

This Week's Citation Classic

Knox W E. Two mechanisms which increase *in vivo* the liver tryptophan peroxidase activity: specific enzyme adaptation and stimulation of the pituitary-adrenal system. *Brit. J. Exp. Pathol.* **32**:462-9, 1951.
[Moltano Institute, University of Cambridge, Cambridge, England]

The amount of an enzyme, now called L-tryptophan oxygenase, was greatly increased in liver by treatment of living rats with the enzyme's specific substrate. Some unrelated compounds caused lesser increases by a different mechanism, but only if the adrenal glands were present. The first mechanism was like the substrate induction of enzymes known in microorganisms. The second mechanism was a way hormones could act to affect metabolism by altering the amounts of specific enzymes in cells. [The *SCP* indicates that this paper has been cited over 250 times since 1961.]

W. Eugene Knox
Department of Biological Chemistry
Harvard Medical School
and Cancer Research Institute
New England Deaconess Hospital
Boston. MA 02215

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"This paper was often cited, perhaps, because it left so much more to be done in the field of metabolic regulation that it started. It described the first inductions of increased amounts of an enzyme in animal tissues by a substrate or by glucocorticoid hormones. It was a big surprise in 1951 that enzyme concentrations in cells did change.

"How hormones in trace amounts could influence metabolism was then a major question for enzyme physiology. Most experimentation envisioned them as a kind of participant in the enzyme reaction, something like the role of coenzymes that had recently explained the actions of vitamins. Typical experiments were always additions of hormones *in vitro* to cell-free enzyme preparations.

"Hormones offered one way to alter metabolism, and parallel alterations of appropriate enzyme reactions could be expected. Our approach differed from the fashionable one only in that we produced the alterations in the animal and not in the test tube.

"We had sought and found something analogous to the enzyme adaptation (induction) of microorganisms: a great elevation in the amount of an enzyme that degraded tryptophan whenever excess tryptophan was administered to an animal.

However, control experiments showed that administration of certain nonsubstrate compounds also increased the enzyme. Such nonspecific noxious stimuli were known to release ACTH from the pituitary and cause the adrenal glands to pour out cortisone (the 'stress response'). Cortisone was not yet available, so a biological test was necessary to find whether the nonsubstrate elevators of the liver enzyme acted through the pituitary-adrenal system to release cortisone. Adrenalectomy interrupted the pituitary-adrenal system and proved both mechanisms: it eliminated the effects of the nonsubstrate compounds but preserved that of tryptophan. In addition to the biologically primitive regulation of an enzyme by its substrate, the higher animals also regulated tissue enzyme amounts by their hormones.

"Some citations were inevitable during the cleanup of numerous problematic aspects left from the discovery in this paper. Did the adrenal hormone itself actually cause the enzyme change? Was the metabolic machinery of animal cells so plastic that environmental conditions could alter the proportions of its enzymes? If so, could other examples of regulated enzymes be found? Several years had already been expended on the properties and assay of this admittedly complex enzyme, the first of the oxygenases to be recognized, and one whose mechanism is still unclear. We could distinguish between merely more activity of the unchanged enzyme and an increased amount of the enzyme. But, inexplicably, for a decade biochemists did not readily assimilate the fact that enzyme concentrations in cells might change. We plumped for a straightforward regulation by hormones of the then still mysterious synthesis of specific proteins.

"Protein synthesis by living cells was apparently necessary for hormones to act 'not by affecting the enzyme reaction itself, but by altering the amount of the enzyme. In spite of the paper's apparently large readership, fruitless experiments seeking *in vitro* actions of hormones in cell-free systems that could not synthesize proteins continued to be published for a decade. Then the problem of hormone actions evaporated. Suddenly it had become a part of common sense, known even to schoolboys, that hormones changed enzyme concentrations in cells."