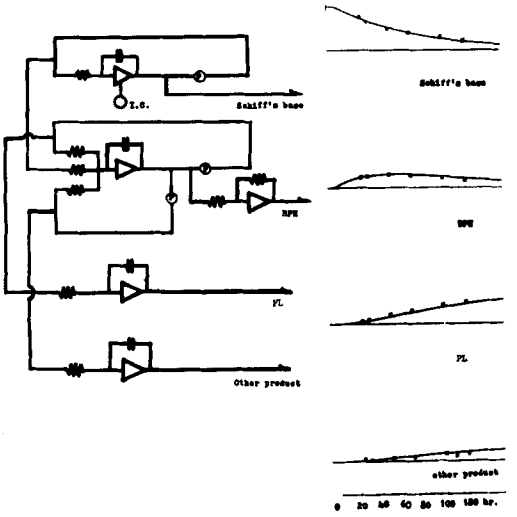

Fig. 2—Block diagram and computer curves obtained by computer analysis. Key: ——, obtained curve; •, experimental value

The agreement between the obtained and experimental values supports the idea that the reaction proceeds on the strength of the above mechanism represented by the simplified scheme and that BPH is the most important intermediate in the conversion route of Schiff's base into PL in the presence of a great excess of PE-NH.

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Keyphrases

Dehydroacetic acid
Schiff's base transformation *N*-phenethyllutidone
Aminolysis reaction
UV spectrophotometry—kinetic analysis
TLC—analysis

Thiry-Vella Dog as a Biologic Model for Evaluation of Drug Absorption from the Intestinal Mucosa

By R. G. SAMPLE, G. V. ROSSI, and E. W. PACKMAN

The Thiry-Vella fistula dog was found to provide a quantitative and reproducible pharmacometric system for the evaluation of drug absorption from the intestinal mucosa. Instillation of buffered solutions of *N*-acetyl-*p*-aminophenol (acetaminophen) into the *in situ* jejunal loop resulted in rapid and essentially complete absorption. Within the range examined (75, 150, 300, and 450 mg.), an increase in dose was reflected by a commensurate increase in the plasma concentration of *N*-acetyl-*p*-aminophenol. Plasma concentration curves for each dosage level were parallel over a 2-hr. postadministration period. The Thiry-Vella fistula provides a stable, readily accessible segment of intestinal mucosa with blood, nerve, and lymph supplies intact. The chronicity of the fistula preparation permits each animal to serve as its own control and to be used repeatedly to compare the absorption characteristics of the same drug at different dosage levels, as well as different drugs at the same or alternate dosage levels.

A WIDE VARIETY of *in vivo* and *in vitro* techniques have been employed in the study of intestinal absorption depending fundamentally on the objectives and preferences of the investigator. Although many methods have been introduced, there has not been developed a relevant and reproducible biologic model for the study of factors relating to drug absorption from the intestinal mucosa, and for evaluation of concepts established principally in *in vitro* systems.

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The Thiry-Vella dog (1) provides a chronic *in situ* loop preparation consisting of a segment of small intestine, isolated with its blood, nerve, and lymph supplies intact, in which both the oral and aboral ends of the segment are exteriorized through the abdominal wall and sutured in place. Thus there is formed a readily accessible U-shaped loop of intestine having considerable physiologic integrity. With proper postsurgical care the fistula remains patent, enabling each animal to be used repeatedly and to serve as its own experimental control, thus providing a high degree of stability and reproducibility. Previous investigations utilizing the Thiry-Vella loop have been concerned primarily with the study of intestinal secretions (2, 3), the absorption of nutrient factors (4–8), and the effect of drugs on the motility of intestinal smooth muscle (9). The basic objective of the research described in this report was to explore the feasibility of the Thiry-Vella preparation as a biologic model for the evaluation of the absorption of drugs from the intestinal lumen.

EXPERIMENTAL

Preparation of Thiry-Vella Fistulae—Five male and 7 female mongrel dogs (9–13.6 Kg.) were used in the various phases of this investigation. Eight of these animals were surgically prepared with a Thiry-Vella fistula according to the procedure described by Markowitz (10). A postsurgical period of not less than 3 weeks elapsed prior to investigational use of each animal. All animals were maintained on a standard laboratory diet, and housed in temperature-, humidity-, and, light-controlled quarters.

Intestinal Instillation—Solutions for intestinal instillation contained either 75, 150, 300, or 450 mg. of *N*-acetyl-*p*-aminophenol (SKF ISO No. 141819) in 25 ml. of phosphate buffer at pH 5.8. Following anesthetization (sodium pentobarbital, 35 mg./Kg., intravenously) of the test animal, previously fasted for 18–24 hr., the fistula was washed with normal saline solution, warmed to 37° to remove any secretion or particulate matter. Washing was continued until the effluate was clear. Thirty minutes after termination of washing, clamped polyethylene tubing was inserted 2 cm. into each end of the fistula. One clamp was opened and a 25-ml. volume of *N*-acetyl-*p*-aminophenol buffered solution, warmed to 37°, was introduced into the fistula. The tubing was again clamped. Care was taken to insure that none of the instilled fluid was lost through leakage. Venous blood samples were obtained prior to and 6, 12, 18, 24, 30, 60, and 120 min. after instillation, and analyzed for total *N*-acetyl-*p*-aminophenol in the plasma.

Oral Administration—Gelatin capsules, containing either 75, 150, 300, or 450 mg. of *N*-acetyl-*p*-aminophenol, were administered orally. Immediately after swallowing the capsule, the test animal was anesthetized. Blood samples were obtained prior to and 15, 30, 60, 120, 180, 240, 300, and 360 min. after oral administration and analyzed for total *N*-acetyl-*p*-aminophenol in the plasma.

Intravenous Administration—Solutions for intravenous administration contained either 75, 150, 300, or 450 mg. of *N*-acetyl-*p*-aminophenol in 25 ml. of 0.9% saline. After anesthetization of the test animal, 25 ml. of the solution, previously warmed to 37°, was injected over a period of 30 sec. Blood samples for analysis were obtained prior to and 15, 30, 60, 120, 180, and 240 min. after injection.

Analytical Procedures—The blood plasma volume of each animal was determined by means of an isotope dilution technique using radio-iodinated (^{131}I) serum albumin (11). A modification (12) of the method of Brodie and Axelrod (13) was employed in assaying the total *N*-acetyl-*p*-aminophenol content of plasma samples.

RESULTS AND DISCUSSION

A critical consideration in the laboratory evaluation of new drugs and new dosage formulations intended for oral administration is the availability of feasible and relevant methodology with which to assess gastrointestinal absorption. Although various *in vitro* and *in vivo* techniques have contributed fundamentally to an understanding of the physicochemical principles involved in the absorption process, the applicability, and predictive value of current methodology in this field leaves much to be desired. Theoretical considerations led us to explore the Thiry-Vella dog as a potential pharmacometric system and, in our opinion, this research has provided support for the utility of the fistula preparation as a biologic model for the evaluation of drug absorption from the intestinal mucosa.

One of the technical problems requiring resolution was the method of administration of drug solution into the *in situ* intestinal loop. Constant rate perfusion of drug solution through the Thiry-Vella loop was found to produce swelling and distention of tissue which did not occur following introduction of relatively small fixed volumes into the isolated intestinal segment. Furthermore, pressure exerted on the intestinal wall, as occurs during perfusion, may artificially facilitate drug absorption. Comparative studies indicated that maintenance of the physiological integrity of the intestinal mucosa could best be achieved by the instillation of fixed volumes of solution.

Selection of *N*-acetyl-*p*-aminophenol as a tool for studying the feasibility of the Thiry-Vella dog as a biologic model for evaluation of drug absorption was based primarily on two considerations: availability of a quantitative procedure for the determination of *N*-acetyl-*p*-aminophenol in biological fluids and the predicted physical state of the compound at the pH of the mucosal segment. The pH of the jejunum in the dog has been reported (2) to range from pH 4 to 7.6. Under the conditions of this study, the pH of the Thiry-Vella jejunal loop was found to be pH 5.3 to 6.3. *N*-Acetyl-*p*-aminophenol has a pKa in excess of 12 and therefore would not be appreciably ionized at pH 5.8, the pH selected for the buffered solution of drug to be instilled in the Thiry-Vella loop. In view of the well-established concepts of the relative lipid solubility of the unionized moiety, it could be anticipated that the compound would be effectively absorbed from the jejunal loop at pH 5.8.

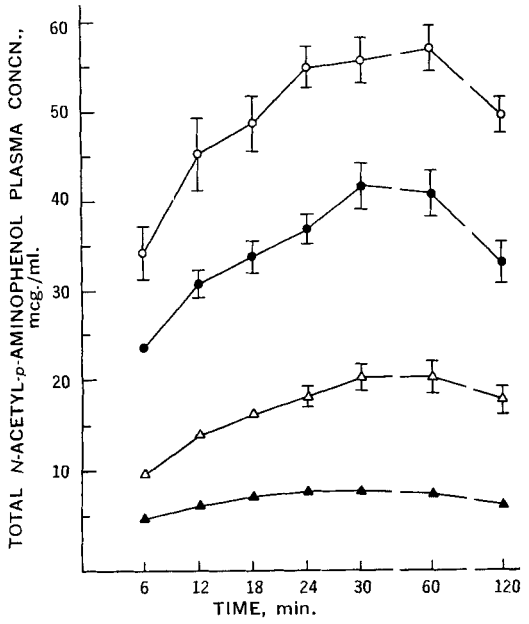

Fig. 1—Total N-*acetyl*-p-*aminophenol plasma concentration (mcg./ml.) in Thiry-Vella dogs following intestinal instillation of designated doses. Key:* ▲, *75 mg.;* △, *150 mg.;* ●, *300 mg.;* ○, *450 mg. in 25 ml. of phosphate buffer (pH 5.8). Each point represents the mean (± S.E.) response of 2 trials in each of 4 dogs. Where not indicated, the S.E. values are equivalent to less than 2 mcg./ml.*

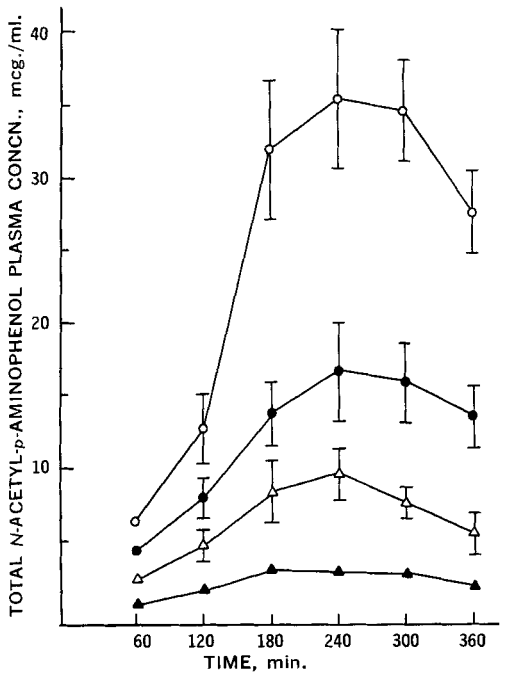

Fig. 2—Total N-*acetyl*-p-*aminophenol plasma concentration (mcg./ml.) in dogs without a Thiry-Vella fistula following oral administration of designated doses. Key:* ▲, *75 mg.;* △, *150 mg.;* ●, *300 mg.;* ○, *450 mg. Each point represents the mean (± S.E.) response of 2 trials in each of 4 dogs. Where not indicated, the S.E. values are equivalent to less than 2 mcg./ml.*

Since absorption was estimated as a function of the concentration of drug in the plasma, and since the blood plasma volume percent was essentially identical (4.35 ± 0.25%) in those animals prepared for this investigation, the experimental drug was administered on a mg./animal basis rather than on a mg./Kg. basis.

Following intestinal instillation of buffered solution of *N*-acetyl-*p*-aminophenol into the chronic Thiry-Vella fistula dog, absorption was initiated within the first 6 min., and the plasma concentration reached a peak within 30–60 min. Graphical representation of the data (Fig. 1) shows that within the dosage range examined, an increase in dose was reflected by an increase in the concentrations present in the plasma commensurate with the dose increments, and that the curves representing the plasma concentrations at the various posttreatment time periods for doses of 75, 150, 300, and 450 mg. were parallel. Therefore, as the dose of the experimental drug was increased the amount absorbed into the blood was increased indicating that the model quantitatively reflects drug absorption from the intestinal mucosa.

The apparent differences in the resulting plasma concentrations of *N*-acetyl-*p*-aminophenol among the various dosage levels were established by analysis of variance as being statistically significant (P = 0.01–0.001). Statistical analysis also indicated no lack of parallelism among the time-response curves.

Analysis of the plasma samples following intravenous injection revealed that the rate of disappearance of *N*-acetyl-*p*-aminophenol from the blood was relatively slow. A comparison of the plasma concentrations following intestinal instillation and those occurring subsequent to intravenous injection of identical doses indicated that, for the dosage range examined, from 80 to 98% of the drug introduced into the intestinal fistula was absorbed.

The characteristics of the Thiry-Vella fistula suggested that drug absorption from this relatively standardized mucosal segment would be subject to fewer variables than encountered upon exposure of the drug to the entire gastrointestinal tract. Therefore, it was of interest to compare absorption following instillation of the experimental drug into the *in situ* loop with that occurring after oral administration. Plasma levels subsequent to the oral administration of *N*-acetyl-*p*-aminophenol to dogs without a Thiry-Vella fistula evidenced (Fig. 2) substantially greater variability than those observed after instillation of identical doses of drug into the intestinal loop.

The applicability of the Thiry-Vella dog to the study of the kinetics of drug absorption is apparent. Following instillation, simultaneous sampling of the blood and the contents of the intestinal loop may be performed to determine the extent of retention, if any, of test agent in the intestinal compartment. Further, the drug may be injected intravenously and periodic samplings taken subsequently from the *in situ* loop. Thus, by determining the concentration of drug in the blood, that which remains in the loop, and that which may be transferred from the blood into the intestinal loop, it may be possible to

establish the kinetics of the absorption phenomena in this biologic model.

Although major abdominal surgery is required, the chronicity of the fistula dog permits each animal to serve as its own control. The same animal can be used repeatedly to compare the absorption characteristics of the same drug at different dosage levels, as well as different drugs at the same and alternate dosage levels. It may also be possible to evaluate absorption from various dosage formulations such as solutions, suspensions, single dose, and sustained-release solid dosage forms. Investigations of the interactions of drug combinations with regard to intestinal absorption are also feasible because the animal is serving as its own control. This is in contrast to other techniques, such as the ligated rat intestine, where the viability of the isolated segment is in question after a relatively short period of time.

Furthermore, the animal and *in situ* loop may be considered essentially identical for each experiment. The uniformity of the absorbing surface (*i.e.*, the loop) does not vary in a given animal as contrasted with the use of different animals. The mucosal epithelium, the blood, lymph, and nerve supplies remain essentially constant from one experiment to another.

The absorbing surface of the intestinal mucosa of the dog is closely related to that of man. Thus, preliminary investigations using Thiry-Vella dogs can provide data from which at least semi-quantitative predictions may be made of the absorption of a compound in man.

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Keyphrases

Drug absorption—intestinal mucosa
Thiry-Vella fistula dog—biologic model
Jejunal loop—Thiry-Vella fistula
N-Acetyl-*p*-aminophenol—absorption
Plasma-*N*-acetyl-*p*-aminophenol—analysis

Factors Affecting the Dissolution Rate of Medicaments from Tablets I

In Vitro Dissolution Rate of Commercial Phenobarbital Tablets

By JAMES T. JACOB* and ELMER M. PLEIN

An *in vitro* dissolution test method is described and 42 lots of commercial phenobarbital tablets produced by 24 manufacturers were evaluated by means of the test. Thirteen lots of tablets showed incomplete dissolution within 30 min., six of which failed to release 100 percent of the drug in 1 hr. The dissolution rate data are compared with those obtained by the USP XVII tablet disintegration procedure. No correlation existed between the two tests.

THE IMPORTANCE of determining physiological availability of medicaments from tablets was recognized only recently. Even though the USP tablet disintegration test is not designed to show a measure of physiological availability of medicament, this test is used by manufacturers, as well as drug control agencies, as one of the criteria in determining the quality of tablets. There is evidence in the literature that this test leaves much to be desired as a control test in the production of tablets (1–3).

Nelson (4) reported that dissolution rate was the rate-determining factor in absorption of

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