process obeyed apparent first-order kinetics. The results obtained from three animals are shown in Table I.

Preliminary experimentation indicated that half-lives obtained from different jejunal segments in the same dog did not vary from one another by more than 15%. These results are thus suggestive of a meaningful relationship between intestinal blood perfusion and drug absorption rate. In most instances, a 40–60% reduction in mesenteric blood flow resulted in a dramatic increase in the absorption half-life for sulfaethidole.

Mesenteric circulation is subject to alteration from a wide variety of sources, and some of these will be examined and discussed in a future publication. However, it is obvious that adequate precautions should be taken to assure that significant differences in intestinal blood perfusion rates do not exist among the different animals used in a particular absorption study. Unless such precautions are taken, comparisons of the drug absorption data obtained from the various animals should be viewed with a degree of caution.

Although the data reported herein do confirm that the intestinal drug absorption process is hindered by a decrease in vascular perfusion, additional studies must be designed to quantitate the effects of fasting on intestinal blood flow before ascribing a causative role to this factor in our previous experiments.

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William Crouthamel
James T. Doluisio
Rolley E. Johnson
Louis Diamond
University of Kentucky
College of Pharmacy
Lexington, KY 40506

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Spectrophotometric Analysis of Acetylcholine Levels in Plasma

Keyphrases □ Acetylcholine levels, plasma—determination □ Plasma—acetylcholine determination □ Colorimetric analysis—spectrophotometer

Sir:

The interest in determining acetylcholine levels in biological media before and after angiotensin-II administration parenterally in turtles led to the search for an acceptable method of analysis. The bioassay of isolated guinea pig ileum contractions against known acetylcholine concentrations was initially considered (1), but the sensitivity of the test was proven unacceptable. The bromocresol purple method of Woods (2) and the methyl orange method of Brodie (3, 4) are general methods for determining organic bases and are limited in their usefulness for biological media, which normally contain interfering organic bases.

The bromophenol blue method of Auerbach (5), modified by Mitchell and Clark (6), was chosen because it bypassed this difficulty of interfering bases and increased the sensitivity of the test 10-fold.

The interaction involves the formation of a color complex between anionic bromophenol blue and the quaternary cation, acetylcholine. The reaction occurs in an alkaline medium with the resulting color complex formed being quantitatively extracted, utilizing organic solvents. The advantage of this method is that organic bases and unreacted dye do not interfere with the extraction. Auerbach (5) also has tested 50 tertiary amines with negative results.

Procedure—Three map turtles heparinized with 100 USP units were utilized for the analysis. Each turtle was treated with 20 mg./kg. of physostigmine salicylate 5 min. prior to withdrawal of the blood sample. One milliliter of blood was removed from each turtle and added to 0.5 ml. of a 0.5% physostigmine solution. The resulting mixture was centrifuged for 15 min. at 2000 r.p.m., and a 0.5-ml. plasma sample was used for analysis. Following the addition of a buffer (0.3 g. of K_2HPO_4 and 0.3 g. of Na_2CO_3) to pH 9, the indicator, bromophenol blue, was added in a 0.5-ml. volume (0.08% in 30% K_2HPO_4). Fifteen minutes of shaking with organic solvents (washed ethylene dichloride and 4% isoamyl alcohol) completed the extraction of the dye–acetylcholine complex. The organic phase was read at 600 mμ against an ethylene dichloride–isoamyl alcohol blank because none of the other reagents absorbs. It is mandatory that the analysis take place within a 1-hr. time span because of the rapid fading of the indicator after this period. All absorbances were read on the Coleman (Hitachi 124) double-beam spectrophotometer. The procedure was then repeated following angiotensin-II administration. The differences in absorbance are due to increased acetylcholine levels. This was again repeated, using turtles with both vagi surgically severed. In the turtles with intact vagi, the following values were read: plasma sample plus physostigmine, 0.040, 0.055, and 0.043—mean = 0.046 ± 0.008 *SD;* plasma sample plus physostigmine after administration of angiotensin, 0.395, 0.410, and 0.380—mean = 0.395 ± 0.014 *SD*. The difference in absorbance, 0.349 mμ, is attributed to increased acetylcholine blood levels and corresponds to a concentration of 9.1 mcg./0.5 ml. on the standard curve. When this was repeated in vagotomized turtles, the following values for absorbance were read: plasma sample plus physostigmine, 0.050, 0.040, and 0.045—mean = 0.045 ± 0.004 *SD;* plasma sample plus physostigmine in angiotensin-treated turtles, 0.290, 0.285, and 0.305—mean = 0.293 ± 0.010 *SD*. The difference in absorbance between the two means, 0.248 mμ, is attributed to increased acetylcholine blood levels and corresponds to

a concentration of 6.0 mcg./0.5 ml. on the standard curve.

Standardization—The experimental values for acetylcholine were standardized against known concentrations of acetylcholine chloride by utilizing a standard curve prepared from the following. A concentration of 20.0 mcg./0.5 ml. gave the following values in three samples: 0.750, 0.760, and 0.769 mμ with a mean of 0.759 mμ; a concentration of 10.0 mcg./0.5 ml. gave the following values in three samples: 0.335, 0.343, and 0.352 with a mean of 0.343 mμ; a concentration of 6.6 mcg./0.5 ml. gave the following: 0.230, 0.243, and 0.250 with a mean of 0.241 mμ; a concentration of 5.0 mcg./0.5 ml. gave the following values: 0.175, 0.187, and 0.199 with a mean of 0.189 mμ; a concentration of 4.0 mcg./0.5 ml. gave the following values: 0.134, 0.145, and 0.156 with a mean of 0.141 mμ.

The results of this analysis support the hypotheses that angiotensin-II administration causes the release of acetylcholine at neuroeffector sites in the turtle plasma and that this release is not dependent upon the presence of intact vagus innervation (7).

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Michael A. Barletta
Charles O. Ward
St. John's University
College of Pharmacy
Department of Pharmacology
Jamaica, NY 11432

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BOOKS

REVIEWS

Pharmaceutical Enzymes and Their Assay. Edited by R. Ruyssen. Universitaire Pers afd. Uitgoeverij van N. V. Universitaire Boekhandel 12, St.-Amandstraat, Ghent, Belgium, 1969. 151 pp. 15.7 × 24.5 cm. Price $11.00. (*French* and *English*)

In 1961, the Fédération Internationale Pharmaceutique created the Commission for the Standardization of Pharmaceutical Enzymes, the primary mission of which was the establishment of uniform international standards for enzymes having pharmaceutical applications.

The lack of uniformity in the methods used to express the catalytic activity of pharmaceutical enzymes prompted the Commission, as its first resolution, to adopt, insofar as is practicable, an international unit system which defines an enzyme unit as that amount which catalyzes the transformation of 1 micromole of substrate per minute (or where more than one bond of a more complex substrate is attacked, one microequivalent of the group concerned per minute) under well-defined, usually external, optimal conditions. Since a unit system of this kind requires that either new methods be developed or existing methods be modified for the assay of virtually every enzyme, the attention of the Commission has thus far been devoted largely to assay methods and the many problems associated with them.

This book is a hard-cover publication of a symposium held by the Commission at the University of Ghent in May 1968. The selected topics, which are all expertly discussed in this series of seven papers, include: (1) reactions of organofluorophosphate-sensitive enzymes; (2) assay of proteinases; (3) determination of the components of the human plasma fibrinolytic system; (4) action of streptokinase on purified human plasminogen; (5) some aspects of the biochemistry of cellulases and hemicellulases; (6) microbial enzymes and their industrial applications, and (7) assay methods of the F.I.P. Commission on enzymes. Papers 4 and 5, previously mentioned, are written in French; the rest of the book is in English.

The long seventh paper contains an excellent review of the problems encountered and the progress made thus far by the Commission toward its goal. Also discussed are the many requirements which must be met by an assay method if it is to be accepted on an international level. Included herein is a detailed presentation of the Commission's proposed methods of assay for trypsin, chymotrypsin, papain, trypsin-inhibitor, pepsin, pancreatic amylase, pancreatic protease, enterokinase, and pancreatic lipase.