BOOK REVIEWS

Biological Transport. By HALVOR N. CHRISTENSEN, University of Michigan, Ann Arbor, Michigan. W. A. Benjamin, Inc., 2465 Broadway, New York 25, N. Y. 1962. viii + 133 pp. 16.5 × 23.5 cm. Price, $6.50.

Since the early fifties there has been a large development of research directed toward studying the processes of transport in biological tissues. Although there are a few rather good reviews and monographs summarizing this extensive literature, there is no suitable introductory text for graduate students or scientists in other areas interested in biological transport. In his preface, the author states:

"This presentation grew from a short series of lectures to an advanced biochemistry class, attended by graduate students of pharmacology, physiology, microbiology, genetics, and other sciences. It should be interpreted more as bibliographical syllabus for that instruction than as a review." Written by an acknowledged expert with this explicit orientation, the combination was ideal for developing a useful text in this area.

The first half of the book, which is comprised of three chapters, deals with the scope of the problem, concepts and terms, and the kinetic approach to transport. The rest of the book deals with the specificity of transport systems, attempts at isolation of the reactive sites, the evidence about the transport mechanisms gleaned from the associated physical and chemical fluxes, the nutritional and genetic approaches to characterizing these processes, and the effects of various hormones on transport processes. The book ends with a summary and some speculations on the future course of transport research.

The style is distinctive. The exposition is in the form of a survey balanced with the considered judgments and reflections of the author spiced with quotations from savants of the field. It is clear that any interested student will be provoked to read the original literature by this book. One acknowledged omission in this survey is the field of ion transport which is only referred to in a sketchy manner in a few scattered places. The figures and examples have been chosen to illustrate the points discussed by the author without introducing complexities. In general, the book is attractively produced.

In summary, this book represents a discriminate introduction to the field of biological transport processes.

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Enzyme Histochemistry and Its Application in the Study of Neoplasms. By M. S. BURSTONE, National Cancer Institute, National Institutes of Health, Bethesda, Md. Academic Press Inc., 111 Fifth Avenue, New York 3, N. Y. 1963. 16 × 23.5 cm. 621 pp. Price, $22.50.

This new addition to the growing list of textbooks on histochemistry is in some respects a valuable compilation and in others has serious shortcomings. Chapter 1 dealing with the principles of fixation, freeze-drying, and freeze-substitution, is a well written account of these techniques, although too much emphasis is placed on freeze drying, which is still largely unnecessary for histochemists. Chapters 2 and 3 dealing with naphthalene derivatives, substrates, diazonium compounds, azo dyes, and synthetic routes to substrates are valuable summaries for the biologists who wish to gain familiarity with this historically important field of dye chemistry. A major criticism is the inclusion of too many compounds that have not been demonstrated to be especially useful in histochemistry either historically or practically. Some compounds are included on the basis that someday someone may be able to make them useful in histochemistry. With this criterion for inclusion, the tables could have been made much larger. In chapters 5 and 6 the author presents a large number of naphthol-AS derivatives, none of which has been shown clearly to offer real advantages over naphthol-AS itself or over one another. The colored plates and figures in the book are no more convincing of histochemical superiority of histochemical methodology with these agents than they were in his original publications. The presentation and illustrations leave the reader with no single agent of preference among the naphthol-AS derivatives. It appears that he cannot select the most appropriate reagent, perhaps because he is fond of them all. It is only when the reagents of other investigators are under scrutiny in comparison with some of his own, that he has no difficulty in selecting the best, clearly, decisively, and sometimes derisively. In one instance on page 304, he had the following to say about the histochemist who first introduced analogs of naphthol-AS acetate for esterase. "Pearse (1954, 1960 p. 466) indicated that the naphthol AS-OT (AS-D) acetate was superior to the original AS acetate of Gomori. Since the only difference between AS acetate and AS-D acetate is the presence of a methyl group, this minor alteration would scarcely be expected to improve the quality of localization." Is it possible that the author is unaware of the fact that the same comment can be made of some of the naphthol-AS derivatives he presents in chart 2 of chapter 5 and in chapter 6?

In chapter 4 on principles of histochemical localization he takes some serious liberties with the history of the subject. On page 152 he has one set of investigators in 1957 confirming conclusions "previously outlined" by another group in 1958. Although he accepts the conclusion of the former investigators that discrete deposition of dye within a cell does not necessarily represent a more accurate location of all enzymes than does the widespread dispersion of the dye throughout cells, and illustrates this with figure 5 which is similar to a figure published by Nachlas, *et al.*, in 1957, on page 150, he places in italics the statement that "histochemists trained as histologists or pathologists should always expect to see clear cut microscopic localizations provided proper techniques have been employed." Unfortunately all the enzymes in cells do not present a configuration desirable by Dr. Burstone's specially defined category of histochemist.

He goes to some pains to relate the controversy that occurred in the early 1950's, when azo dye methods were being rapidly expanded and better understood. The differences of opinion as to the nuances of the then young vigorous developing science have been largely resolved by now and the dust of battle of another decade has settled. It is, therefore, with some consternation that I behold Dr. Burstone in 1963, flogging a dead horse with a zeal that will cause the eyebrows of those of us who remember this historical episode to rise a little.

Although it is proper to warn the reader that the demonstration of phosphatases and esterases may be accomplished with less danger of diffusion artefacts when the derivatives of naphthols less soluble than 2-naphthol are used, the warning against its use in starch gel electrophoresis (page 552) is fatuous and it should not be necessary to resort to the conceit of showing a photograph (page 141), obtained by using a fresh frozen section of rat kidney and attributing the diffusion cloud that results to naphthol diffusion, when in fact it had been shown by Nachlas, *et al.*, in 1957, that this cloud is due to diffusion of the soluble and diffusible lyoenzyme. In that same paper a companion photograph showed that the cloud of dye could be prevented by merely incubating the section in saline for 30 min., during which time diffusible enzyme was readily removed. Modern methods of fixation remove most if not all of the lyoenzymes and therefore give sharper localizations, not necessarily more accurate localizations. The artefacts so produced may be of considerable interest nonetheless. If the author took the trouble to repeat this experiment with naphthol-AS substrates on fresh tissue sections, he would have discovered that dye also accumulates in the surrounding medium, although at a slower rate due to slower enzymatic hydrolysis of these substrates. This tendency to ignore facts in the literature which conflict with what he wants to believe is a major disappointing feature of several chapters of the book. It would have been in better taste if the first azo dye methods, that opened histochemical doors for many investigators as well as for Dr. Burstone, were presented in their proper historical perspective.

The chapters devoted to each class of enzyme includes a section on biochemistry, usually handled thoroughly if a bit disorganized; a section on historical development, usually deficient in the rationale and often slanted toward Dr. Burstone's own contributions; a section on methodology, usually biased for his own modifications; and a section on application, including application to tumors. The last section consists mainly of a list of tumors in which the enzyme has been found to be present or absent. In the case of aminopeptidase (chapter 10) he devotes considerable space to defending, in less extravagant terms than formerly, his original theory that the aminopeptidase activity in the stroma of tumors was related to their malignant and invasive properties. Since Monis, *et al.*, in 1959, have pointed out that the fibroblast as well as other connective tissue cells have enough enzymatic activity to explain all the findings in tumor stroma as well as in nonmalignant lesions with fibroblastic activity, the burden of proof for his theory rests upon Dr. Burstone. Proof has not been forthcoming, and the extent to which he has been willing to backtrack may be indicated by the following quote from page 414, "With reference to the role of aminopeptidase, in view of the fact that the biological function of this enzyme has not been delineated, statements to the effect that the enzyme is completely unrelated to tumor invasion have not been completely established. This may also be said of attempts to associate the enzyme with tumor invasion, although in this instance there is

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