Table 1 Effects of starvation and dehydration of *Hadrurus arizonensis* on sodium and potassium concentrations of centrifuged ileal contents

Ions in ileal fluid	Freshly fed*	Starved and partially dehydrated†	Starved and severely dehydrated‡
Na	123.3±17.8 (5)	177.0±38.6 (3)	468.0±112.4 (7)
K	274.9±41.5 (5)	131.8±58.1 (3)	83.2± 24.3 (7)

Ion concentrations are expressed in mM (mean±s.e.m.) and numbers in parentheses represent sample sizes.
*Scorpions fed crickets the day before sampling.
†Scorpions maintained without food at 25 °C and at about 30% relative humidity for 2 weeks before sampling.
‡Scorpions maintained without food at 35 °C and about 30% relative humidity for 4 weeks before sampling.

0.24 μl cm^{-2} min^{-1} (n=36), which is not significantly different from zero (P>0.05). Figure 1 also indicates that when ilea were perfused with Na-free medium (normal medium on serosal side), the net flux of water from mucosa to serosa was eliminated (J_v= +0.28±0.22 μl cm^{-2} min^{-1}; n=44; 0.20>P>0.10). An analysis of variance test comparing J_v for the control group and net water flux for the Na-free preparations showed the two groups to be significantly different (P<0.005).

When potassium ion was eliminated from the saline perfusate and sodium ion remained normal (281.5 mM) net water flux from the mucosal to serosal side of the preparation increased significantly (P<0.005) above the control value of +1.79±0.26 (n=30) to 4.92±0.29 (n=42) μl cm^{-2} min^{-1} (Fig. 2, top). An increase in luminal potassium concentration from 8.0 to 50.0 mM at constant luminal sodium concentration (281.5 mM) resulted in a significant decrease (P<0.005) in mucosal to serosal J_v from a control value of +1.95±0.23 (n=23) to +0.43±0.14 (n=43) μl cm^{-2} min^{-1} (Fig. 2, bottom). The latter value is still significantly greater than zero (P<0.01). Control J_v values from the above three experiments, compared using analysis of variance, were not significantly different (P>0.05).

Scorpions that had eaten the day before sampling illustrated ileal sodium and potassium concentrations of 123.3±17.9 mM (n=5) and 274.9±41.5 mM (n=5), respectively (Table 1). Starving and dehydrating animals at temperatures up to 35 °C and for periods up to 4 weeks significantly increased the ileal sodium concentration (n=15; P<0.05) and significantly decreased the potassium concentration (n=15; P<0.01). In addition, the ratio of sodium to potassium was reversed. In freshly fed scorpions, Na/K was 0.46, whereas in starved and dehydrated scorpions Na/K was 5.6 (an ionic ratio that produced a net flux of water from lumen to blood in Fig. 2, bottom).

This is the first detailed study of a water transport mechanism in the ileum of a terrestrial arthropod, although Wigglesworth and Ramsay have suggested[9,10] that some water absorption occurs in this portion of the insect gut. Our data suggest that the ileal absorption of water in the desert scorpion occurs through a sodium-dependent, potassium-inhibited transport process. This mechanism is clearly adaptive as dehydration and starvation lead to intraluminal sodium and potassium concentrations which favour the uptake of water. In freshly fed animals excessive hydration could be reduced by the inhibitory interaction of potassium ion on the sodium-dependent water movement. Water balance in these organisms may depend on the relative sodium/potassium ratio present in the ileum. This will require further study.

The anatomical location of the inhibitory effect of potassium on sodium-dependent water movement remains unclear. The scorpion ileum, in contrast to that of insects, is unchitinised and has a tall columnar epithelium[11]. Therefore, a standing osmotic gradient[12-14] in the long intercellular spaces created by an electrogenic, energy-requiring lateral membrane cation pump could satisfactorily generate the transmural water fluxes observed. The rate of sodium efflux by the cation pump would be a function of the intracellular sodium concentration, which, in turn, would depend on the luminal concentration of this ion. Potassium ion, derived from the ileal lumen could compete with sodium for attachment to this lateral membrane cation pump and convert its electrogenicity into a simple K–K exchange involving no net ionic fluxes. A reduction or elimination of net transmural water flow would then result from a decrease in the intercellular standing osmotic gradient. In contrast, competition between sodium and potassium for a shared carrier process in the apical membrane, followed by active sodium efflux across the lateral membrane could equally well account for the effects of ions on water flow described here. Further studies of ion fluxes in the scorpion ileum are clearly needed to establish more thoroughly their role in transmural water movements.

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Fast block to polyspermy in sea urchin eggs is electrically mediated

THE prevention of polyspermy in sea urchin eggs is accomplished in two steps: a "fast" block, occurring within a few seconds of the entry of the fertilising sperm, and a "slow" block, associated with the cortical reaction (compare refs 1–4 with 5 and 6). Results presented here confirm the existence of the fast block and demonstrate that it is caused by the electrical depolarisation of the egg plasma membrane that accompanies the entrance of the fertilising sperm.

Eggs of *Strongylocentrotus purpuratus* were hand centrifuged and agitated mechanically with a jet of seawater, to remove their jelly coats. Some of the dejellied eggs adhered to the bottom of Falcon plastic Petri dishes, such that a microelectrode could be inserted. All experiments involved eggs with a stable resting potential of greater than −60 mV (Table 1). These values of resting potential are close to that calculated from independent ion tracer flux studies done on these eggs (my work in preparation with K. R. Robinson). A single microelectrode was used for both recording voltage and passing current, by means of a conventional bridge circuit. Experiments were done at 15 °C, in natural or artificial seawater, Tris-buffered at pH 8. In all experiments, the concentration was about 10^6 sperm per ml, which is within the range that can produce polyspermy in some batches of eggs, but not in most. Polyspermy was determined by observation of first cleavage: monospermic eggs divide into two equal cells, whereas polyspermic eggs

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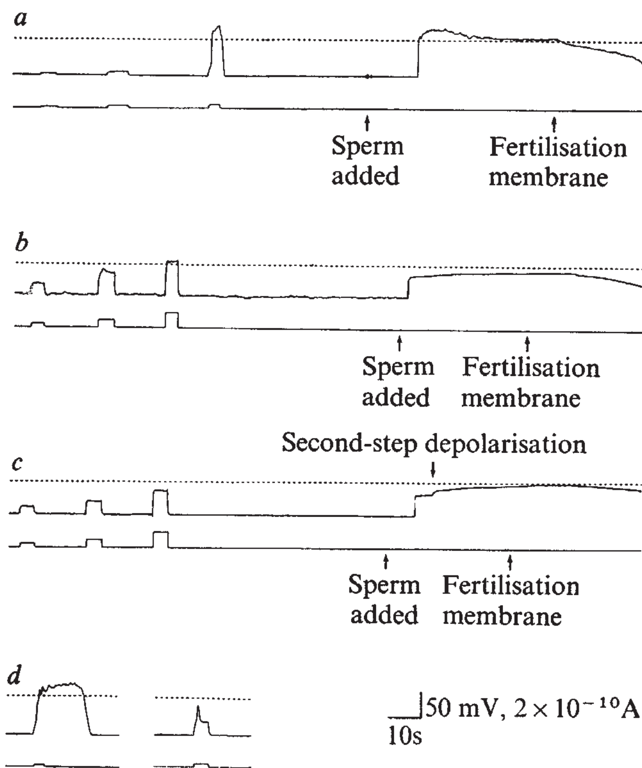

Fig. 1 Action potentials and activation potentials in *S. purpuratus*. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. Before addition of sperm, each egg membrane was tested for electrical excitability by applying a series of small depolarising current pulses. *a*, Egg with regenerative response to current pulse; monospermic activation potential. *b*, Egg with non-regenerative response; monospermic activation potential. *c*, Egg with non-regenerative response; polyspermic activation potential. *d*, Other examples of the regenerative response; a strong response outlasting the stimulus, a weak response.

cleave abnormally, dispermic eggs usually producing three or four cells at once[7,8].

Figure 1 shows intracellular electrical records from three eggs before, during and after addition of sperm. The fast depolarisation after the addition of sperm has been called the "activation potential"[9–15]. Within 3–30 s after the introduction of sperm near the egg, the egg membrane depolarises to a plateau at -30–$+20$ mV, and then after about 1 min begins to repolarise.

An interesting correlation was found between the plateau level of the activation potential (measured 3 s after its rise) and the occurrence of polyspermy (Table 1). Eggs with activation potentials which reached a plateau more positive than 0 mV were never polyspermic (none out of eight cases) and those which reached a plateau less positive than -10 mV were sometimes polyspermic (seven out of 13 cases). These results suggest that the entry of extra sperm is prevented by the more positive-going activation potential.

Figure 1*c* shows an activation potential in a polyspermic egg. About 5 s after the initial rise, there was a distinct second step depolarisation (see arrow). Eleven polyspermic activation potentials were observed (seven listed in Table 1, four not shown). All 11 showed this second step, and sometimes a third step. Ten monospermic activation potentials, as in Fig. 1*b*, were observed; of these, eight definitely did not show a second step. Two cases did show a second step; their nature is obscure. (In cases like that in Fig. 1*a*, with an oscillating plateau level, the steps could not be counted.) These results indicate that the second step depolarisation is associated with the entrance of a second sperm. The voltage at which this second step occurs is always more negative than -10 mV, suggesting that when the egg membrane is depolarised beyond -10 mV, sperm penetration is less probable.

Figure 2*a* shows that when current was applied to hold the unfertilised egg membrane at a potential more positive than $+5$ mV, sperm did not fertilise the egg. In spite of the presence of many active sperm adhering to the egg surface, no fertilisation membrane formed, and no electrical event resembling an activation potential occurred. In some experiments, the potential was held for more than 5 min, with repeated additions of sperm; but still no fertilisation occurred in the voltage-clamped eggs. (Surrounding eggs formed fertilisation membranes within 1 min of the first addition of sperm.) As soon as the current was turned off, fertilisation occurred: the egg depolarised, showed a typical activation potential, formed an apparently normal fertilisation membrane with the usual time course, and, if the activation potential was of the one-step form, first cleavage was normal, indicating that only one sperm had entered the egg. The same result was obtained with holding potentials ranging from $+5$ to $+130$ mV. In other experiments, the potential was held for about 10 min, without addition of fresh sperm, after which most sperm had become less active and incapable of fertilising eggs. When the current was turned off, the egg membrane returned to its original resting potential and showed the electrical properties characteristic of the unfertilised state. Morphologically, there was no sign of activation, and no development occurred. If more sperm were added, fertilisation and development proceeded in the usual way.

Fertilisation is prevented at $+5$ mV; but it occurs at -10 mV, as determined by the experiments reported in Table 2. The egg was depolarised enough to block fertilisation, and sperm were added. After 3–5 min, the voltage was lowered to a point where fertilisation occurred. Each experiment sets an upper and a lower limit on the threshold voltage for suppressing sperm entry. This is between $+5$ and -10 mV; at intermediate potentials, entry of sperm may depend on their concentration and activity, and on individual variation among eggs. This threshold is consistent with the following observations on activation potentials. (1) Eggs whose activation potential plateau (measured 3 s after the initial rise) was more positive than 0 mV, are

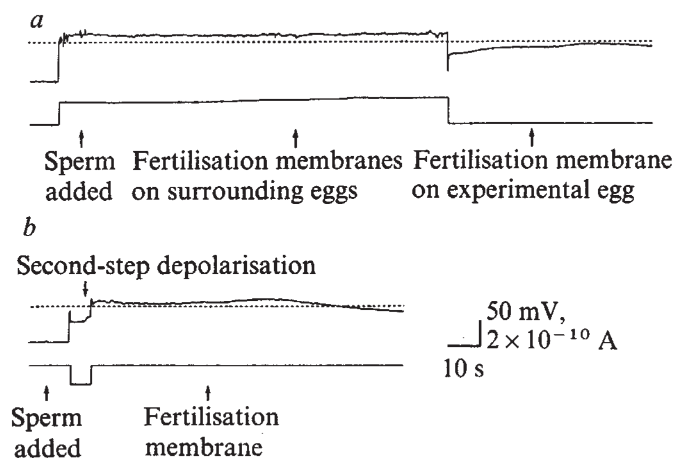

Fig. 2 Demonstration of potential dependence of sperm entrance. The top trace is voltage against time; the bottom trace is current against time. The dotted line indicates 0 mV. *a*, Suppression of fertilisation by positive holding potential. *b*, Induction of polyspermy by negative holding potential. In (*a*) the amount of current required to maintain the holding potential increased about 10% during the first 1–2 min after the current was turned on. This increase was seen even if sperm were not added. In some experiments, sperm were not added until this initial adaptation of the egg membrane was complete. The addition of sperm did not cause any change in the amount of current required to maintain the potential.

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Table 1 Activation potentials for monospermic and polyspermic eggs

Egg	Resting potential (mV)	Regenerative response	Activation potential plateau (mV)	First cleavage	No. of steps in rise of activation potential	Voltage at second step (mV)
A	−75	Strong	+23	M	—	—
B	−69	Strong	+17	M	—	—
C	−68	Strong	+15	M	—	—
D	−76	Strong	+14	M	—	—
E	−77	Strong	+12	M	—	—
F	−74	Strong	+10	M	—	—
G	−71	Strong	+ 6	M	—	—
H	−71	Weak	0	M	—	—
I	−71	Weak	−12	P	2	− 9
J	−74	NT	−13	M	1	—
K	−68	Weak	−14	M	1	—
L	−75	Weak	−15	M	1	—
M	−56	NT	−17	P	2	−15
N	−60	None	−19	M	1	—
O	−63	None	−20	M	1	—
P	−79	Weak	−20	P	2	−14
Q	−71	Weak	−22	P	2	−22
R	−64	None	−23	P	2	−22
S	−70	Weak	−26	M	2	−27
T	−65	None	−29	P	2	−26
U	−78	NT	−35	P	3	−35, −24

The activation potential plateau was measured 3 s after the initial rise. Eggs were classified as monospermic (M) if first cleavage produced two equal cells; otherwise, they were classified as polyspermic (P). The regenerative response of the unfertilised egg was classified as strong if in response to a 5-s current pulse of 3×10^{-11} A, the membrane maintained a positive potential for the duration of the pulse. None means that the response was completely, or almost completely, non-regenerative. Weak means that there was some regenerative response, but it was not strong. NT, Not tested.

always monospermic. (2) The entrance of the second sperm in polyspermic eggs occurred at a voltage more negative than −10 mV.

Application of current to bring the plateau of the activation potential to a more negative level facilitated entry of additional sperm (Fig. 2*b*). After the activation potential began, current was applied to hold the potential at −30 mV. A second-step depolarisation occurred, then the current was turned off. Observation of first cleavage showed that this egg was polyspermic, whereas 98% of the surrounding eggs in the dish were monospermic. This experiment was repeated seven times, with holding potentials of −30 ± 5 mV. In all cases, experimental eggs were polyspermic and surrounding eggs were monospermic.

These experiments demonstrate that the electrical depolarisation occurring at fertilisation constitutes a fast block to polyspermy. It was frequently observed that when the electrically monitored egg was polyspermic, other eggs in the dish were also polyspermic (except in cases where current was applied to make an egg polyspermic). Therefore, the lack of a fast block to polyspermy is a property of a batch of eggs. Loss of the capacity to block polyspermy might be a symptom of egg deterioration, either in the adult, or after removal from the adult.

The rise of the activation potential can occur within 3 s of insemination. Part of this time is necessary for the sperm to swim to the egg surface and to attach to the vitelline membrane. Only then does the sperm fuse with the egg plasma membrane. Therefore, it can be concluded that the fast block to polyspermy is established in less than 3 s after fusion of the fertilising sperm. Quite possibly, the rise of the activation potential is a direct consequence of the disturbance of the egg membrane by sperm fusion. If this is the case, it follows from the rate of rise of the activation potential that the fast block to polyspermy is established in less than 1 s after fusion. (The rise from resting level to −10 mV takes between 0.1 and 1 s.) The membrane remains in the depolarised state for about 1 min, after which the cortical reaction has occurred, thus providing a permanent block to polyspermy.

In each example in Fig. 1, a series of small depolarising current pulses was applied to the egg before sperm were added. In the case shown in Fig. 1*a*, the stimulus pulse induced a regenerative response of the egg membrane. This response showed a threshold at about −50 mV, and sometimes outlasted the stimulus (Fig. 1*d*); it has been observed in other echinoderm oocytes and eggs[15–17] and has been studied in sea urchin eggs by K. Takahashi (personal communication). It is an action potential of the sort seen in nerve and muscle (as distinct from the activation potential). Figure 1*b* and *c* illustrates eggs which did not show a regenerative response to depolarisation. These eggs showed less positive-going activation potentials after sperm addition, compared with the egg in Fig. 1*a*. This relationship between the presence of the regenerative response and the height of the activation potential is also shown in Table 1. The correlation indicated that the activation potential is caused partly by a regenerative response of the egg membrane. The data in Table 1 suggest that the sperm fusion depolarises the egg to a level of about −30

Table 2 Voltage threshold for suppression of fertilisation

Egg	Suppressing potential (mV)	Non-suppressing potential (mV)
A	+6	−6
B	+3	−11
C	+2	−10
D	+1	−10
E	+5	−7
F	+3	−12
G	+5	−6
H	+6	−5
I	—	−2

The egg membrane was held at the suppressing potential and sperm were added. About 1 min later, all the surrounding eggs had formed fertilisation membranes. 3–5 min after addition of sperm, the potential was reduced to the non-suppressing level: within 1–2 min, the experimental egg formed a fertilisation membrane. After removal of the electrode, balance of the WPI bridge circuit was tested by applying a current pulse of the same magnitude used in the experiment. If the imbalance was greater than 2 mV, data were not used. Error associated with the single microelectrode technique was small, because of the small currents used ($0.3–3 \times 10^{-10}$ A), and the high input resistances of the cells (200–2,000 MΩ, depending on the degree of regenerativeness of the egg's response), compared with the resistances of the microelectrodes (40–80 MΩ).

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to −20 mV. Then, in eggs which have the capacity to produce a regenerative response, the sperm-initiated depolarisation is amplified, bringing the egg membrane potential to a level which excludes additional sperm.

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Translation of RNA from unfertilised sea urchin eggs does not require methylation and is inhibited by 7-methylguanosine-5′-monophosphate

AT fertilisation, the rate of protein synthesis in the sea urchin embryo becomes several times greater than that observed in unfertilised eggs[1–4]. This increase does not require the synthesis of messenger RNA (mRNA), since it occurs both in the presence of actinomycin D (ref. 5) and in enucleated parthenogenetically activated egg fragments[6]. These observations led Spirin[7] to postulate the existence of preformed "maternal" mRNA in the eggs. Recent experiments have directly demonstrated the presence of mRNA in sea urchin egg cytoplasm by isolating it from postribosomal cytoplasmic fraction and translating it in a heterologous cell-free system[8].

The mechanism(s) responsible for the increased rate of protein synthesis at fertilisation is still not understood. Some investigators have attributed this increased rate to the removal of an inhibitor of protein synthesis from the egg ribosomes[9,10]. Clegg and Denny[11], however, have reported that ribosomes isolated from unfertilised eggs translate rabbit globin mRNA as efficiently as ribosomes isolated from zygotes when supplied with ascites cell postribosomal supernatant and reticulocyte initiation factors. These authors concluded that the low protein synthetic activity in unfertilised eggs is not attributable to the presence of inhibited ribosomes[11].

Activation of mRNA by adenylation may also be important in the increased rate of protein synthesis[12,13]. The mRNAs coding for histones are never adenylated[14], however, but make up a considerable proportion of maternal mRNA[8,15]. Adenylation thus does not have any role in the translational activation of this class of mRNAs, although it may be involved in increasing the stability of other mRNAs[16]. In this paper we discuss two other mechanisms for the activation of maternal mRNA which involve the methylation of the 5′-terminal nucleotide or the addition of 5′-terminal 7-methylguanosine.

The 5′ termini of many viral and cellular mRNAs have been shown to contain a 7-methylguanosine residue linked, by way of a triphosphate, to the 5′-hydroxyl of the adjacent nucleotide ($m^7G^{5'}ppp^{5'}Xp$) (ref. 17). Removal of the 5′-terminal $m^7G^{5'}p$ from globin mRNA prevents its translation[18]. Moreover, Both *et al.*[19] have shown that the presence of the methyl group in the 5′-terminal guanosine is required for translation of some viral RNAs. These RNAs can be obtained without a methyl group in the 5′-terminal guanosine by synthesising them *in vitro* in the presence of an inhibitor of methylation, S-adenosylhomocysteine (SAH)[20]. Wheat germ extracts can methylate these RNAs specifically to yield the structure $m^7G^{5'}ppp^{5'}G$ in the case of reovirus RNA, which can then be translated[19]. In the presence of SAH, however, the RNA is not methylated and cannot be translated[19,20]. Both *et al.*[19] considered the possibility that methylation may be involved in the activation of maternal mRNA at fertilisation.

The assay used so far to translate maternal mRNA could not discriminate between mRNAs methylated in the 5′-terminal

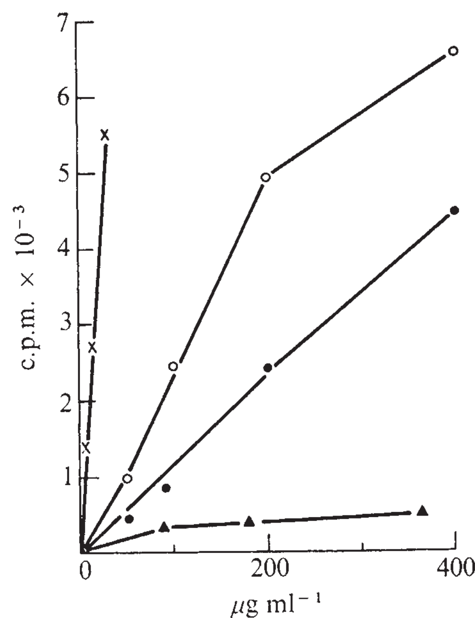

Fig. 1 Stimulation of protein synthesis in a wheat germ cell-free system by RNA isolated from unfertilised sea urchin eggs. Eggs were collected from *Lytechinus pictus* by injection of KCl and washed as described previously[8]. About 10 ml of pelleted eggs were homogenised at 0 °C with 10 strokes of a loose fitting Dounce homogeniser in 35 ml of a buffer containing 200 mM NaCl, 5 mM magnesium acetate, and 20 mM Tris, *p*H 7.6. A post-mitochondrial fraction was obtained by centrifugation at 17,000*g* for 15 min. This fraction was adjusted to contain 1% sodium dodecyl sulphate and 10 mM EDTA, then warmed to room temperature and deproteinised with phenol–chloroform–isoamyl alcohol (25:24:1) (ref. 28). RNA was recovered by ethanol precipitation and separated into poly(A)+ and poly(A)− fractions by chromatography on oligo(dT)–cellulose[22]. A portion of the poly(A)− RNA was further fractionated by sucrose gradient centrifugation. The RNA sedimenting in the 6–12S region of the gradient was recovered by ethanol precipitation. All RNA preparations were reprecipitated from ethanol three times before translation in a wheat germ cell-free system[21]. Each assay contained in 25 μl:7.5 μl of wheat germ extract, 1.2 mM ATP, 0.25 mM GTP, 15 mM creatine phosphate, 0.34 units of creatine phosphokinase (Sigma), 22 mM HEPES–KOH buffer *p*H 7.1, 3 mM magnesium acetate, 0.1 M KCl, 2.6 mM DTT, 1.8 mM mercaptoethanol, 50 μM unlabelled amino acids minus lysine, and 2.5 μCi of ^{3}H-lysine (38 Ci mmol^{-1}). RNA was added to give the concentrations indicated. The incubation was carried out for 60 min at 24 °C and 5-μl samples were taken in duplicate for counting as described previously[21]. A background of 800 c.p.m. obtained in an incubation without added RNA was subtracted from each value. Nonspecific stimulation resulting from the addition of ribosomal RNA (rRNA) was monitored by the addition of 16S RNA isolated from *E. coli* ribosomal subunits by phenol extraction and sucrose gradient centrifugation. ×, Poly(A)+ (total); ○, poly(A)− (6–12S); ●, poly(A)− (total); ▲, *E. coli* 16S rRNA.

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