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H. SUMMARY OF DISCUSSION AND COMMENTARY

E. BUEDING

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Bülbring mentioned another effect of epinephrine (E) which takes place without the activation of phosphorylase. E exerts its normal physiological effects on intestinal smooth muscle (taenia coli), *i.e.*, cessation of spontaneous spike activity and relaxation, in a glycogen-depleted tissue and in the absence of glucose, provided β-hydroxybutyrate, a substrate giving rise to oxidative phosphorylations, is supplied. Therefore, E can produce its usual physiologic effects on taenia coli in the complete absence of carbohydrate from the tissue and from the medium. Furthermore, in the nondepleted tissue and in the presence of glucose, epinephrine-induced relaxation of taenia coli is not associated with an activation of phosphorylase nor with changes in the glycogen and hexose phosphate levels. On the other hand there is a positive correlation between the inhibitory effect of E and increases in the concentration and the turnover of ATP and creatine phosphate. It is concluded that the physiological effects of E have no obligatory requirement for glycolysis or glycogenolysis, but that they are dependant on metabolic processes generating energy-rich phosphate compounds.

Brody stated that he has observed a significant increase in phosphorylase *a* with E concentrations as low as 5×10^{-9} M in the estrogen-primed rat uterine muscle. The tissue was assayed for phosphorylase activity after freeze-clamping. In guinea pig taenia coli, control values for phosphorylase *a* were 0 to 1 %, and after E they were 3 to 5%. The earliest changes were observed after 1 min and maximum effects 2 min after catecholamine addition. No increases in phosphorylase *a* were detected 15 sec after amine addition. It should be pointed out that the technique used by Brody does not exclude the possibility of an artifactual phosphorylase activation (Nature, **196:** 944, 1962). Clamping can produce disruption of cell compartments resulting in a diffusion of cyclic 3′,5′-AMP from the membrane into the cytoplasm, where activation of phosphorylase kinase could take place. In any case, even with this technique no activation of phosphorylase was found 15 sec after addition of E, while cessation of spike activity and relaxation occurs already when the tissue is exposed to the catecholamine for 3 to 5 sec. Therefore, phosphorylase activation cannot account for the physiological action of E on intestinal smooth muscle.

Schild drew attention to a problem concerned with assessing the relevance of the cyclase system for adrenergic activity because of a difference between the actions of the heart and on smooth muscle. Heart muscle reacts to a conducted electrical impulse by an all-or-none response. Conceivably, cyclic 3′,5′-AMP, generated by catecholamines, could facilitate such a response. On the other hand in smooth muscle catecholamines react with receptors and these initiate a response which may result in either contraction or relaxation.

Schild also raised the question whether there is any evidence that cyclic 3′,5′-AMP is an *essential link* in the processes of contraction or relaxation initiated by catecholamines. Sutherland replied that there is as yet little evidence available regarding this problem. In the nondepleted taenia coli threshold concentrations of E produce relatively small, but significant and reproducible increases in cyclic 3′,5′-AMP. In the rat uterus this increase is greater. Theophylline, which inhibits the enzymatic inactivation of cyclic 3′,5′-AMP, is known to enhance the bronchodilator effects of E in man. Finally, Bülbring reported that imidazole, which activates the phosphodiesterase, abolishes the physiologic and biochemical effects of E on taenia coli.

Since small doses of E can increase cardiac contractile force without activation of phosphorylase (Mayer *et al.*, J. Pharmacol. **139:** 275, 1963), Crout raised the question whether a dose-response relationship has been established with cyclic 3′,5′-AMP. Williamson replied that with small doses of E the increases in contractile force were not associated with changes in cytoplasmic DPNH, a circumstance that indicates a dissociation between contractile force and glycogenolysis; in addition there is the reported temporal separation at higher concentrations of E. In these experiments the tissue fluorescence and contractile force were measured in the same heart at the same time; hence this particular assay system has a high degree of sensitivity. Similar studies involving determination of cyclic 3′,5′-AMP concentrations at different dose levels of E have not yet been carried out.

Williamson also commented on the problem whether or not cyclic 3′,5′-AMP affects phosphofructokinase (PFK) activity. He has reported recently that in the perfused rat heart cyclic 3′,5′-AMP and contractile force increase *prior* to the activation of PFK. In fact, cyclic 3′,5′-AMP decreases rapidly during the period of PFK activation. In this connection Cori called attention to the large differences in the concentrations of cyclic 3′,5′-AMP required to activate PFK *in vitro* and the levels of this nucleotide present in the tissue after exposure to E. Mansour stated that he had found an activation of partially purified guinea pig heart PFK with considerably lower concentrations of cyclic 3′,5′-AMP than those which he reported in this symposium and that cyclic 3′,5′-AMP is about 10 times more active than ADP and AMP. However, as was pointed out recently by O. Lowry at another symposium (Metabolic Control Mechanisms, University of Pennsylvania, Philadelphia, 1965), it is unlikely that E-induced changes in the levels of the cyclic nucleotide would affect PFK activity *in vivo* because the tissue concentrations of ADP and AMP are several magnitudes higher than those of cyclic 3′,5′-AMP.

In view of the multiplicity of the known effects of cyclic 3′,5′-AMP, which suggest functions in the nature of a cofactor, the physiological actions of catecholamines are not necessarily accounted for by an activation of phosphorylase *b* kinase, the first enzyme found to be susceptible to the cyclic nucleotide. In fact, there is a good deal of evidence that there is no causal relationship in the case of cardiac and smooth muscles. On the other hand, the data reviewed by Sutherland

strongly suggest that phosphorylase activation by cyclic 3′,5′-AMP is primarily responsible for the hyperglycemic action of catecholamines, although gluconeogenesis, an effect demonstrated by Park, could be a significant contributory factor. Furthermore, the time course of phosphorylase activation by E in frog skeletal muscle, reported by Helmreich, could explain the known effect of E in preventing or reducing muscular fatigue on repetitive stimulation.