rats the time taken to reach the goal was not different between the two groups in the straightaway (sham-operated rats 4 84±0 93 sec, embolized rats 5 13±0 72 sec) In the water maze, the swimming time to reach the goal was gradually shortened by training in both groups of rats, and no significant difference was observed between the two groups in ANOVA, $F(1,28)=2$ 0, $p>0$ 1 The embolized rats could also learn to reach the goal with a few number of errors, however, the mean number of errors was more in the embolized than in the sham-operated rats, $F(1,28)=17$ 3, $p<0$ 01, in all trials (Fig 6B) In the final trial, 10 out of 15 sham-operated but only 4 out of 15 embolized rats reached the goal with no errors

DISCUSSION

Cerebral embolized rats, produced by injecting microspheres into the left internal carotid artery, had impaired acquisition of the passive avoidance response Retention of the avoidance response, acquired in the intact state before the embolization, was also disturbed by the embolization The impairment was proportional to the number of microspheres injected In addition, acquisition of the active avoidance response in a shuttle box was also disturbed in the embolized rats

Emotional response to an electric shock is an important factor in learning tasks that use an aversive stimulus for conditioning The embolized rats showed a temporary decrease in sensitivity to the electric shock, but the thresholds for the responses were normal 7 days after the embolization, indicating that the impairment in the passive or active avoidance task is not due to a decrease in sensitivity to the electric shock As shown by the normal ambulation in the open-field test, general activity in the rats was not disturbed 7 days after the embolization This result also indicates that a secondary factor (motor disturbance) did not cause the impairment in the passive and active avoidance tasks Therefore, the disturbances in learning tasks in these rats seem to be due to dysfunction of the memory process induced by the cerebral embolization

Learning behavior in the embolized rats was also studied in another learning task without electric shock Although their ability to swim was not impaired, the rats' performance in the water maze was significantly disturbed they had an increased number of errors This result suggests that the disturbance in water maze performance is not due to a deficit in swimming ability This assumption is also supported by the fact that there was no difference betwen two groups in performance time in the maze learning Sham-operated rats stopped at the choice points and then chose the correct pathway, whereas the embolized rats swam toward the goal quickly, often entering into dead ends without hesitating at the choice points Thus, sham-operated rats took as long as the embolized rats to reach the goal but they made fewer errors

The hypothesis that the unilateral injection of microspheres leads to bilateral damage is supported by reports that unilateral embolization of the cerebral hemisphere produces dysfunction of the opposite hemisphere (diaschisis [19]), and that some microspheres injected unilaterally move to the opposite side [4] We also found that a slight edema occurred not only in the ipsilateral but also in the contralateral cerebral hemisphere after unilateral injection (unpublished data)

To assess recovery from the disturbances of learning behavior in the embolized rats, we studied the effects of cerebral embolization on acquisition of passive avoidance response at various times after the operation The impaired acquisition of passive avoidance response gradually improved and disappeared 8 weeks after the embolization However, the effects of cerebral embolization on the function of the central nervous system have not completely disappeared 8 weeks later, because cerebral edema was still observed in the left cerebral hemisphere at this time As the one-trial passive avoidance task is a simple one for learning, recovery from impairment in the passive avoidance task may be observed in the present experiment If we had used a more complex task, a longer-term disturbance might have been revealed in the embolized rats

In conclusion, the results of the present experiment demonstrate that rats with the cerebral embolization showed impairment in three different types of learning tests, and suggest that the embolized rat may be useful as an animal model for the vascular type of dementia

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