$$\% \text{ absorbed} = \frac{\frac{1}{k_1}\frac{dC}{dt} + \left(1 + \frac{k_2}{k_1}\right) C_T + k_2 \text{ area}\Big|_{t=0}^{t=T}}{A_\infty} \times 100 \quad \text{(Eq. 10)}$$

where A_∞ is the asymptotic value obtained by the procedure described.

It is of interest to point out that when the rate of biotransformation of the absorbed species is very rapid (large k_1), and when the ratio k_1/k_2 is also large, Eq. 9 takes the form of the equation previously presented (1) which was based on the use of blood level *versus* time data on the absorbed species.

(1) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **52**, 610(1963).
(2) *Ibid.*, **53**, 1392(1964).

EINO NELSON

School of Pharmacy
State University of New York at Buffalo
Received April 12, 1965.
Accepted for publication May 4, 1965.

Paper Chromatographic Method for Detecting Phenethicillin Contamination in Drugs

Sir:

Recently promulgated Federal regulations (1) dealing with possible cross-contamination of other drugs manufactured on the same premises or with the same equipment as penicillin products have created a multitude of problems for the pharmaceutical industry. Among these problems is the necessity of testing a large backlog of samples representing batches of drugs produced prior to the advent of the new regulations.

Microbiological methods thus far proposed (2) are expensive, and many small laboratories are not equipped to conduct microbiological assays. Chemical methods currently in use for assaying the penicillins lack the sensitivity required for detecting the minute amounts specified by the regulations (not more than 0.5 unit of penicillin contamination per maximum single oral dose, and not more than 0.05 unit per maximum single parenteral dose).

To meet this problem, the authors have developed a quick and inexpensive paper chromatographic method for traces of phenethicillin. Sensitivity is of the order of 5 units, so that the sample spotted on the paper strip must represent approximately 10 maximum single oral doses or 100 maximum single parenteral doses. The method is applicable mainly to oral dosage forms, although the efficiency of the extraction procedure employed makes it feasible for many parenterals. Preliminary experimentation has shown that penicillins G and V are detected equally as well as phenethicillin by this test.

Reagents.—*Phosphate Buffer, pH 2.5, 10% Stock Solution.*—Dissolve 100 Gm. of monobasic potassium phosphate in 800 ml. of distilled water, adjust to pH 2.5 with concentrated hydrochloric acid (about 0.2 ml.), and dilute to 1000 ml. with distilled water.

Phosphate Buffer, pH 2.5, 1% Solution.—Dilute the 10% stock solution 1:10 with distilled water, and readjust to pH 2.5, if necessary.

Mobile Phase for Chromatographic Development.—Equilibrate 2 parts *n*-butanol and 1 part 2% aqueous oxalic acid. Use upper layer.

Spray Reagent.—0.02 *M* iodine, prepared by diluting 0.1 *N* iodine test solution (U. S. P. XVI, p. 1080) 1:5 with distilled water.

Other Solutions and Solvents.—1 *N* hydrochloric acid, ammonium hydroxide, chloroform, and methanol.

All reagents and glassware must be free of penicillin contamination.

Preparation of Test Sample.—Assuming one tablet or capsule per maximum single oral dose pulverize 20 tablets (or combine contents of 20 capsules) and dampen the powder with a small amount of distilled water. Dissolve or suspend in sufficient distilled water to make 18 ml. Filter through a prewetted filter paper, and transfer 9 ml. of the filtrate to a separator. Adjust to pH 2.5 with 1 *N* hydrochloric acid, add 1 ml. of 10% phosphate buffer, pH 2.5, and extract with two 20-ml. portions of chloroform. Combine the chloroform extracts and wash with one 20-ml. portion of 1% phosphate buffer, pH 2.5. Transfer the chloroform layer to a beaker and air dry. Wash the residue into a 15-ml. centrifuge tube with two 1-ml. portions of methanol. Take the methanol solution to dryness with forced air and a warm water jacket. Dissolve the residue in 0.1 ml. of methanol.

Preparation of Control Sample.—Repeat the above procedure with an additional 20 tablets or capsules from a batch of the same formulation known to be penicillin-free, adding 10 μl. of phenethicillin standard (10 units of activity) to the powder prior to dampening with water.

Preparation of Phenethicillin Standard.—Dissolve an accurately weighed amount of

potassium phenethicillin in distilled water, so that each 5 μl. contains 5 units of phenethicillin activity.

Paper Strips and Equipment.—Prepare paper strips of suitable size from Whatman No. 1 chromatography paper, and mark them for application of the samples approximately 1 in. from the bottom. Use a 5-μl. pipet for application of samples to the spots. Any closed container, adapted for ascending paper chromatography, may be used as a developing tank. We use a 2-L. large-mouth, round, amber bottle with a screw cap. The strips are held in place by strings and clips inserted through holes in the cap and secured to the cap with adhesive tape. Pressurized Chromatosprayers, purchased from Research Specialties Co., Richmond, Calif., are used to apply the color reagent after development of the strips.

Procedure.—Apply the entire 0.1 ml. of test sample to one spot on the origin line of the strip, and the 0.1 ml. of control sample to an adjacent spot. Do not let the spot diameters exceed 0.8 cm. To a third spot, apply 5 μl. of phenethicillin standard (5 units). Allow the spots to dry, and then expose the strip to ammonia fumes in a closed container for 10 min. Remove and air dry. Place in the developing tank so that about 1/4 in. of the bottom of the strip is immersed in the mobile phase. Allow to develop to the 15-cm. mark, remove, and air dry. Again, place the strip in ammonia vapor for 10 min., remove, air dry, and spray *once lightly* with 0.02 *M* iodine solution. White spots on an iodine-colored background indicate the presence of phenethicillin, R_f approximately 0.55. The size and intensity of the spot produced by the control sample should be similar to those of the spot produced by the phenethicillin standard. Any sign of phenethicillin in the test sample indicates contamination, the degree of which is estimated by comparison of the three spots.

Discussion.—Treatment of the paper strip with ammonia fumes serves to split the lactam ring of the phenethicillin molecule, producing the thiazolidine derivative. The latter reacts with iodine, whereas the intact molecule does not (3). This step is carried out prior to development of the paper strip, because the thiazolidine derivative has a lower and more desirable R_f value than does phenethicillin itself. The authors have found that unwanted residues are carried along closer to the solvent front, and they tend somewhat to reduce the R_f value of phenethicillin and to distort the shape of the spot.

Some formulations provide excessive amounts of residues that thwart detection of phenethicillin. In some such cases, it has proved advantageous to add another extraction step, as follows.

Reduce the chloroform extract to 20 ml., and extract with two 10-ml. portions of 1% phosphate buffer, pH 6.0, to remove the phenethicillin. Combine the extracts, adjust them to pH 2.5 with 1 *N* hydrochloric acid, add 2 ml. of 10% phosphate buffer, pH 2.5, and extract with two 40-ml. portions of chloroform. Wash the chloroform extracts with 20 ml. of 1% phosphate buffer, pH 2.5, take to dryness with air and a warm water jacket, and proceed with the methanol extraction steps given under *Preparation of Test Sample.*

This method has been used successfully for a variety of pharmaceutical formulations, both simple and complex. It is considered a tentative procedure, because the authors realize that it will need modification in certain instances. It provides a quick inexpensive screening method and a starting point for further refinement.

(1) *Federal Register*, 30, 932(1965). Amendment to 21 CFR Part 133, "Drugs—Current Good Manufacturing Practice in Manufacture, Processing, Packing or Holding," paragraph 133.11(h).
(2) Wilner, J., *et al.*, "Tentative Procedures for Detecting and Measuring Penicillin Contamination in Drugs," Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C., February 1965.
(3) Vasilev, B., *Compt. Rend. Acad. Bulg. Sci.*, 16, 369 (1963).

WILLIAM H. COX
BEN E. GREENWELL

Control Department
Rowell Laboratories, Inc.
Baudette, Minn.

Received April 21, 1965.
Accepted for publication May 5, 1965.

Linear Free Energy Relationship Among Analgesic *N*-Substituted Phenylpiperidine Derivatives. Method of Detecting Similar Modes of Molecular Binding to Common Receptors

Sir:

If compounds in two different series of analgesics are exerting their effect by interacting in a similar way with a common analgesic receptor, then identical changes in a portion of the molecule belonging to both series should produce parallel variation in activity. Thus, identical changes in the *N*-substituent in two series of analgesics