Klein D C & Weller J L. Indole metabolism in the pineal gland: a circadian rhythm in N-acetyltransferase. *Science* 169:1093-5, 1970.
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This was the first report of the endogenously generated ≈ 100-fold daily rhythm in rat pineal N-acetyltransferase (NAT) activity. It was postulated that the rhythm in NAT activity generates the rhythm in the production and release of melatonin, thus outlining the unique role that this enzyme plays in vertebrate photoneuroendocrine transduction. [The *SCI®* indicates that this paper has been cited in over 465 publications.]

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I was midway through a postdoctoral fellowship with Larry Raisz at the University of Rochester, working on the hormonal control of bone metabolism, when I started to look for a new research area in which to work. The pineal gland fascinated me, and I became interested in determining how melatonin production was regulated. Julius Axelrod and Richard Wurtman had just published evidence that norepinephrine stimulated melatonin production severalfold in organ culture. They thought that large changes in the last enzyme in melatonin synthesis (serotonin→N-acetylserotonin→melatonin), hydroxyindole-O-methyltransferase (HIOMT), probably caused large changes in melatonin production. I picked up this line of investigation and was able to confirm the *in vitro* effect of norepinephrine on melatonin production, but I was never able to obtain any *in vivo* or *in vitro* evidence indicating that HIOMT regulated large changes in melatonin production.

This left me wondering about the regulation of melatonin production, and I turned to thin-layer chromatography to obtain a more detailed picture of the effects of norepinephrine on serotonin metabolism *in vitro*. I found that increased melatonin production was always associated with increased production of the precursor N-acetylserotonin. This indicated that changes in the rate of serotonin N-acetylation might regulate large changes in melatonin production.

When I came to the National Institute of Child Health and Human Development of the National Institutes of Health in July 1969, Joan L. Weller and I developed an assay for serotonin N-acetyltransferase (NAT) activity. By using this assay we found that when norepinephrine or dibutyryl cyclic AMP increased melatonin production in organ culture, NAT activity also increased.[1] We realized that if NAT regulated indole metabolism physiologically then a large day/night rhythm in activity should exist, with high values occurring at night when melatonin was high and serotonin was low. We found that this was the case, and we soon published the report of an *in vivo* rhythm, which became this *Citation Classic*. The report also contained evidence that the rhythm in NAT activity is regulated by an endogenous mechanism that is influenced by light and proposed that NAT regulates melatonin synthesis through a mass action effect involving N-acetylserotonin, thus outlining the unique role that this enzyme plays in photoneuroendocrine transduction.

The report has been frequently cited in part because it indicated that the NAT rhythm was driven by an endogenous clock. In subsequent studies with Bob Moore I used the NAT rhythm to discover that the circadian clock driving the rat pineal gland is in the suprachiasmatic nucleus,[2] which now appears to be the master circadian clock in mammals. In addition, the NAT rhythm has been used to show that the avian pineal gland and amphibian retina also contain circadian clocks.[3,4] Other citations reflect the role of NAT in melatonin production, use of the pineal gland as a model neural system, and investigation of the chemistry of NAT.[5,6]

1. **Klein D C, Berg G R & Weller J L.** Melatonin synthesis: adenosine 3',5'-monophosphate and norepinephrine stimulate N-acetyltransferase. *Science* 168:979-80, 1970. (Cited 190 times.)
2. **Moore R Y & Klein D C.** Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. *Brain Res.* 71:17-33, 1974. (Cited 320 times.)
3. **Binkley S A, Riebman J B & Reilly K B.** The pineal gland: a biological clock *in vitro*. *Science* 202:1198-201, 1978. (Cited 80 times.)
4. **Besharse J C & Iuvone P M.** Circadian clock in Xenopus-Laevis eye controlling retinal serotonin N-acetyltransferase EC-2.1.1.4. *Nature* 305:133-5, 1983.
5. **Klein D C.** Photoneural regulation of the mammalian pineal gland. (Short R, ed.) *Photoperiodism, melatonin and the pineal.* London: Pitman, 1985. p. 38-56.
6. **Namboodiri M A A, Dubbels R & Klein D C.** Arylalkylamine N-acetyltransferase (mammalian pineal). *Meth. Enzymology* 142:583-90, 1986.

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