ported here and summarized in Table I indicate that 3′,5′-AMP likewise inhibits the incorporation of amino acids into the proteins of rat-liver slices incubated in a phosphate–saline medium, to at least the same extent as does glucagon.

In other experiments not described here, little or no effect of 3′,5′-AMP on the incorporation of amino acids into proteins was observed when either rat- or rabbit-liver slices were incubated in a Krebs–bicarbonate medium, whereas glucagon and adrenalin were inhibitory under these conditions[6]. Neither has it been possible to reproduce with the nucleotide the effects of the glycogenolytic agents on the incorporation of acetate into fatty acids or cholesterol in liver slices incubated in a Krebs–bicarbonate medium. However, 3′,5′-AMP also does not stimulate glucogenolysis in this medium, and it seems very probable, therefore, that the nucleotide does not penetrate sufficiently rapidly within the cells or is too rapidly inactivated to be effective in a Krebs–bicarbonate medium.

In view of the results obtained in phosphate–saline, it is reasonable to conclude that 3′,5′-AMP is a common mediator in the various metabolic effects exerted by glucagon and adrenalin on the liver. On the other hand, it seems unlikely that the actions of the cyclic nucleotide on lipid metabolism could be secondary consequences of the activation of phosphorylase[3]. Various experiments have led to the same conclusion with respect to the effect of the glycogenolytic agents on protein synthesis. It appears, therefore, that 3′,5′-AMP must influence one or more additional enzyme systems besides that involved in the activation of phosphorylase. The nature of these systems raises interesting problems.

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The nature of the fluorescence of an enzyme-reduced coenzyme-reduced substrate complex

This paper presents evidence that L-glutamate decreases the dissociation of the glutamic dehydrogenase–TPNH complex, and that the increase in the intensity of fluorescence of that complex caused by the addition of L-glutamate is due solely to the increase in the amount of TPNH bound rigidly to the enzyme surface.

Abbreviations: TPNH, DPNH, reduced tri- and diphosphopyridine nucleotides; GAD, glutamic dehydrogenase.

We have shown previously that the intensity of fluorescence of TPNH (or DPNH) is increased whenever the molecule is bound on any surface which decreases the flexibility of the reduced coenzyme molecule, and have suggested that such increases in fluorescence intensity of reduced coenzymes (referred to hereafter as Δf), which occur upon addition of their apoenzymes, are explained by such binding[1]. We have also shown that the Δf caused by the addition of GAD is a direct measure of the GAD–TPNH complex[2]. From the fact that α-ketoglutarate is not bound to the enzyme in the absence of the reduced nicotinamide ring, from the obligatory order of addition of reactants, and from stereochemical considerations, it was concluded that the α-carboxyl group of α-ketoglutarate is bound to the face of the reduced nicotinamide ring of TPNH. WINER AND SCHWERT[3] had already shown that the Δf of TPNH, caused by the addition of GAD, is further increased by glutamate, which may be presumed to form a ternary complex with its α-carboxyl group bonded to the reduced nicotinamide ring in a manner similar to that of α-ketoglutarate.

The above considerations predicted the possibility that glutamate so bonded could suppress the dissociation of the GAD–TPNH complex. That this predicted suppression actually occurs is shown in Fig. 1. The negative reciprocal of the apparent dissociation constant is, of course, the intercept of the curve on the abscissa. It is apparent that, at least within this range of experimental conditions, increasing the

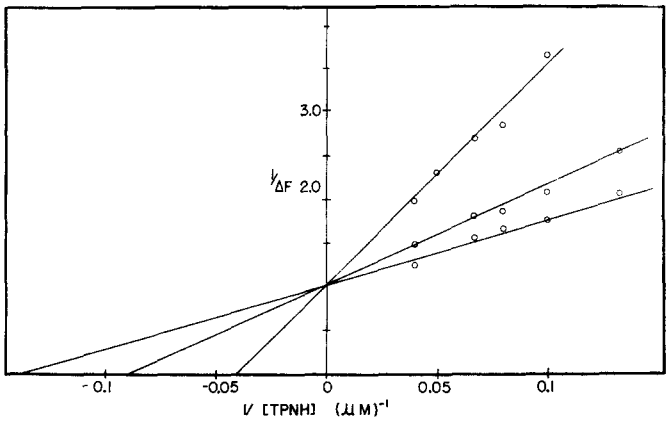

Fig. 1. Lineweaver–Burk plots of complex formation of TPNH at different concentrations of L-glutamate. Each cuvette contained 0.8 mg/ml GAD, TPNH as indicated in 0.16 *M* phosphate buffer, pH 7.7. L-glutamate concentrations were: (A) 0; (B) 0.3 m*M*; (C) 0.85 m*M*. The exciting wavelength was 370 mμ. Δf was measured as the fluorescence intensity at the wavelength of maximum fluorescence corrected for the separately measured fluorescence intensities of TPNH and GAD at the same wavelength.

concentration of L-glutamate progressively lowers the apparent dissociation constant of TPNH.

Although such suppression could be considered intuitively as simply a lowering of the rate of the dissociation of TPNH from the enzyme, the formal kinetics of the proposed mechanism provide us with additional information. Consider the following system:

$$T + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_1 \qquad (1) \qquad\qquad C_1 + G \underset{k_4}{\overset{k_3}{\rightleftharpoons}} C_2 \qquad (2)$$

where T = TPNH, E = free enzyme, C_1 = enzyme–TPNH complex, G = L-glutamate, and C_2 = enzyme–TPNH–glutamate complex. If we now make the critical assumption that the molar coefficient of fluorescence is identical for C_1 and C_2, we obtain the following expression for $\Delta f = ([C_1] + [C_2])$ in arbitrary units:

$$\frac{1}{\Delta f} = \frac{1}{[E_t]} + \frac{K_T}{[T]\,[E_t]} \left(\frac{1}{1 + \frac{[G]}{K_G}} \right) \tag{3}$$

where $K_T = k_2/k_1$; $K_G = k_4/k_3$; E_t = total enzyme. In the absence of glutamate, the equation reduces to:

$$\frac{1}{\Delta f} = \frac{1}{[E_t]} + \frac{K_T}{[T]\,[E_t]} \tag{4}$$

Equations (3) and (4) represent straight lines with identical intercepts on the ordinate ($1/[E_t]$) but different intercepts on the abscissa. The data shown in Fig. 1 are therefore consistent with this model, and the assumption that the molar fluorescence intensity of the binary complex and of the ternary complex are identical is not only justified, but required.

Where $1/\Delta f = 0$, eqn. (3) simplifies to: $K_G = \dfrac{-[T]\,[G]}{K_T + [T]}$ (5)

Using the value of K_A from curve A (eqn. 4), and the value of (T) obtained from the intercepts on the abscissa of curves B and C at their respective values of (G), we can calculate values for K_G. This calculation gives values for K_G of $2.6 \cdot 10^{-4}$ and $3.6 \cdot 10^{-4}$ M respectively. The agreement of these values, within the experimental error of their determination, provides a totally independent check on the agreement of the data with the mechanism proposed. We are led, then, to two conclusions:

1. L-glutamate forms a ternary complex which hinders TPNH leaving, thereby suppressing the dissociation of the enzyme–TPNH complex.

2. L-glutamate does not change the fluorescence of the enzyme–TPNH complex itself, but only causes an increase in the fluorescence intensity of the solution by increasing the number of molecules of enzyme-bound TPNH in that solution.

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Sur la structure chimique de la partie peptidique de la cire D d'une souche humaine de *Mycobacterium tuberculosis*

Les souches humaines de *M. tuberculosis* contiennent un peptido-glycolipide appelé cire D, qui peut remplacer les Mycobactéries dans l'adjuvant de FREUND[1]. L'activité

Abbréviations: DAP, acide diaminopimélique; Glu, acide glutaminique; Ala; alanine; DNP, dinitrophényl-.