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Neural Network as Crosstalk Models, an Example in Adrenaline and Insulin Signalling Pathways

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***Abstract* – Since 1960, scientists have observed unexpected interactions between proteins of distinct pathways or molecules in the cells. These interactions have been termed as crosstalk and increasing evidence portrays crosstalk as an important component for a cell's robustness. In this study we use the insulin and adrenaline pathway as an example while employing machine learning techniques to study the crosstalk between the two pathways. Insulin and adrenaline are two important regulators of glucose metabolism and other physiological processes in rat skeletal muscles. While adrenaline's effects require cAMP and PKA and insulin's effects require PKB, recent evidence indicates possible crosstalk between the two pathways via Epac (Exchange protein directly activated by cAMP) in some cell types.The results show that the model can explain the crosstalk consistent with the biological finding.**

I. INTRODUCTION

Over the past decades, many signalling molecules (proteins, lipids and ions) and the way through which they communicate via signalling pathways have been identified and elucidated. Extracellular cues trigger multiple sequential events in which signalling proteins are physically and chemically modified; e.g. covalent modifications (phosphorylation), recruitment, allosteric activation or inhibition and binding of proteins; affect subsequent proteins and culminate in a specific phenotypic cellular response [1]. It is now apparent that signalling does not necessarily occur only in parallel linear pathways, but rather through a large and complex network of interacting signalling networks [2]. With signalling proteins from different pathways interacting directly (e.g. phosphorylation) or indirectly (e.g. via regulation of gene expression), it is now understood that interpathway *cross-talk* can reflect underlying complexities within a cellular signalling network causing the output of a signalling pathway to depend non-linearly on the input [3], [4], [5].

Crosstalk is generally described in biochemistry and molecular biology as indirect influences between signalling pathways. The term encompasses positive and negative signalling, layered changes in gene expression and feedback between signalling proteins [6]. Crosstalk can also be described as specific interactions between proteins of more than one signalling pathway. Crosstalk events can be observed when there is a shared component between two or more different pathways or in protein-protein interactions. This general and specific description implies that crosstalk acts to balance signal specificity (e.g. one output for one specific input) and signal integration (e.g. one output for many inputs).
[1]

The specificity of biological responses is largely generated by the combinatorial integration of pathway crosstalk and the versatility of component functions [7]. Since 1960, biological studies of crosstalk have increased exponentially suggesting that crosstalk is an important phenomenon in cell signalling; which poses the question: to what extent does the consequence of crosstalk affect the robustness of a signalling cell? Fig 1 shows the number of published articles in PubMed since 1960.

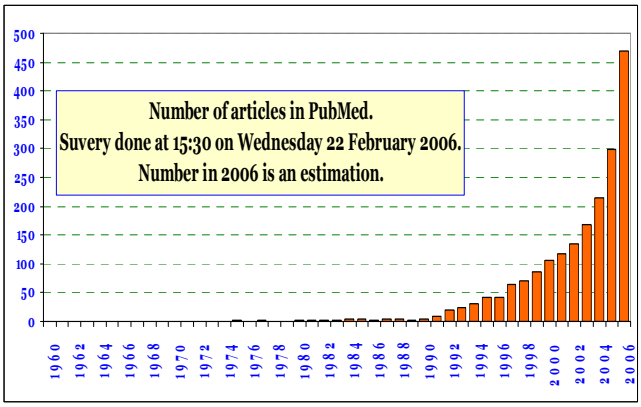

Fig 1: Experimental studies on crosstalk since 1960 till February 2006.

There have been two main streams of research in signaling network complexity. The first is to use mathematical modeling approaches like differential equations systems. The second is to use graphic models or Bayesian net approaches [8].In recent research, machine learning methods have been applied to gene expression data for study and discovery of genetic, regulatory pathways [9], [10] as well as to protein data to understand signal-response cascade relationships [8] and find casual relationships among biological pathways with success. Machine learning techniques have a rich history in bioinformatics studies. They can represent complex non-linear relationships among multiple interacting molecules; they can accommodate noise which is inherent in biological data and describe statistically meaningful direct as well as indirect

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influences that proceed through addition unobserved components.

Previous crosstalk models of MAP Kinase pathways have been studied using linear models with considerable success [11].In the present study we have investigated the crosstalk between adrenaline on the insulin signalling pathway in rat skeletal muscles Insulin and adrenaline are two of the most important regulators of physiological processes like glucose metabolism, ion transport and protein synthesis in skeletal muscles [12]. Adrenaline has well characterised effects in muscle which via binding β-adrenergic receptor activates cAMP and PKA resulting in the breakdown of glycogen[13].On the other hand, insulin's effects on a wide range of processes, simulation of glycogen synthase, GLUT4 glucose transporter translocation, protein synthesis, and gene expression, require the PI3-kinase dependent activation of PKB[12].Previous studies have shown cAMP elevating agents to activate PKB in some cell types [14] and PKB activation in others[15],[16]. Recent evidence indicates cAMP can regulate PKB in some cell types via Epac (Exchange protein directly activated by cAMP). This suggests possible crosstalk between insulin and adrenaline signaling in muscle. In this study we use using the logistic categorical model and neural network model to investigate the crosstalk between the two pathways.

II. SYSTEMS AND METHOD

A. Dataset

The biological measurements used in the study are from a published dataset [12].Figure 2 shows the two distinct pathways of adrenaline and insulin where cAMP-mediated PKB activation requires the presence of the GTPase exchange factor Epac (exchange protein directly activated by cAMP) to regulate crosstalk. The dataset is created to study the effect of adrenaline on the insulin pathway via its effect on protein kinase B (PKB) with the aid of antagonist and agonists. The dataset consists of 89 data points. The following are the antagonists and the agonists used in study: the effect of adrenaline on insulin-stimulated PKB phosphorylation was blocked by timolol (β-blocker), whereas phentolamine blocked the α-receptors. The β -agonist isoprenaline imitated the effect of adrenaline on PKB phosphorylation and cell permeable cAMP analogue (db-cAMP) mimicked the effect on PKB phosphorylation.

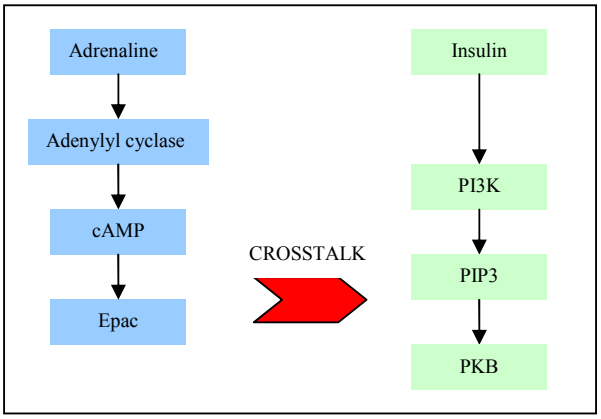

Fig 1: The figure denotes the two signalling pathways Adrenaline and Insulin. In this study of skeletal muscle cells, adrenaline crosstalks with the Insulin pathway via cAMP elevating agent Epac. The crosstalk is mirrored in the amplification of PKB activation.

B. Experimental design

The results are achieved by using a k-fold cross validation. The data is divided into k subsets. Each time one of the k subsets is used as the validation set, while the remaining k-1 subsets form a training set. The advantage of this method is that it matters less how the data gets divided, every data point gets to be in a test set exactly once, and gets to be in a training set k times. The average error across 5 trials is computed and the variance of the resulting estimate reduces with a high K value. We employ the 5 fold cross validation in this study.

The regularisation constant takes 7 values, i.e., 1.0E+00,1.0E-01, ,1.0E-02, ,1.0E-03, ,1.0E-04, ,1.0E-05, ,1.0E-06

C. *Algorithms*

We investigate two models in this study. The first is referred to as a logistic categorical model while the second is a neural network model.

Model A: logistic categorical model

We denote by $\mathbf{x}_n$ an input vector, where each element of $\mathbf{x}_n$ is binary indicator indicating if a specific activator or inhibitor is present as well as if a protein is phosphorylated. We denote by t_n a corrupted observation from a real model parameterised by a weight vector $\mathbf{w}$,

$$y_n = f(\mathbf{x}_n, \mathbf{w}) \quad (1)$$

In a logistic categorical model, we have the model as

$$y_n = \frac{1}{1+\exp(-\mathbf{x}_n \cdot \mathbf{w})} \quad (2)$$

This model introduces a limited nonlinearity. The error function is defined as

$$e_n = t_n - y_n \quad (3)$$

The objective function with a regularisation term is defined as

$$O_L = \frac{1}{2}\left[\mathbf{e}^{\mathrm{T}}\mathbf{e} + \lambda\, \mathbf{w}^{\mathrm{T}}\mathbf{w}\right] \quad (4)$$

Here λ is called a regularisation constant, $\mathbf{e} = (e_1, e_2, \cdots, e_\ell)^{\mathrm{T}}$ and $\mathbf{w} = (w_1, w_2, \cdots, w_\ell)^{\mathrm{T}}$. The first derivative of O_L with respect to $\mathbf{w}$ is

$$\nabla O_L = \lambda \mathbf{w} - \mathbf{X}^{\mathrm{T}}\mathbf{Q}\mathbf{e} \tag{5}$$

Here

$$\mathbf{X} = \{x_{nk}\}_{1\le n\le \ell, 1\le k\le d} \tag{6}$$

and

$$\mathbf{Q} = \mathrm{diag}\{y_n(1-y_n)\}_{1\le n\le \ell} \tag{7}$$

The second derivative O_L with respect to $\mathbf{w}$ is

$$\mathbf{\Lambda} = \nabla\nabla O_L = \lambda \mathbf{I} + \mathbf{X}^{\mathrm{T}}\mathbf{Q}(\mathbf{Q}-\mathbf{A})\mathbf{X} \tag{8}$$

Here $\mathbf{\Lambda}$ is called the Hessian matrix and

$$\mathbf{A} = \mathrm{diag}\{e_n(1-2y_n)\}_{1\le n\le \ell} \tag{9}$$

The weight update is then defined as

$$\Delta\mathbf{w} = -\mathbf{\Lambda}^{-1}\nabla O_L = -(\lambda \mathbf{I} + \mathbf{X}^{\mathrm{T}}\mathbf{Q}(\mathbf{Q}-\mathbf{A})\mathbf{X})^{-1}(\lambda \mathbf{w} - \mathbf{X}^{\mathrm{T}}\mathbf{Q}\mathbf{e}) \tag{10}$$

The significance of each signalling component and external-cues can be assessed by the Z score. First, the standard deviation of each weight is

$$SE(w_k) = \hat{\Lambda}_{kk} \tag{11}$$

Here $\hat{\Lambda}_{kk}$ is the kth diagonal element of $\mathbf{\Lambda}^{-1}$. The Z score is defined as

$$Z_k = \frac{w_k}{SE(w_k)} \tag{12}$$

It can be seen that the Z score is used to test the hypothesis that w_k is zero. If $|Z_k|$ is large, the hypothesis is denied, hence the kth input (signalling component or external-cue) is significant.

Model B: neural network model

In neural network model, we introduce a hidden neuron representing the function of signalling proteins in the Adrenaline pathway. Fig 3 shows a corresponding neural network structure, where one hidden neuron is introduced for all the signalling components in adrenaline signalling pathway. Doing this is for simplification as introducing more neurons in this signalling pathway may not introducing meaning information but computational cost. We treat the insulin signalling pathway in the same way omitting all the signalling components because we are only interested in the target signalling component (Akt) to see if it is affected by the adrenaline signalling pathway.

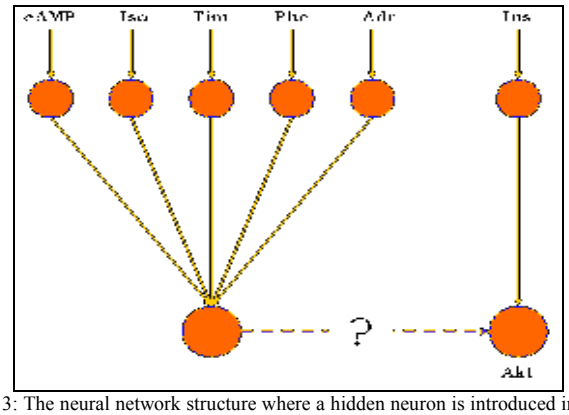

Fig 3: The neural network structure where a hidden neuron is introduced in the adrenaline pathway.

We denote by $\mathbf{x}_n^A$ an input vector for the adrenaline signalling pathway including db cAMP, isoprenalin, timolol, phentolamine and adrenaline.

$$h_{n1} = \frac{1}{1+\exp(-\mathbf{x}_n^A \cdot \mathbf{w}_A)} \tag{13}$$

We denote by h_{n2} (we use H rather X for convenience here) the input to the insulin signalling pathway (insulin) and by w_{H2} the weight of h_{n2}, an unknown hidden effect. We denote by w_{H1} the weight of the hidden neuron. The Akt activity is then model as

$$y_n = \frac{1}{1+\exp(-\mathbf{h}_n \cdot \mathbf{w}_H)} \tag{14}$$

Here $h_n = (h_{n1}, h_{n2})^{\mathrm{T}}$ and $\mathbf{w}_H = (w_{H1}, w_{H2})^{\mathrm{T}}$. We also denote by t_n a corrupted observation from a real model. The regularised objective function is

$$O_N = \frac{1}{2}\left[\mathbf{e}^{\mathrm{T}}\mathbf{e} + \lambda\left(\mathbf{w}_A^{\mathrm{T}}\mathbf{w}_A + \mathbf{w}_H^{\mathrm{T}}\mathbf{w}_H\right)\right] \tag{15}$$

The first derivative of O_N with respect to $\mathbf{w}_H$ is

$$\nabla O_N(\mathbf{w}_H) = \lambda \mathbf{w}_H - \mathbf{H}^{\mathrm{T}}\mathbf{Q}\mathbf{e} \tag{16}$$

Here $\mathbf{Q}$ is defined in equation (7) and

$$\mathbf{H} = \{h_{nk}\}_{1\le n\le \ell, 1\le k\le 2} \tag{17}$$

The second derivative of O_N with respect to $\mathbf{w}_H$ is

$$\mathbf{\Lambda}_N(\mathbf{w}_H) = \nabla\nabla O_N(\mathbf{w}_H) = \lambda \mathbf{I} + \mathbf{H}^{\mathrm{T}}\mathbf{Q}(\mathbf{Q}-\mathbf{A})\mathbf{H} \tag{18}$$

Here $\mathbf{\Lambda}_N(\mathbf{w}_H)$ is called the Hessian matrix and A is defined in equation (9). The weight update equation for $\mathbf{w}_H$ is then defined as

$$\Delta\mathbf{w}_H = -\mathbf{\Lambda}_N^{-1}(\mathbf{w}_H)\nabla O_N(\mathbf{w}_H) = -(\lambda \mathbf{I} + \mathbf{H}^{\mathrm{T}}\mathbf{Q}(\mathbf{Q}-\mathbf{A})\mathbf{H})^{-1}(\lambda \mathbf{w}_H - \mathbf{H}^{\mathrm{T}}\mathbf{Q}\mathbf{e}) \tag{19}$$

The first derivative of O_N with respect to $\mathbf{w}_A$ is

$$\nabla O_N(\mathbf{w}_A) = \lambda \mathbf{w}_A - w_{H2}\mathbf{X}_A^{\mathrm{T}}\mathbf{Q}\mathbf{R}\mathbf{e} \tag{20}$$

Here Q is defined in equation (7),

$$\mathbf{X}_A = \{x_{nk}^A\}_{1\le n\le \ell, 1\le k\le d-1} \tag{21}$$

and

$$\mathbf{R} = \mathrm{diag}\{h_{n1}(1-h_{n1})\}_{1\le n\le \ell} \quad (22)$$

The second derivative of O_N with respect to $\mathbf{w}_A$ is

$$\mathbf{\Lambda}_N(\mathbf{w}_A) = \nabla\nabla O_N(\mathbf{w}_A) = \lambda \mathbf{I} + w_{H1}(\mathbf{X}_A^{\mathrm{T}}(\mathbf{Q}-\mathbf{A}-\mathbf{B})\mathbf{X}_A) \quad (23)$$

Here **A** is defined in equation (9) and

$$\mathbf{B} = \mathrm{diag}\{e_n(1-2h_{n1})\}_{1\le n\le \ell} \quad (24)$$

The weight update equation for $\mathbf{w}_A$ is then defined as

$$\Delta\mathbf{w}_A = -\mathbf{\Lambda}_N^{-1}(\mathbf{w}_A)\nabla O_N(\mathbf{w}_A) = -(\lambda \mathbf{I} + w_{H1}\mathbf{X}_A^{\mathrm{T}}\mathbf{Q}(\mathbf{Q}-\mathbf{A}-\mathbf{B})\mathbf{X}$$

The standard deviation of each weight (all weights) is

$$SE(w_k) = \hat{\Lambda}_{kk} \quad (26)$$

Here $\hat{\Lambda}_{kk}$ is the kth diagonal element of $\mathbf{\Lambda}^{-1}$ (both $\mathbf{\Lambda}_N^{-1}(\mathbf{w}_A)$ and $\mathbf{\Lambda}_N^{-1}(\mathbf{w}_H)$). The Z score is defined as

$$Z_k = \frac{w_k}{SE(w_k)} \quad (27)$$

It can be seen that the Z score is used to test the hypothesis that w_k is zero. If $|Z_k|$ is large, the hypothesis is denied, hence the kth input as well as the crosstalk component (the hidden component) is significant.

III. RESULTS AND DISCUSSION

Signalling through the insulin pathway is critical for the regulation of intracellular and blood glucose levels. Insulin binds to its receptor leading to phosphorylation of the β-subunits and the tyrosine phosphorylation of insulin receptor substrates (IRS).IRS activates phosphoinositide 3-kinase (PI3K) through its SH2 domain, thus activating the intracellular concentration of PIP_2 and PIP3. PIP3 in turn activates phosphatidylinositol phosphate-dependent kinase-1 (PDK-1), that subsequently activates Akt/PKB This results in the translocation of the glucose transporter (GLUT4) from cytoplasmic vesicles to the cell membrane [17].Adrenaline on the other hand is a hormone that is part of the fight or flight mechanism to protect the body in situations of acute stress. Adrenaline binds to its β-adrenergic receptor activating cAMP and PKA.

The primary objective of this study is to investigate the weights associated with the input cues as well as to study the crosstalk between adrenaline and insulin signalling pathways via the activation of PKB (protein kinase B). The logistic categorical model and the neural network model is employed in this study. Fig 3 shows the R-squares for two models with different regularisation values. It can be seen that two models show very similar performance, where the neural model slightly outperforms the logistic model when $1.0e-4 \le \lambda \le 1.0e-2$.

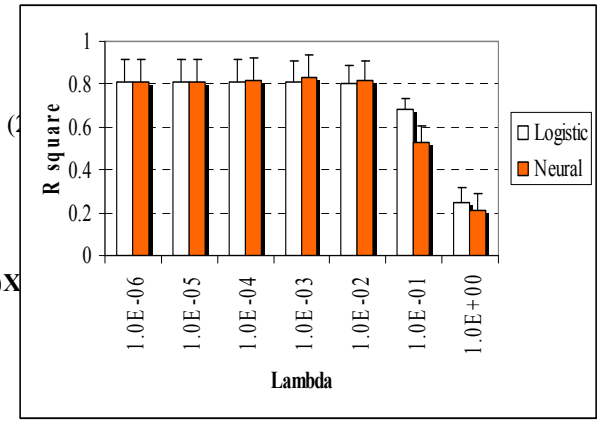

Fig 3: The R squares for the neural network model and the logistic categorical model.

The weights, standard error and the Z scores are shown in Table I. It can be seen that except for Phentolamine, all the other indicator play an equal role for Akt phosphorylation. Phentolamine in fact has a lower magnitude then the other inputs used. It is interesting to note that in the biological experimental study while the effect of adrenaline on insulin-stimulated PKB phosphorylation was blocked by timolol (β-blocker), the blockade of α-receptors with phentolamine was without effect[12]. The negative weights for timolol and phentolamine are indicators of their inhibitive association with PKB activation. The logistic model is represented as

$$y = \rho(1.66\times \mathrm{Adr} - 1.58\times \mathrm{Tim} + 1.72\times \mathrm{Iso} - 0.06\times \mathrm{Phe} + 1.83\times \mathrm{cAMP} + 4.01\times \mathrm{Ins} - 4.85)$$

0.51 0.76 0.77 0.59 0.73 1.95 1.98

TABLE I
STIMULATION RESULTS FOR THE LOGISTIC MODEL

	Weight	Standard error	Z score
Adr	1.66	0.51	3.27
Tim	-1.58	0.76	-2.08
Iso	1.72	0.77	2.24
Phe	-0.06	0.59	-0.10
cAMP	1.83	0.73	2.51
Ins	4.01	1.95	2.06
Bias	-4.85	1.98	-2.45

The table shows the weights, standard errors and Z scores in the logistic model..The inputs and outputs are abbreviated as follows Adr for Adrenaline, Tim for Timolol, Iso for Isoprenaline, Phe for Phentolamine,cAMP for cAMP, and Ins for Insulin.The weights for timolol and phentolamine are negative depicting their association with PKB activation in the insulin pathway.

The weights, standard error and the Z scores are shown in Table 2. It can be seen that except for Phentolamine, all the other agonist, antagonists and proteins indicate an equal role for Akt phosphorylation. .Again, it is interesting to note that in the biological experimental study the blockade of α-receptors with phentolamine had no effect on the crosstalk between adrenaline and insulin pathways [12]. It is also very interesting to see that the crosstalk between the Adrenaline and Insulin signalling pathways have been confirmed by the weight associated with the hidden neuron (3.72) with the Z score as

4.31 meaning that the null hypothesis that the crosstalk between two signalling pathways has been strongly denied. The neural model is presented as below.

$$A = \underset{0.25}{\rho}(\underset{0.25}{2.09}\times \mathrm{Adr} - \underset{0.39}{2.14}\times \mathrm{Tim} + \underset{0.39}{2.08}\times \mathrm{Iso} - \underset{0.31}{0.12}\times \mathrm{Phe} + \underset{0.37}{2.22}\times \mathrm{cAMP} - \underset{0.19}{1.49}) \quad (4)$$

$$y = \underset{0.86}{\rho}(\underset{1.88}{3.72}\times \mathrm{A} + \underset{1.94}{4.00}\times \mathrm{Ins} - 5.53)$$

where A is Adrenaline

TABLE II
RESULTS FOR THE HIDDEN NEURON IN THE NEURAL NETWORK MODEL

	Weight	Standard error	Z score
Adr	2.09	0.26	8.12
Tim	-2.14	0.39	-5.53
Iso	2.08	0.39	5.32
Phe	-0.12	0.31	-0.38
cAMP	2.22	0.37	6.01
Bias	-1.49	0.19	-7.76

The table shows the weights, standard errors and Z scores for the hidden neuron in the Neural network model.The inputs and outputs are abbreviated as follows Adr for Adrenaline, Tim for Timolol, Iso for Isoprenaline, Phe for Phentolamine,cAMP for cAMP, and Ins for Insulin.The weights for timolol and phentolamine are negative depicting their association with PKB activation in the insulin pathway.

TABLE III
RESULTS FOR THE OUTPUT NEURON IN THE NEURAL NETWORK MODEL

	Weight	Standard error	Z score
Adrenaline(crosstalk)	3.72	0.86	4.31
Insulin	4.00	1.88	2.13
Bias	-5.53	1.94	-2.85

The table shows the weights, standard errors and Z scores for the output neuron in the Neural network model.

IV. CONCLUDING REMARKS

It is now a well-established fact that signaling pathways do not function in isolation and increasing evidence for the complex signalling topology suggest non-linear inter-pathway crosstalk in a cell. Crosstalk between proteins can be quite complex depending on type of ligand, ligand concentration and intensity of signalling. As an extension of previous work using linear statistical models [15], this paper employs the use of non-linear methods like neural networks and logistic categorical model to discuss the crosstalk between signaling pathways using adrenaline and insulin signaling pathways as an example. We employ the extra cellular cues and inhibitors to aid in capturing the crosstalk interaction between the two pathways. While both models show very similar performance, the neural network model outperforms the logistic model when model when $1.0e-4 \le \lambda \le 1.0e-2$.The crosstalk is confirmed by the weight associated with the hidden neuron with the Z score as 4.31.While this preliminary work using non-linear methods has captured the non-linearity between the two pathway ,future work will focus on developing the machine learning methods used to increase sensitivity to the data as well as employ new datasets of other pathways.

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