Adrenergic Stimulation and Sodium Transport*

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Epinephrine is known to influence electrical and, therefore, ion transport phenomena in cell membranes in various tissues, including cardiac pacemaker (Hutter and Trautwein, 1956) and contractile cells (Webb and Hollander, 1956), smooth muscle (Bulbring, 1960) and skeletal muscle (Dockry et al., 1966). Also, administered catecholamines and sympathetic nerve stimulation alter renal excretion of electrolytes (Kruhoffer et al., 1960), although it has been postulated that these changes are related primarily to hemodynamic alterations rather than to a direct effect of adrenergic stimulation on renal tubular ion transport mechanisms. However, Koefoed-Johnsen et al. (1953) reported that epinephrine produced changes in sodium transport and electrical properties of isolated frog skin.

Ahlquist (1948), comparing the potency of various catecholamines, postulated the presence of two types of adrenergic receptors (alpha and beta) in effector cells such as smooth muscle, cardiac muscle, and salivary glands. Since that time, adrenergic blocking agents which specifically block alpha or beta stimulation have been developed (Ahlquist, 1965). The studies to be presented here were undertaken to clarify further the nature of the alterations in sodium transport in isolated frog skin (a model system for study of ion transport), particularly in relation to the Ahlquist concept of alpha- and beta-adrenergic receptors. Previous studies in living animals suggest that the frog skin is responsive to both alpha and beta stimulation (Watlington, 1965).

The skin model used here (Fig. 1) is based on the work of many investigators, and its application is particularly well exemplified by the studies of Curran and co-workers (Curran et al., 1963; Cereijido et al., 1964). Sodium transport inward is thought to be by a two-step process across the epidermis, both steps being rate limiting. As shown in figure 1, there is passive diffusion across the epidermal permeability barrier into the active transport site followed by active transport inward into the internal medium. Outflux, which is small relative to influx (thus accounting for net flux or net transport of sodium), may be primarily a passive process. The mucous glands offer a possible pathway for sodium movement although they are not felt to be important in the unstimulated isolated skin.

Methods

In most of the sodium flux experiments single pieces of abdominal skin of frogs (*Rana pipiens*, 40 to 60 gm) were used. Sodium influx and sodium outflux were determined on different pieces of skin. Influx was measured by placing Na^{22} on the epidermal side and sampling the inside or corium side. In outflux experiments Na^{22} was placed on the corium side, and samples were removed from the epidermal side. The estimation of rate coefficients (see "Results") was performed on paired skins using Na^{24}. The skin pairs were obtained by dividing the belly skin of larger frogs (70 to 90 gm) into halves. Determination of rate coefficients also requires knowledge of the skin extra-cellular space as measured from the outside solution; C^{14} inulin was used for this purpose. Na^{22} and Na^{24} activity was measured in a crystal scintillation counter. C^{14} activity was measured by liquid scintillation counting.

The skins were mounted between two conical Lucite chambers, placed one on each side of the skin. The chambers were filled with Ringer's solution of the following composition: NaCl, 110 mM; KCl, 10 mM; $NaHCO_3$, 4 mM; Na_2HPO_4, 1.3 mM; pH 8.1. The solutions were aerated and mixed by bubbling with moisturized air. Each chamber was provided with Ringer agar bridges connected to

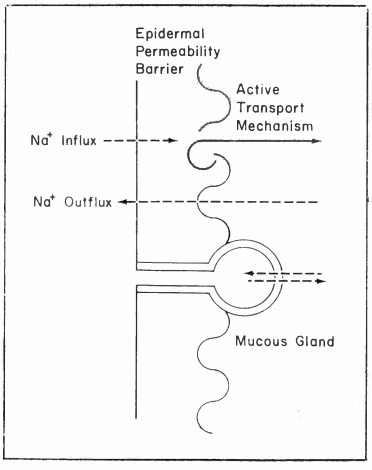

Fig. 1—Pathways for sodium transport across frog skin.

* Supported by Research Grant AM-06481 from the National Institute of Arthritis and Metabolic Diseases.

† Recipient of Public Health Service Special Fellowship 5F3 AM-18, 664.

calomel half cells so that during the flux experiments the skins could be continuously short-circuited by the method of Ussing and Zerahn (1951). This experimental approach simplifies the study of active ion transport by eliminating the two major passive forces across the skin which influence rates of ion movement, namely those resulting from chemical and electrical potentials. Use of identical Ringer's solution on each side eliminates a chemical potential gradient of ions. Short-circuiting the skin eliminates the spontaneous skin potential difference (P.D.) or the electrical potential gradient. The short-circuit current which flows across the skin under the above conditions is measured and its equivalent in ionic flow, the short-circuit current equivalent (SCCE), is then calculated. The SCCE is considered to be the sum of all ions flowing across the membrane and the result of active transport by the membrane, since the major passive forces have been eliminated. Figure 2 depicts the relationship between sodium flux and SCCE. In the non-stimulated skin, there is active transport of sodium only. In this case, sodium influx minus sodium outflux (which is net flux) is equal or equivalent to the SCCE. If active transport of another ion or ions occurs, net sodium flux is not equal or not equivalent to the SCCE. In other words, non-sodium current appears.

Table 1 lists the drugs and the concentrations used for adrenergic stimulation. All compounds were placed in the solution in contact with the inside of the skin. Catecholamines were administered at the end of a one hour control flux period. Epinephrine was used for combined alpha and beta receptor stimulation, as it has both types of effects. Alpha stimulation was achieved with epinephrine, administered one-half hour following blockade of its beta effect with pronethalol. Beta stimulation was produced with isoproterenol which

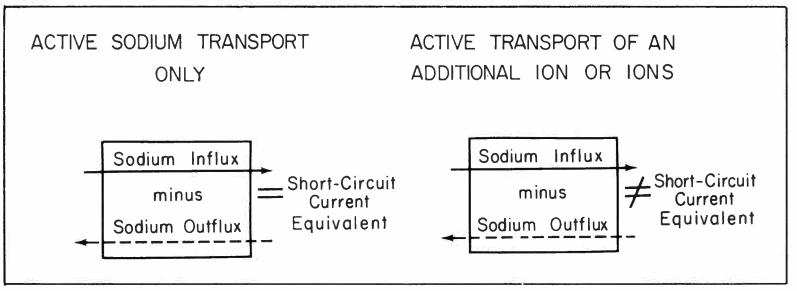

Fig. 2—Relationship between sodium flux and SCCE in the isolated frog skin in the absence of electrical and chemical gradients across the skin.

TABLE 1.
Drugs used for adrenergic stimulation. See text for time of administration.

Receptor Type	Drugs
Alpha and Beta	Epinephrine (3.9×10^{-6}M)
Alpha	Epinephrine (3.9×10^{-6}M) Beta blockade-Pronethalol (1.7×10^{-4}M)
Beta	Isoproterenol (0.78×10^{-6}M)

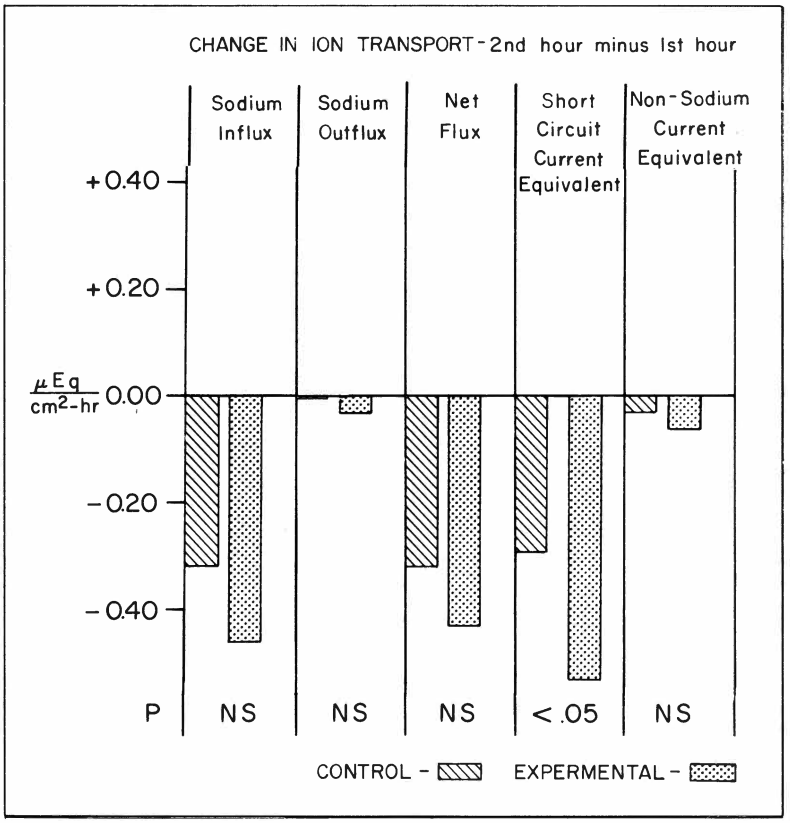

Fig. 3—Effect of alpha-adrenergic stimulation (pronethalol plus epinephrine) on sodium flux and short-circuit current. *P* refers to *t* test comparing change in experimental group to change in control. *P* values greater than .05 are considered not significant.

exerts little if any alpha-adrenergic effect (Ahlquist, 1965). Isoproterenol is 3 to 10 times as potent as epinephrine in beta stimulation effect (Mayer and Moran, 1960); therefore isoproterenol was used in one-fifth the concentration of epinephrine. Eight influx and eight outflux experiments were performed concomitantly in each of these categories, as well as in a control series in which two sequential one hour flux periods were performed without administration of catecholamines.

Results

Figure 3 compares the average changes in sodium flux and short-circuit current produced by alpha stimulation to the average changes in the control series. Epinephrine was placed in the inside chamber at the beginning of the second hour flux periods (30 min after beta blockade with pronethalol). The resulting changes in fluxes and current are compared to the changes that occurred spontaneously during this time period in the control group of experiments. After alpha stimulation, average sodium influx, outflux, net flux, and SCCE all decreased more than in the control experiments. The greater decrease in SCCE was significant and was nearly accounted for by the greater decrease in net sodium flux. Thus, little if any non-sodium current equivalent appeared, indicating that the SCCE change was due to sodium transport change only.

The decrease in net sodium transport produced by alpha-adrenergic stimulation could be caused by an alteration in either of the two steps for sodium transport. Therefore, the rate coefficients for the permeability step, k_{12}, and the active transport step, k_{23}, were estimated with eight paired skins obtained as discussed in "Methods." The control skins of each pair were exposed to pronethalol (1.7×10^{-4}M). Pronethalol alone produced no alteration in sodium transport at the drug concentration used. Alpha stimulation was produced with pronethalol plus epinephrine (1.6×10^{-6}M). The permeability rate coefficient, k_{12}, is significantly reduced ($P < .05$) by alpha stimulation when compared to control (Fig. 4). There is no significant difference in the rate coefficients for active transport, k_{23}, in the two groups. These findings indicate that alpha stimulation depresses sodium transport by decreasing permeability in the epidermal pathways for sodium transport.

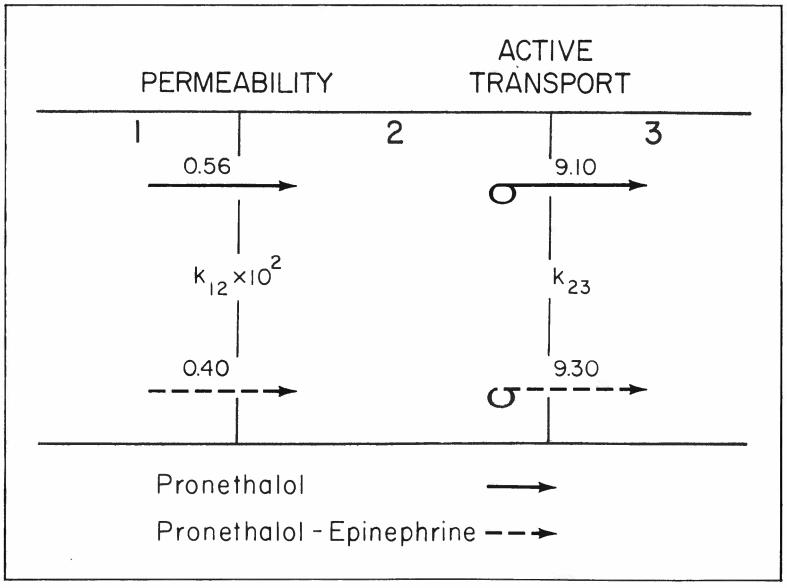

Fig. 4—Effect of alpha-adrenergic stimulation (pronethalol plus epinephrine) on the rate coefficients (hr^{-1}) for the epidermal sodium transport system. *1* and *3* represent the outer and inner bathing solutions respectively, and *2* represents the epidermal sodium transporting compartment.

The results with beta-adrenergic stimulation with isoproterenol were quite different (Fig. 5). Sodium influx and outflux increased greatly. It is of particular interest that the differences between the spontaneous change in the control experiments and the change in the beta stimulation experiments (relative change), which is an index of the magnitude of flux change attributable to beta stimulation, are approximately equal for influx and outflux. This difference or relative change is shown at the bottom of each column. The influx increased 0.65 μEq/cm^2 $\times$ hr relative to the control, and the outflux increased 0.56 μEq. This resulted in a net flux change which is approximately that of the control experiments. In other words, beta stimulation did not alter net sodium transport.

The large and equal increase in influx and outflux suggests that beta stimulation produces an increase in sodium permeability. However, if this increase in permeability occurred in the epidermal system for sodium transport previously discussed, an alteration in net flux or net transport should occur, for sodium permeability is a rate limiting factor in epidermal sodium transport inward (*see* Fig. 4). Therefore, the change in sodium flux must occur in another pathway for sodium movement. Beta stimulation causes mucous discharge by the skin (Watlington, 1965), and it may be that the mucous glands are the pathways for the increased sodium movement. It should also be noted (Fig. 5) that after beta stimulation, non-sodium current develops, and it has been postulated that this is the result of active chloride transport outward by the mucous glands (Koefoed-Johnsen et al., 1953).

The effects of epinephrine, which

produces both alpha and beta stimulation, is shown in Figure 6. The results are compared to the sum of the effects of alpha stimulation (pronethalol plus epinephrine, Fig. 3) and beta stimulation (isoproternol, Fig. 5). The changes in sodium flux, short-circuit current, and non-sodium current are all very similar in magnitude so that the separate alpha and beta effects well account for the changes produced by the combined alpha and beta stimulation produced by epinephrine.

Discussion

Figure 7 presents a hypothetical scheme of the nature of the alpha- and beta-adrenergic influences on sodium transport in frog skin. Alpha receptor stimulation decreases permeability of the epidermal system for sodium transport and hence reduces influx and outflux. The result is a decrease in net sodium transport. Beta-adrenergic receptor stimulation produces increased permeability of another pathway for sodium movement which is not involved in active transport of the ion, so that equal increases of influx and outflux occur. The effects with epinephrine, then, would be the resultant of these two opposing alterations in rates of sodium ion movement.

Alpha and beta stimulation produce opposite effects on smooth muscle contraction in most systems studied, including the arterioles (Ahlquist, 1965). Insulin release by the pancreas has been reported to be altered in opposite directions by the two stimuli (Porte, 1966). It is conceivable that opposing alterations in membrane permeability are fundamental to these and other physiologic changes produced by alpha- and beta-adrenergic receptor stimulation.

The possible role of cyclic 3′,5′-AMP, in the action of catecholamines on ion transport, merits discussion. This substance had been shown to be the mediator of epinephrine-induced activation of liver phosphorylase and has been proposed as the mediator of the action of catecholamines on other systems, as recently discussed by Sutherland and Robison (1966).

Strong evidence has been presented to implicate cyclic 3′,5′-AMP as the mediator of the increase in sodium and water permeability produced by vasopressin in toad bladder (Orloff and Handler, 1962; Handler et al., 1965). Vasopressin produces similar permeability changes in isolated frog skin (Koefoed-Johnsen and Ussing, 1953). An increase in tissue concentration of cyclic 3′,5′-AMP has been found in isolated frog skin treated with vasopressin, epinephrine, or isoproterenol (Watlington, Butcher, and Sutherland, unpublished). Beta blockade prevented the rise following the administration of epinephrine or isoproterenol. This suggests that the increase in cyclic 3′,5′-AMP concentration in tissue with catecholamines is a beta-adrenergic effect. It is probable that the increase in sodium and water permeability in frog skin produced by vasopressin is related to cyclic 3′,5′-AMP, as in toad bladder, in view of the marked similarities in the two tissues in regard to ion and water transport. The coincidence of an increase in sodium permeability and a rise in cyclic 3′,5′-AMP with beta-adrenergic stimulation in frog skin also suggests a causal relationship, although as previously discussed, the site of beta-adrenergic stimulation probably is other than that influenced by vasopressin.

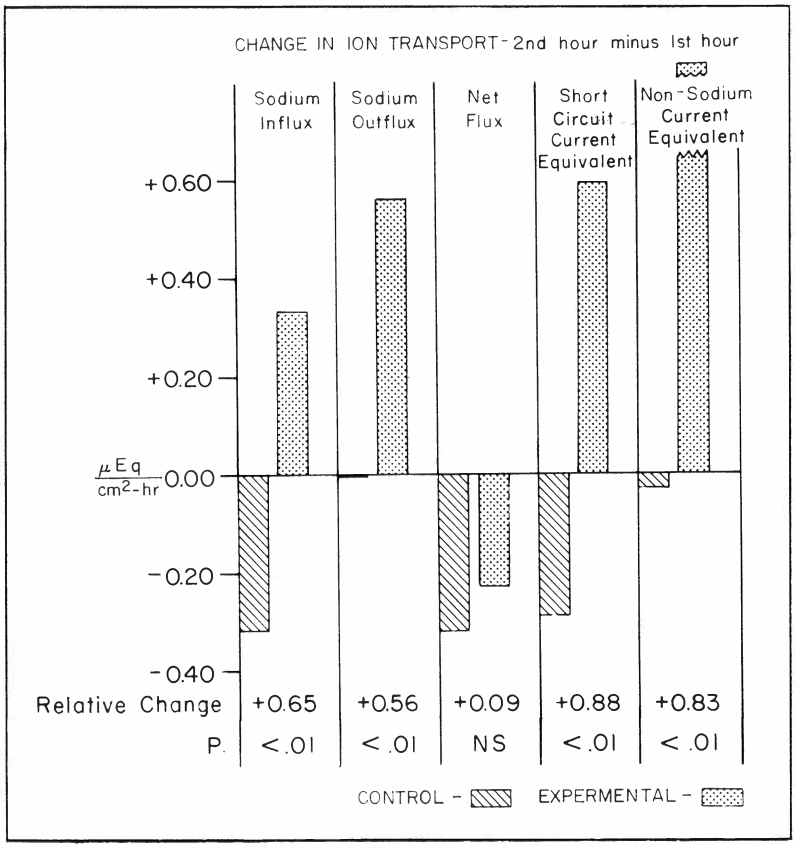

Fig. 5—Effect of beta-adrenergic stimulation (isoproterenol) on sodium flux and short-circuit current. *P* refers to *t* test comparing change in experimental group to change in control. *P* values greater than .05 are considered not significant. See text for meaning of "Relative Change."

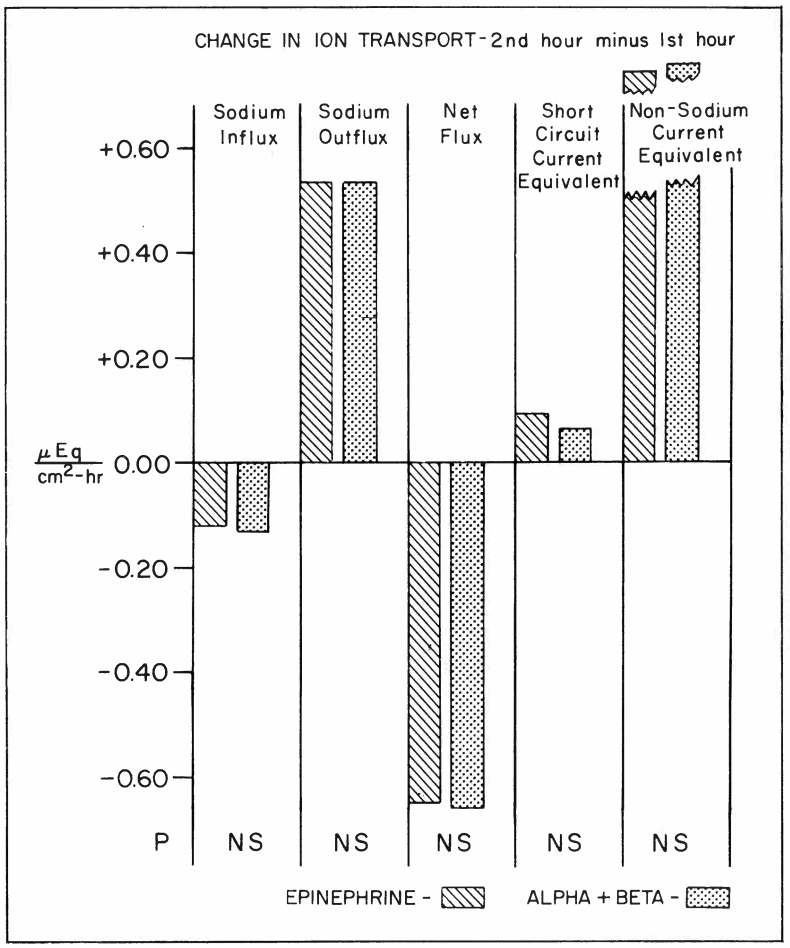

Fig. 6—Comparison of the effect of epinephrine and the algebraic sum of alpha and beta stimulation, separately produced, on sodium transport and short-circuit current. *P* refers to *t* test comparing change in experimental group to change in control. *P* values greater than .05 are considered not significant.

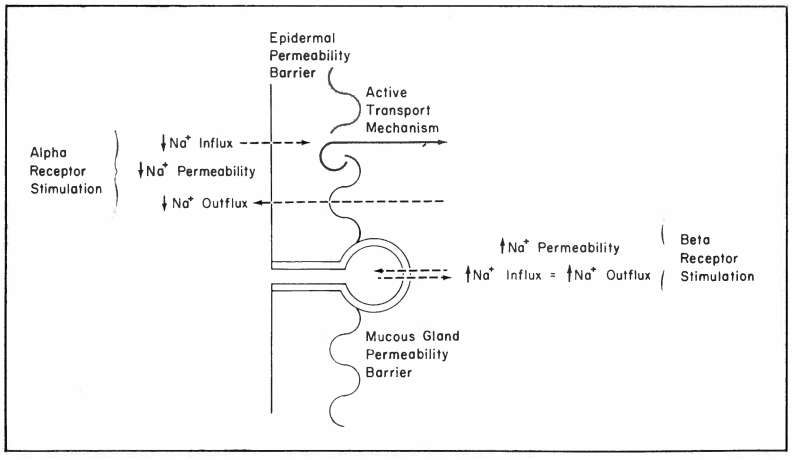

Fig. 7—Tentative scheme of the mode of action of alpha- and beta-adrenergic stimulation on sodium transport in isolated frog skin.

In view of the opposite effect of alpha-adrenergic stimulation on sodium permeability, it is conceivable that the permeability decrease is induced by a decrease in cyclic 3′, 5′-AMP levels. Sutherland (1963) previously considered the possibility that alpha-adrenergic stimulation may decrease tissue cyclic 3′,5′-AMP levels. Alonso et al. (1965) demonstrated a 50% decrease in short-circuit current following treatment of toad bladder with imidazole. This substance increases the tissue concentration of the phosphodiesterase which degrades cyclic 3′,5′-AMP. Thus, a decrease in tissue cyclic 3′,5′-AMP may have occurred and resulted in the decrease in short-circuit current secondary to a reduced sodium permeability of the system for active sodium transport.

Summary

The effect of catecholamines and a beta-adrenergic blocking agent on sodium flux and short-circuit current was evaluated in isolated frog skin. Alpha stimulation (pronethalol plus epinephrine) decreased net sodium flux and short-circuit current to an equivalent degree. Kinetic studies during alpha stimulation demonstrated a decrease in rate coefficient for entry into the skin transporting compartment but no change in the rate coefficient presumed to be related to active transport. Beta stimulation (isoproterenol) produced an equivalent increase in sodium influx and outflux with no change in net flux, and development of non-sodium current. The results suggest opposing effects of alpha- and beta-adrenergic stimulation on sodium permeability, although on different pathways for sodium movement, i.e., alpha stimulation decreases sodium permeability of the epidermal pathways for active transport, and beta stimulation increases permeability to sodium via another pathway. The beta effects may be related to mucous gland stimulation.

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