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ENZYME AMPLIFIER KINETICS

by

Sumner W. Levine

Research sponsored by the Air Force Office
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ABSTRACT

Enzyme amplifier systems have been implicated in such diverse physiological phenomena as vision and blood coagulation. The present report considers such systems from a general point of view and introduces the concept of steady state gain of the enzyme amplifier. Expressions for the latter are obtained and several of the main factors influencing gain are discussed.

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In recent years it has been suggested that certain important biological processes involve a sequence of enzyme-proenzyme reactions which exhibit a net chemical gain. Thus, MacFarlane⁽¹⁾ has proposed that blood clotting constitutes such an enzyme sequence. In this instance surface activation of comparatively few molecules of Hageman factor serves to further activate a series of some six additional factors which culminates in the conversion of millions of molecules of fibrinogen to fibrin. Wald⁽²⁾ has also considered the possibility that vision may involve enzyme amplification since the optical activation of a single molecule of rhodopsin can trigger a response involving a large number of molecules. Rhodopsin is postulated to be a proenzyme which is activated as a consequence of the absorption of a photon by 11-cis-retinaldehyde resulting in the unmasking of the active site. Once formed the enzyme can in turn activate a large number of molecules of the next proenzyme in the series and so on down the chain with each step providing a gain in the number of participating enzyme molecules.

Both positive and negative feedback probably play a role in the regulation of enzyme amplifiers⁽¹⁾. In the case of blood clotting positive feedback is provided by thrombin which seems to accelerate the reactions associated with the conversion of factors V and VIII. On the other hand fibrin seems to accelerate the disappearance of the other clotting factors.

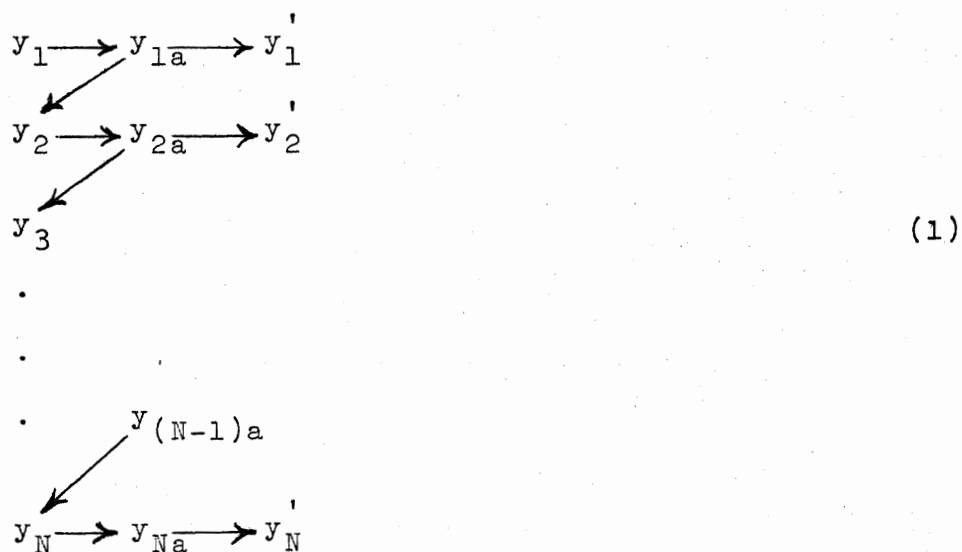
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In order to facilitate the study of such enzyme amplifiers it is helpful to have available a mathematical model of the system. Consequently in what follows such a model will be developed for an amplifier neglecting any feedback effects. In particular we consider a cascaded sequence of reactions for which, at the i th stage, a proenzyme of concentration y_i at initial concentration y_{i0} is converted by an enzyme of the previous stage to the active form of concentration y_{ia} at a rate determined by the constant k_i . The rate at which the activated form is destroyed is determined by the constant K_i (we assume none are equal) while the concentration of the inactivated form is designated by y_i' . This process is considered to be terminated at the N th stage: The sequence may now be represented as follows:



If we assume that the first factor, y_1 , is activated over some time interval $\Delta t = a$ after which the stimulus is terminated then the differential equations describing the system are as follows:

$$\frac{dy_{1a}}{dt} = k_1 y_1 [U(t) - U(t-a)] - K_1 y_{1a}$$

$$\frac{dy_{2a}}{dt} = k_2 y_2 y_{1a} - K_2 y_{2a} \quad (2)$$

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$$\frac{dy_{Na}}{dt} = k_N y_N y_{(N-1)a} - K_N y_{Na}$$

In the above $U(t)$ is the unit step function and hence $[U(t) - U(t-a)]$ is a unit pulse of duration a . It is possible to provide a general solution to (2) if the linear case is considered for which the concentration of proenzyme y_i remains constant at the initial value y_{i0} . This will be approximately so if the amount of activate proenzyme is small in comparison to that initially present or if the precursor is replaced at approximately the same rate as it is converted. The build-up in the active form is given by the solutions to equations(2):

$$y_{ia} = \frac{y_{10} k_1}{K_1} \left\{ U(t) - U(t-a) + e^{-K_1 t} \left[e^{K_1 a} U(t-a) - 1 \right] \right\} \quad (3)$$

and for the pth stage

$$y_{pa} = \frac{y_{10} k_1}{K_1} \frac{y_{20} k_2}{K_2} \dots \frac{y_{p0} k_p}{K_p} \left\{ U(t) - U(t-a) + \sum_{i=1}^p A_i e^{-k_i t} \left(e^{K_i a} U(t-a) - 1 \right) \right\} \quad (4)$$

where,

$$A_i = \frac{1}{\prod_{j=1}^p \left(1 - \frac{K_i}{K_j} \right)} \quad (j \neq i)$$

It is informative to consider the above solution as the product of two factors one of which, $F(t)$, alone depends on time while the other factor, R , depends only on the initial concentrations and on the ratio of the rate constants. For $t \leq a$ the factor $F(t)$ increases from an initial value of zero to a steady state value of unity. We note that the time required to reach the steady state, t_s , is determined by the smallest decay constant K_m and is given by the condition $t_s > K_m^{-1}$. When the system is at steady state we may define the gain G of the enzyme amplifier as

$$G_s = \frac{y_{aN}}{y_{a1}} = \prod_{i=2}^N \frac{y_{i0} k_i}{K_i} \quad (5)$$

It is evident that if the time interval, a , over which y_{10} is activated is not sufficient for the realization of the steady states then the gain of the enzyme amplifier will be less than that indicated by (5). The effect of progressively shorter time intervals, a , on $F(t)$ is shown in figure 1 in the instance of a three stage process. Though the choice of constants is arbitrary the curves make clear the nature of this dependency.

A second point worthy of note with reference to equation 5 is that the gain involves the products of several single stage terms. This means that small shifts in the rate constants or the initial conditions can collectively produce significant overall effects. Thus a decrease of only 2% in k_i and y_{10} together with a corresponding increase in K_i results in an eight stage process, such as blood coagulation, in an overall decrease in gain of nearly forty percent. This sort of effect has bearing on negative and positive feedback which effects several stages simultaneously.

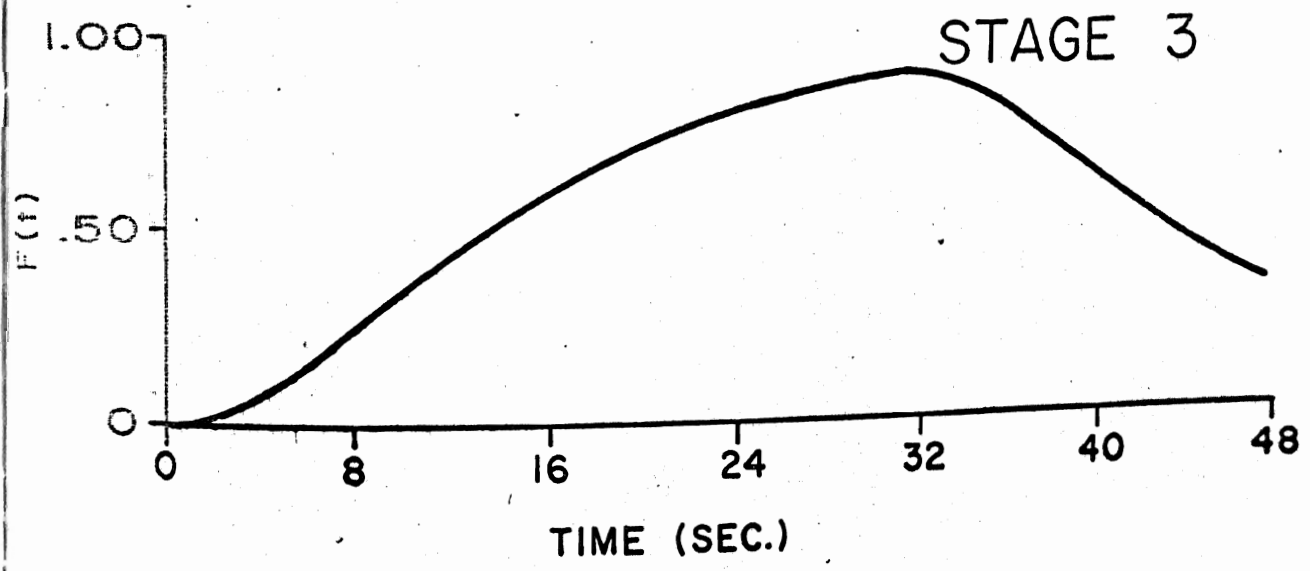
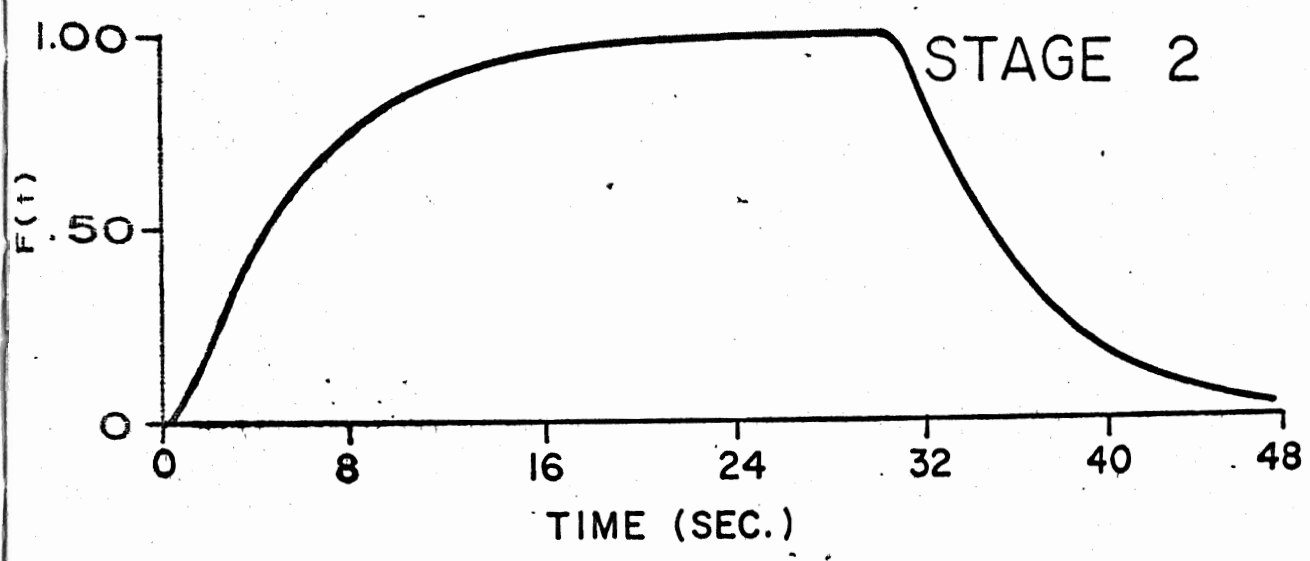
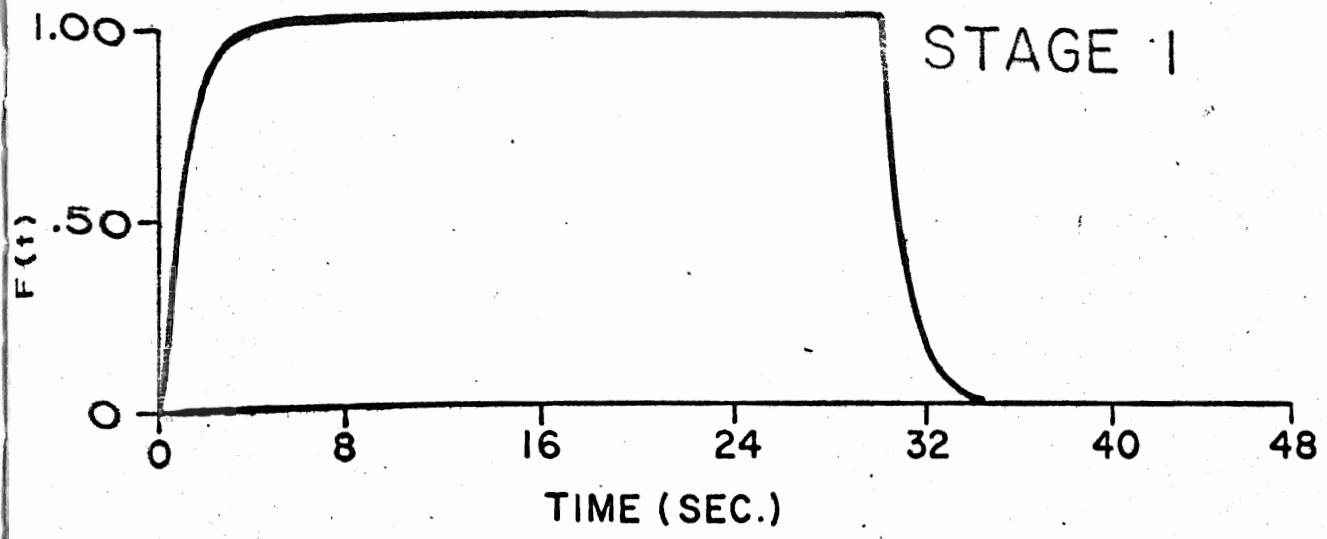
REFERENCES

- 1) R.G. MacFarlane, Nature, 202,498(1964).
- 2) G.Wald, Science, 150,1028(1965).

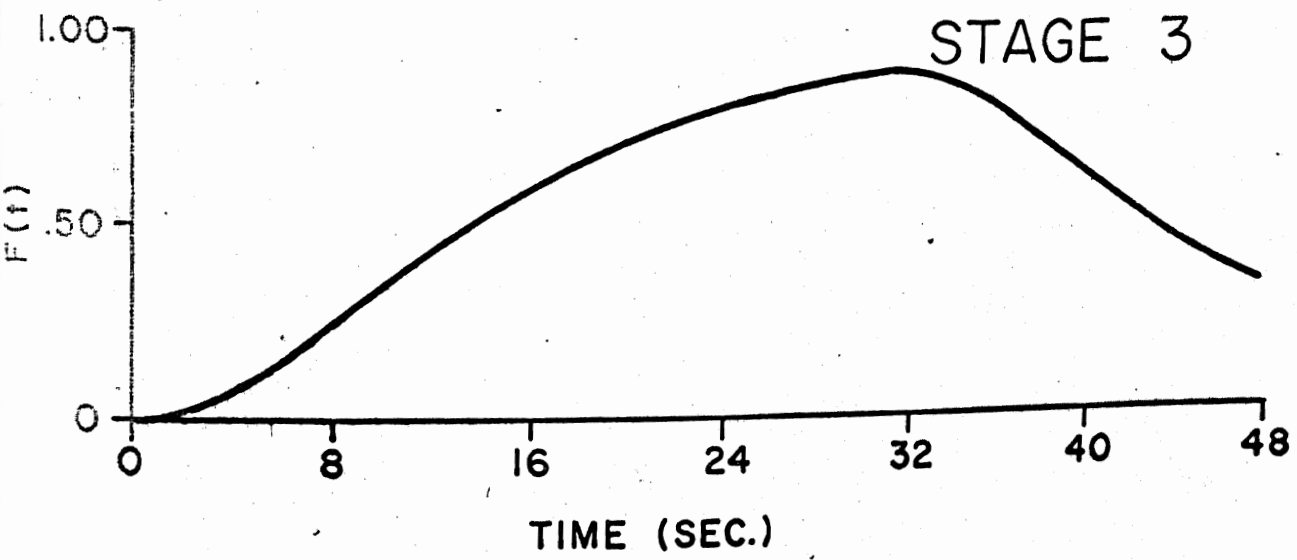
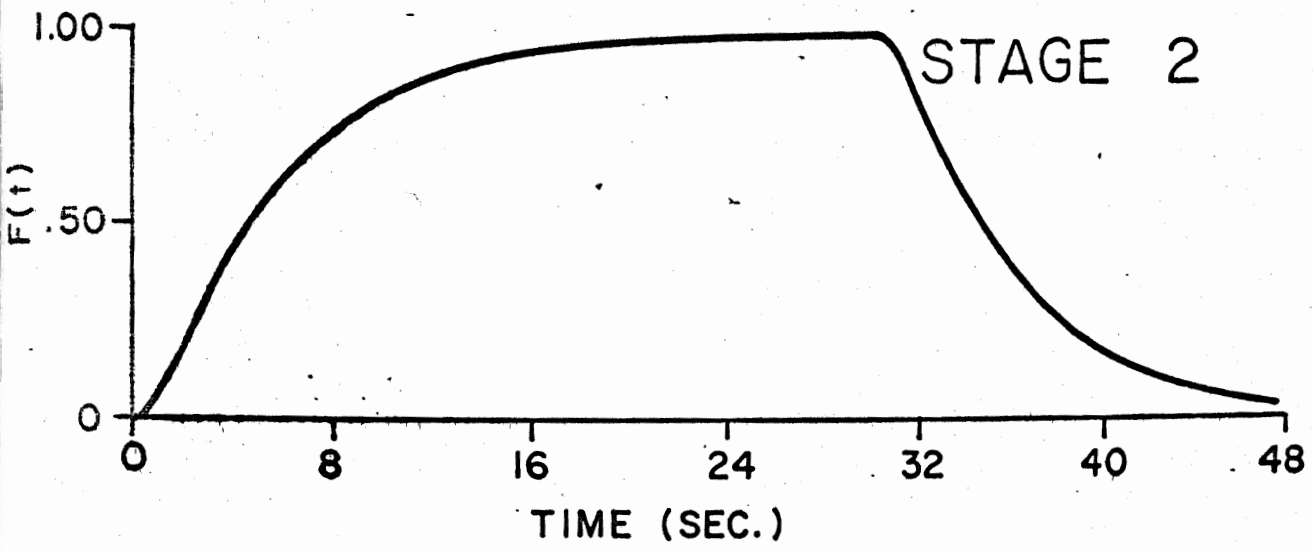
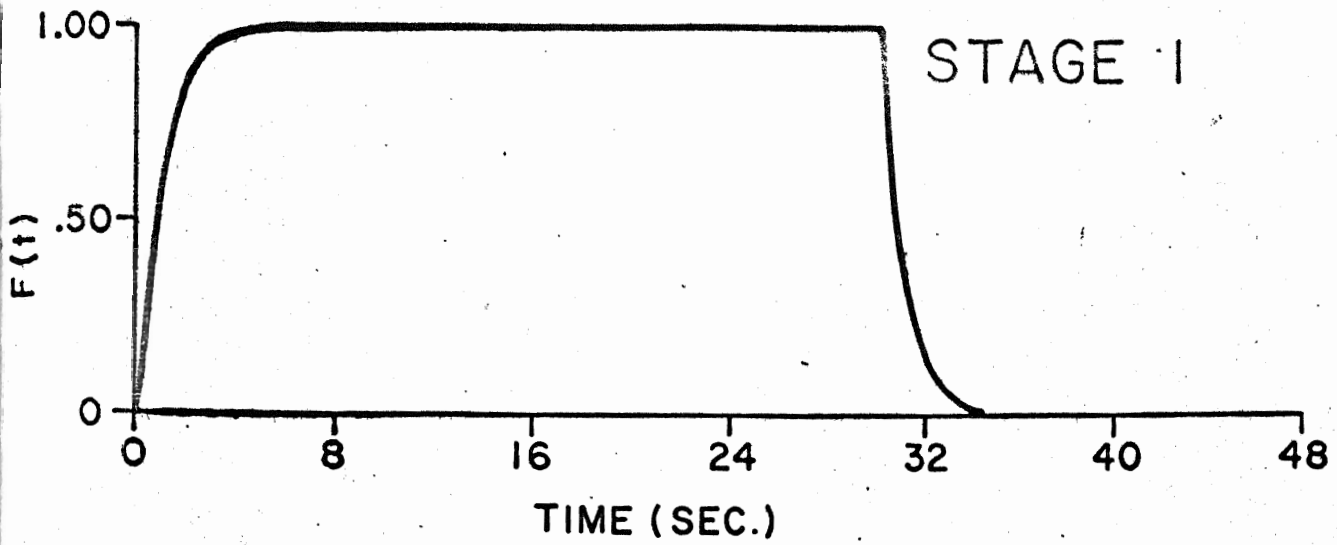
CAPTION

Figures (a), (b), (c)

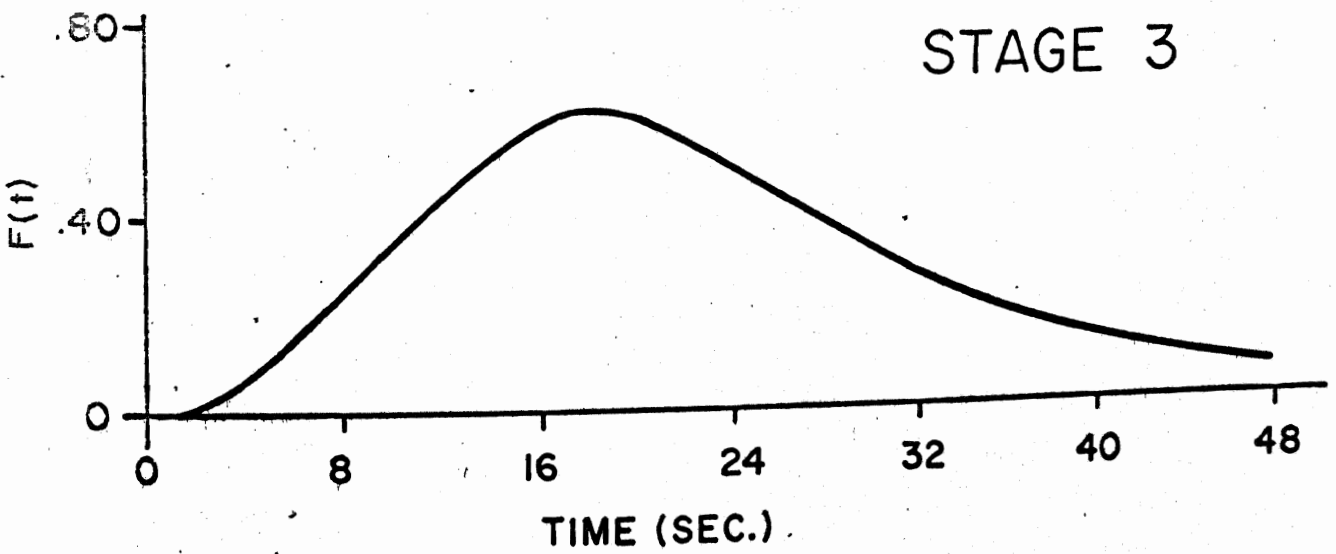
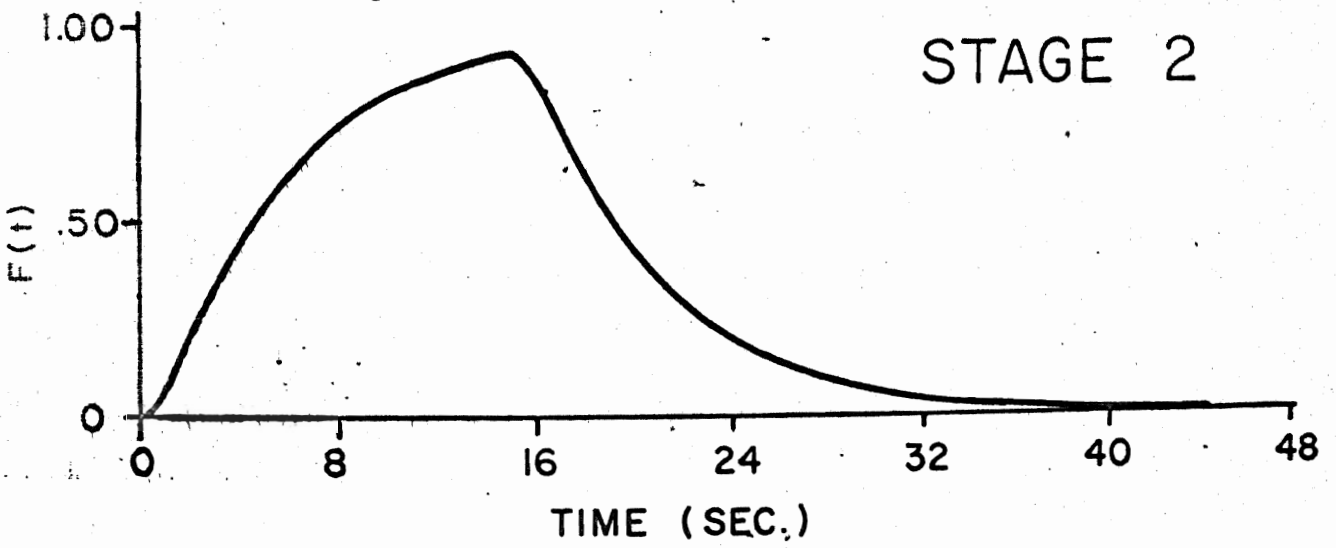
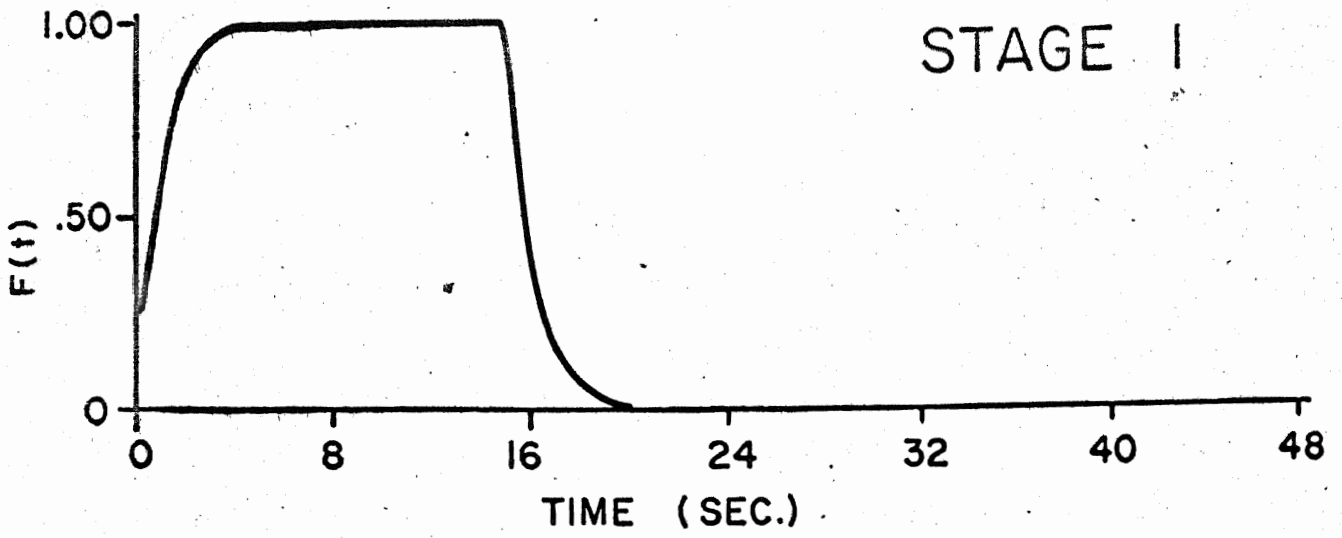
The effect of varying time of activation of stage I of biochemical amplifier on the form and magnitude of the output response, stage 3. The time of activation decreases from 30 units in (a) to 15 units in (b) while (c) corresponds to 3 units.



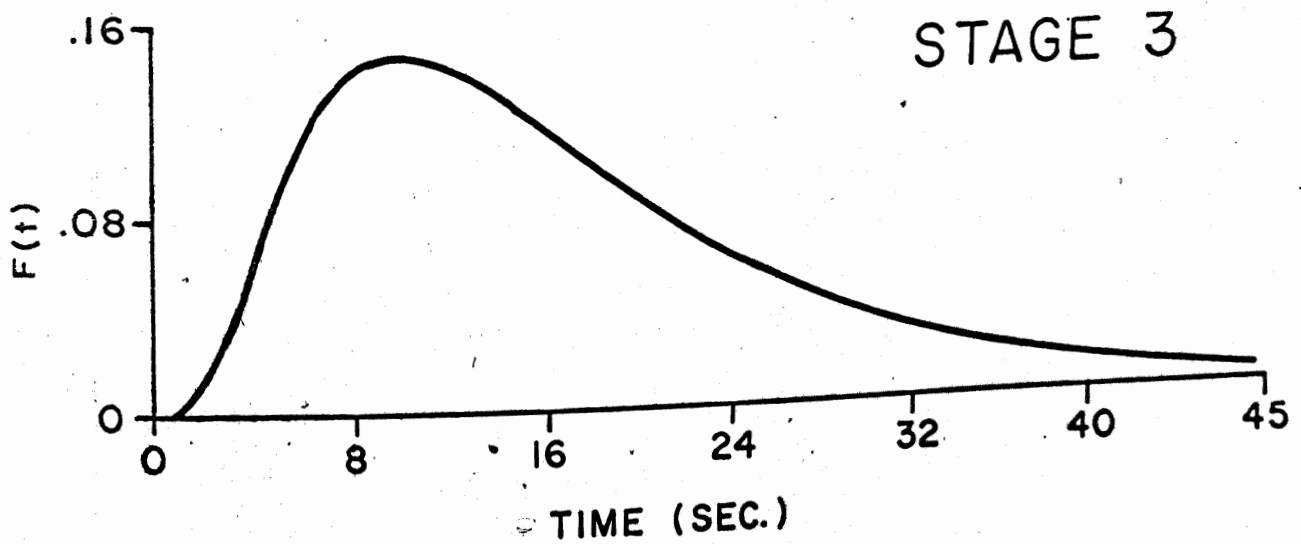
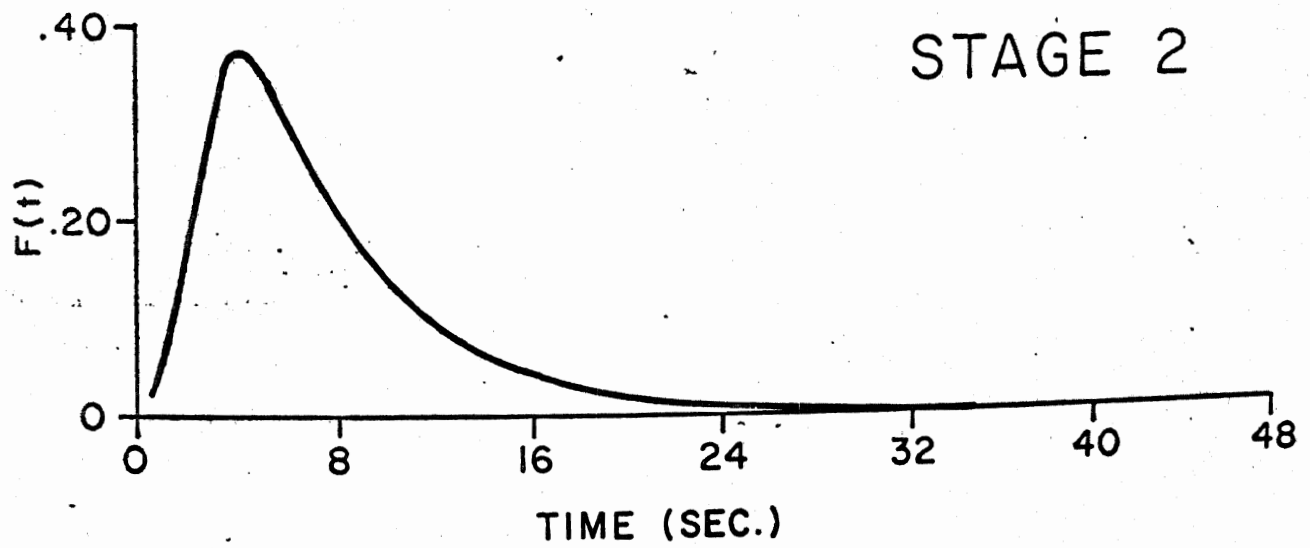
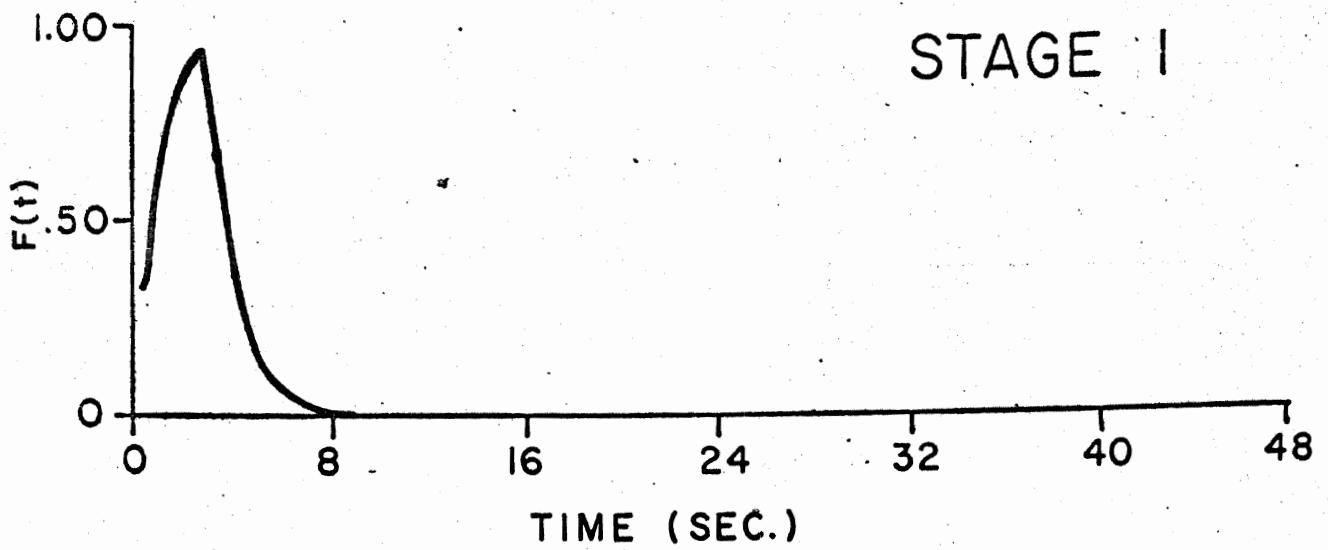
(a)



(a)



(b)



(c)