an appreciable amount of evidence which clearly correlates aminopeptidase activity with tissue breakdown."

On page 148 he points out properly the recent uncritical use of metal salt methods of histochemistry with the electron microscope. But in the same chapter he fails to credit properly the first application in histochemistry of the post-incubation coupling principle and of metal chelation to improve the quality of pigments. That he does not understand the reasons why post-incubation coupling was developed in the first place is revealed by his suggestion that it be used for enzymes that work above pH 7.0 where simultaneous coupling and a rapid capture reaction makes post-incubation coupling unnecessary. On page 301 he suggests that indophenyl acetate may have application in histochemical visualization of esterase, apparently overlooking a report of its failure to do so due to the solubility of the indophenol in histochemical trial published in 1957 by Nachlas, *et al.*

There is little emphasis on the logic and rationale which has been used in the design of reagents for developing and improving histochemical methods. Since his own approach seems to be by empirical testing he tends to underplay or ignore the rational aspect of the work of others in his presentation of their methods. Notable examples are in his handling of the best substrate for the histochemical demonstration of leucine aminopeptidase (L-leucyl-4-methoxy-2-naphthylamide) and in the development of the reagent, Nitro-BT for the dehydrogenases. The following quotation will serve as an example. On page 405 he states: "More recently, Nachlas, *et al.* (1960), reported on the histochemical application of a modified leucyl substrate which contained a methoxy group in the 4-position of the naphthalene nucleus. The enzymatically released 4-methoxy-2-naphthylamine was claimed to couple 40 times as fast as 2-naphthylamine. It is difficult to understand how the introduction of a methoxy group can result in such a remarkable increase in coupling rate, but in any case some of the photomicrographs shown in the paper exhibit diffusion artefacts and there is little evidence at present to recommend the methoxy substrate over the original leucyl or alanyl naphthylamides." If it is really true that the author does not understand how the methoxy group in the 4-position can produce a 40-fold increase in coupling rate, I must conclude that he has missed reading the paper (*J. Histochem. Cytochem.*, **7**, 50 (1959)) in which it was shown by direct measurement of coupling rates and by careful presentation of the application of electronic theory that the coupling rate of naphthols was increased by the introduction of electronegative groups into the diazonium salts, whereas the coupling rate of naphthylamine was greatly increased only by the introduction of an electropositive group into the 4-position of 2-naphthylamine rather than by the selection of diazonium salts with strong electronegative groups. This theoretical study lead to the design and preparation of L-leucyl-4-methoxy-2-naphthylamide, which is a far better substrate for aminopeptidase than the original one. The results with it in frozen sections, using the tetrazonium salt, fast blue B, which favors copper chelation to decrease lipid-solubility, are superior to those with any other method so far proposed. Since amino acid amides of 4-methoxy-2-naphthylamine are now available from Cyclo Chemical Corporation, Los Angeles, Calif., histochemists will be able to judge the merits of the method for themselves and should not accept Dr. Burstone's evaluation based upon his inspection of our published plates and a strong desire to have it otherwise. His handling of this subject raises serious question of his suitability to write an objective review. That the author is capable of perceiving this weakness in other writers is shown in the following quote from page 187. "The metal–salt technique has been both overestimated and overcriticized with reference to quality and precision of localization, and there is a notably high degree of correlation between the type of evaluation and the preference of the individual authors."

In chapter 11, modifications of the original Nadi reaction for demonstrating cytochrome oxidase are given. As usual a large variety of agents which may be used successfully are presented in tables. Why any one compound is to be preferred over another is not mentioned. Just so long as they appear in his tables, it is to be tacitly assumed that they are superior to the reagent 4-amino-1-N,N-dimethylnaphthylamine (ADN), published by Nachlas, *et al.*, in 1958, and shown to give results which proved to be similar to those shown in 1959 by Dr. Burstone with some of the compounds in his tables. An important difference is that Nachlas, *et al.*, mentioned that the pigment obtained as a final product was lipid soluble, lacked substantivity for protein, and faded after several months. Although this is quoted on page 438 (omitting the fact that fading occurred only after several months), the author failed to mention that pigments obtained from *p*-amino diphenylamine are also lipid soluble, lack substantivity for protein, and are not permanent. Furthermore, since *p*-aminodiphenylamine is capable of reacting with itself, some doubt is justified that reaction occurs exclusively with the various naphtholic and methylene compounds which it is claimed may be used for the Nadi reaction. This thought finds some confirmation in the similarity of the two absorption curves reproduced from Person, *et al.*, in figure 2. As a matter of fact, neither method is good enough, as becomes evident when one searches for mitochondrial morphology at suitably high magnification, such as in figures 6 and 7. Figures 8–15 are too low power to serve a useful purpose other than to indicate cytochrome oxidase activity in the cells. There is need for the design of better reagents that will yield pigments with better properties. Until this is done, electron photomicrographs such as the one shown on page 536, will not tell us enough about the relationship of chemical activity and intracellular structure.

In chapter 12 on the dehydrogenases, the presentation is somewhat better. Labeling all the succinic dehydrogenase activity shown in figure 1 as mitochondrial, when it has been shown by Bergman and Walker (1959) that the sarcomeres are also enzymatically active, will only serve to confuse. Figures 2, 4, and 6 hardly do justice at low magnification to what can be shown with Nitro-BT. The reader would be advised to consult the literature for better examples. The rational and evolutionary development of the tetrazolium reagents is played down so that the newcomer to histochemistry reading the chapter will get the impression that these reagents and methods were hit upon by chance rather than by invention. Thus he will miss an exciting chapter in historical development and an important lesson in the design of reagents to fulfill the stringent needs of histochemistry. This is to be regretted since only with the dehydrogenases has the door been opened so far for studying in great detail the enzymatic activity and morphology of intracellular organelles.

Chapter 13 on application of enzyme histochemistry to electron microscopy, gives a fair presentation of the development of this the youngest of the histochemical sciences. The reproductions of the beautiful electron micrographs of Dr. E. Mölbert and Dr. R. J. Barrnett do much to strengthen the chapter. Chapter 14 on electrophoretic procedures is well written and contains useful technical data for those who wish to use these methods. Chapter 15 on the relationship between carcinostatic agents and histochemical substrates will interest some cancer chemotherapists and few histochemists. It is a brief and adequate summary.

The book as a whole will serve as a useful reference especially for recent material not covered in the older texts. Although Dr. Burstone deserves credit for the hard work he has obviously put into its preparation, the book does not attain the standards of scholarship and accuracy to be found in the 1960 edition of the book by Dr. A. G. E. Pearse.

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Physical Methods in Heterocyclic Chemistry, A Comprehensive Treatise in Two Volumes. Volumes I and II. Edited by A. R. KATRITZKY, University Chemistry Laboratory, Cambridge, England. Academic Press, Inc. 111 Fifth Ave., New York 3, N. Y. 1963. 23.5 × 15.5 cm. 346 pp. Price, $12.00.

Recent advances in the development of new physical methods and the often specialized characteristics of the heterocyclic variants of organic structures offer an appropriate point of departure for the compilation of a series of reviews such as are presented in these two volumes. Whether or not the result achieves the reader's expectations may depend somewhat on the approach the reader takes. It can certainly be agreed, however, that the chemist working within this field will be indebted to these authors either for having presented superior analyses of their particular topic or for having indicated the desirability for a more thorough elaboration. The chapters include discussions of ionization constants by A. Albert; heteroaromatic reactivity by J. Ridd; X-ray diffraction studies of heterocyclic compounds by W. Cochran; solubility of heterocyclic compounds by W. Pfleiderer; applications of dipole moments to heterocyclic systems by S. Walker; electrochemical properties in solutions by J. Volke (all in Volume I); and (in Volume II) electronic absorption spectra of heterocyclic compounds by S. F. Mason; nuclear quadrapole resonance by E. A. C. Lucken; nuclear magnetic resonance spectra by R. F. M. White; and infrared spectra (985 references) by A. R. Katritzky and A. P. Ambler. Some of these chapters are thorough and obviously of value; others are so short or so restricted in scope as to be of help mostly in terms of making the reader curious as to why so many data are missing. Since such omission may result in the recognition of presently unappreciated research problems, it is hardly just to hold the author responsible for them as short-comings in the presentation. The reciprocal solubilities and consolute temperatures characteristic of dimethylpyrone and some pyridine bases are not considered in the solubility chapter and the compression of X-ray diffraction studies into fifteen pages seems disproportionate at a time when this technique promises to remake completely the traditional approach to chemical structural

studies of natural products. The tables of oxidation–reduction potentials, ionization constants, and infrared characteristics are extensive and apparently exhaustive. The chapter on electronic spectra presents a theoretical approach, wisely leaving the compilation of data to others. On balance the reviewer concludes that the volumes are a valuable addition to the growing literature on heterocycles and will serve the scientists working with heterocycles very well.

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