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Purity of Synthetic Peptide Preparations

WE strongly endorse Vane's comments[1] on the pitfalls associated with the use of synthetic peptides in biological studies. It is particularly misleading to express results in terms of weight without evidence of homogeneity, because apart from error peptides (which may not be inert), account is seldom taken of salt and solvent content.

The impact of these problems in the field of radioimmunoassay was discussed at some length[2,3] at the recent workshop meeting in Edinburgh. In the case of one commercial synthetic angiotensin already widely used in research, evidence has been recorded of varying stability between batches[4] and apparent variations in the peptide content of different batches[5]. Furthermore, extending Vane's comments on the chemical purity of these peptides, it may not be generally appreciated that rearrangement of the aspartyl residue in angiotensin can occur in solution even at neutral *p*H; the biological activity of the β aspartyl isomer in the rat pressor assay is some 150% that of the α form[6]. The activity of successive batches of peptide could thus differ according to the degree of contamination by the β isomer. If, however, as suggested[1], manufacturers provided comprehensive analytical data for each batch of synthetic peptide, the user would be aware of this possibility.

When precise quantification is needed, the practice of describing results in terms of apparent peptide content is misleading and could be avoided by assay in terms of a suitably characterized standard preparation. Such a preparation of angiotensin II (asp[1] ileu[5] angiotensin II) has been chemically characterized and its stability studied; this preparation (coded 70/302) and also a human renin preparation (coded 68/356) are now available from this division to research workers for the purpose of standardization.

In due course a preparation of human type angiotensin I[7] will also be provided. It is hoped that use of these will make possible valid quantitative comparison of results in the renin/angiotensin field.

We would emphasize that these general comments apply to many of the synthetic peptide hormones used quantitatively in research which include oxytocin, vasopressins, angiotensins I and II, bradykinin, corticotrophins, human calcitonin M and secretin.

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Role of Reduced TPN in the Response to Interstitial Cell Stimulating Hormone

DISCUSSING the mechanism of action of interstitial cell stimulating hormone (ICSH), also called luteinizing hormone, on the ovary, Flint and Denton[1] concluded that the "primary steroidogenic response" of rat lutein tissue to ICSH involves no change in the cytoplasmic concentration of TPNH (NADPH). This argument was based on two observations: (i) glucose added *in vitro* to luteal tissue increased TPNH/TPN$^+$ but not steroid synthesis and (ii) ICSH *in vivo* increased steroid synthesis but not TPNH/TPN$^+$. Steroid synthesis was observed to increase by 0.19 μmol/g/h after injection of ICSH. The interpretation proposed reveals a fundamental misunderstanding of the significance of steady state values of TPNH/TPN$^+$ which this letter attempts to clarify.

In steady state conditions the concentration of luteal TPNH (or the ratio TPNH/TPN$^+$) at any one time is the outcome of a balance between production and destruction of TPNH (effectively between the relative rates of oxidation and reduction of the pyridine nucleotide). Clearly, constancy of steady state value of TPNH (or TPNH/TPN$^+$) does not necessarily mean that the rate of reduction of TPN$^+$ remains constant; the only valid conclusion is that the balance between oxidation and reduction has not changed. If the rate of reduction of TPN$^+$ increased two-fold, steady state values of TPNH/TPN$^+$ would not alter, if at the same time, oxidation of TPNH also increased two-fold.

Steroid-forming organs do not maintain high levels of stored biosynthetic intermediates beyond cholesterol, so that trophic stimulation depletes such stores of cholesterol (refs. 3–5 and unpublished observations). The synthesis of 1 mol of gestagen (progesterone and 20α-dihydroprogesterone) requires at least 2 ml. of TPNH to cleave the side chain of cholesterol[2]. It follows that the administration of ICSH has resulted in the formation of at least 2×0.19 μmol TPNH/g/h above levels in control tissue (no ICSH). The fact that steady state levels of TPNH/TPN$^+$ are unchanged should not obscure this. At least three possible explanations for increased formation of TPNH without change in TPNH/TPN$^+$ following administration of ICSH can be suggested. (i) ICSH increases the rate of production of TPNH to the level required to accelerate steroid production by 0.19 μmol/g/h; the additional TPNH is oxidized