highest intensity, which is about what we would guess for our set-up.

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THE ALLERGENIC PRINCIPLES OF POISON IVY. IV. ON THE MECHANISM OF THE ENZYMATIC OXIDATION OF CATECHOLS[1]

Sir:

Our study of the immunochemistry of the alkylcatechols present in *Rhus toxicodendron* has led us to examine the mechanism of the enzymatic oxidation of these and related substances. The oxidation of catechol itself by tyrosinase has been extensively investigated, and the resulting data have been interpreted in terms of a sequence of reactions by Dawson and Nelson.[2]

When such a sequence of reactions involves chromophoric molecules, in the ideal case the observed absorption D at any wave length λ and at any time t during the process may be expressed as

$$D_{\text{obs.}, \lambda, t} = l\,[e_{\lambda A}(A) + e_{\lambda B}(B) + e_{\lambda C}(C) + \cdots e_{\lambda N}(N)]$$

where l is the length of the cell used, (A), (B), (C), and . . . (N) are the molar concentrations of those components of the sequence which are present in sufficient concentration at time t to contribute a measurable increment to the total absorption, and where $e_{\lambda A}$, $e_{\lambda B}$, $e_{\lambda C}$ . . . and $e_{\lambda N}$ are the molecular extinction coefficients at λ of those components. If each such component and its molecular extinction coefficients be known, the kinetics of the sequence may be determined by repeatedly scanning a range of absorbing wave lengths during the process. If they be unknown, hypothetical mechanisms may be tested by comparing the spectrophotometric kinetics of the total process with those initiated by postulated intermediates.

By scanning the 220–400 mμ absorption region of *catechol* at intervals of four minutes during its enzymatic oxidation[3] it is now found that the postulated formation of hydroxy-*p*-quinone[2] is not detectable. Under conditions optimal for this process[2] the initial maximum absorption of catechol at 275.5 mμ disappeared rapidly with simultaneous development of a new maximum at 380 mμ, that characteristic of the initial absorption of *o-benzoquinone*. Scattering curves then appeared throughout the observed range of both spectra, with inflexions at 260–290 mμ. *Hydroxy-p-quinone* under similar conditions but in the absence of enzyme displayed an intense initial absorption at 260 mμ; this degraded quickly with the formation of a new maximum at 340 mμ. The latter was relatively stable; only in sixteen hours did a final spectrum with maximum at 290 mμ appear. Significantly, in the presence of tyrosinase the initial maximum absorption of *hydroxyhydroquinone* at 287 mμ disappeared rapidly with formation of successive maxima at 260 mμ, 335–340 mμ, and at 285–287 mμ.

On the basis of this evidence, of that in the cited review, and of unpublished analyses of the polymeric oxidation products of catechol, it is probable that the initial product of the enzymatic oxidation of catechol, *o*-benzoquinone, participates in a polymerization to a phenolic chain susceptible to further enzymatic oxidation. Kinetic spectrophotometry of the autoxidation, oxidation in the presence of unsaturated fatty esters, and oxidation in the presence of tyrosinase of 3-*n*-pentadecylcatechol (hydrourushiol) has similarly established 3-*n*-pentadecyl-*o*-benzoquinone as the initial oxidation product in each case. The technique is being extended to related problems.

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(1) For the third paper in this series, see Mason, THIS JOURNAL, **67**, 418 (1945).

(2) Dawson and Nelson, *Advances in Enzymology*, **IV**, 99-152 (1944).

(3) The purified mushroom tyrosinase was generously contributed by Professor Charles R. Dawson.