LETTERS TO THE EDITORS

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Activation of Amino-acids by Soluble Enzymes from Pancreas and Other Tissues

LAST year, Hoagland[1] reported that he had obtained from rat liver a soluble enzyme preparation which catalysed the formation of amino-acid hydroxamates when incubated with a mixture of twelve amino-acids in the presence of adenosine triphosphate and hydroxylamine. He suggested that this type of carboxyl group activation might be a first step in protein synthesis. In a later paper with Keller and Zamecnik[2] he reported partial purification of activating enzyme from liver.

We were able to repeat the work of Hoagland *et al.*, and having regard to the possible importance of these enzymes for protein synthesis we examined the distribution of amino-acid - activating enzymes in several mammalian tissues. Enzyme activity was estimated by measuring the amount of amino-acid hydroxamate formed from a mixture of twelve amino-acids as described by Hoagland, Keller and Zamecnik[2]. The enzyme preparation was the supernatant obtained by centrifuging a homogenate of tissue (1 part) in 0·05 *M* potassium chloride (1·5 parts) at 100,000 *g* for 45 min. Results are given in Table 1.

Table 1. RELATIVE ACTIVITY OF DIFFERENT TISSUE EXTRACTS IN ACTIVATION OF AMINO-ACID CARBOXYL GROUPS

Tissue	Hydroxamate formed (μmole/mgm. protein/hr.)
Rat liver	0·03
Rabbit liver	0·02
Guinea pig liver	0·03
Chicken liver	0·02
Rat kidney	0·01
Rabbit kidney	0·02
Guinea pig kidney	0·01
Chicken kidney	0·02
Rat pancreas	0·04
Guinea pig pancreas	0·05
Rabbit muscle	0·02
Guinea pig muscle	0·02
Guinea pig gut	0·04

From these results, it was clear that amino-acid - activating enzymes are widely distributed in mammalian tissues and that pancreas was likely to be a good raw material for further purification.

It was found that an active enzyme could be obtained from guinea pig pancreas by dilution of the centrifuged supernatant with twelve times its own volume of 0·05 *M* potassium chloride, adjustment of the *p*H to 4·8 and centrifugation. The precipitate was redissolved in *tris*hydroxymethylaminomethane buffer at *p*H 7·6 and tested for its ability to activate single amino-acids or groups of chemically related amino-acids; the results are shown in Table 2.

From these results, it was clear that guinea pig pancreas was rich in enzymes activating serine or threonine and histidine or tryptophan. Tests on the individual amino-acids showed that the *p*H 4·8 enzyme activated serine, threonine and tryptophan but was inactive towards histidine.

By fractionation with ammonium sulphate, the enzyme activating serine and threonine could be partially separated from that activating tryptophan.

Table 2. ACTIVATION OF AMINO-ACIDS BY *p*H 4·8 ENZYME FROM GUINEA PIG PANCREAS

Amino-acid	Hydroxamate formed (μmole/mgm. protein/hr.)
Valine + leucine + *iso*leucine	0·008
Serine + threonine	0·010
Arginine + lysine	0·003
Histidine + tryptophan	0·030
Methionine	0·005
Phenylalanine	0·007
Mixture*	0·104

* The complete mixture contained alanine, valine, leucine, *iso*leucine, phenylalanine, threonine, serine, histidine, arginine, lysine and tryptophan.

Starting with the *p*H 4·8 enzyme, serine–threonine activation was predominant in protein separated between 0·35 and 0·55 saturation with ammonium sulphate (0·125 μmole/mgm./hr. for serine and threonine and 0·09 μmole/mgm./hr. for tryptophan), while the protein precipitated between 0·55 and 0·8 saturation was mainly tryptophan-activating enzyme (0·19 μmole/mgm./hr. for tryptophan and 0·06 μmole/mgm./hr. for serine and threonine).

Working on a larger scale, it has been possible to use sheep or ox pancreas and to prepare a stable acetone powder. By ammonium sulphate fractionation of an extract of this powder, an enzyme preparation has been obtained which is specific for L-tryptophan, stable to dialysis and which requires magnesium ions and adenosine triphosphate.

It seems likely from the results of Keller and Zamecnik[3] that these amino-acid - activating enzymes play a part in protein synthesis, but the distribution of enzyme activity towards different amino-acids seems to be characteristic of the type of tissue used as source of enzyme; thus, Hoagland, Keller and Zamecnik[2], using rat liver, found a quite different distribution of activity from that shown in Table 2; moreover, Demoss and Novelli[4] reported that a similar enzyme preparation from micro-organisms showed no activity towards serine or threonine.

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Competitive Inhibition of Mammalian Tyrosinase by Phenylalanine and its Relationship to Hair Pigmentation in Phenylketonuria

IN Jervis's introductory study, and in subsequent case reports of phenylketonuria (phenylpyruvic oligophrenia), a blonde hair colour and a fair skin have been noted in the large majority of the patients[1–4]. The most striking example described by Jervis was "an idiot baby with blonde hair and blue eyes who belonged to a family of Sicilian extraction, all the members of which, for at least three generations, were of a very dark Mediterranean race"[1]. Three possible hypotheses may be advanced to explain the reduction of melanin formation: absence of the melanin-forming enzyme, tyrosinase; absence or decrease of the melanin precursor, tyrosine; and