it attained 90% avoidance of unconditioned stimulus on that day or until it had received 200 trials. Rats failing to reach the criterion were discarded. Those attaining 90% avoidance were dosed with drugs (randomly selected) the following day.

Drug Session—The day following attainment of criterion the rat was injected with a saline solution of a drug intraperitoneally and immediately placed in the shuttlebox. A series of 140 trials was given, and the data (escape or avoidance and reaction time) were automatically recorded. With the exception of extremely unruly subjects, the rats were completely isolated during the entire drug session. After the drug day, the rats were discarded.

Some animals died during the drug sessions with mescaline hydrochloride at 25 mg./kg. (after Trials 77, 87, and 97) and at 100 mg./kg. (after Trials 60, 61, and 68), and with 3,4-DMA hydrochloride at 12.5 mg./kg. (after Trial 53) and at 50 mg./kg. (after Trials 66, 67, and 73). The animals were not considered in the computation of mean reaction times after death.

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COMMUNICATIONS

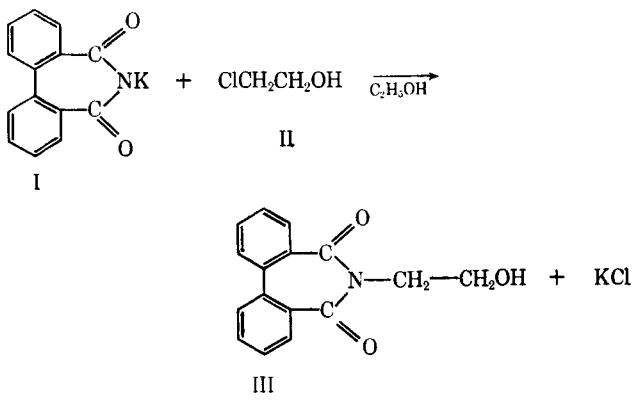
Preparation of Ethyl Diphenamate in Ethanol Using Potassium Diphenimide and 2-Chloroethanol

Keyphrases ☐ Ethyl diphenamate—synthesis ☐ *N*-(2-Hydroxyethyl)-diphenimide synthesis—literature correction ☐ NMR spectroscopy—structure

Sir:

The preparation of *N*-(2-hydroxyethyl)-diphenimide (III, Scheme I) was reported in 1952 by Demers and Jenkins (1). The approach taken in preparing this compound involved the reaction of potassium diphenimide (I) with 2-chloroethanol (II) in ethanol (1). Since this route of synthesis had been used successfully by Moore and Rapala (2) in the preparation of a series of dialkylaminoalkyl phthalimide derivatives, it appeared unlikely that a product other than that reported (III) would be obtained. Elemental nitrogen analysis of Compound III reported by these workers was well within experimental error.

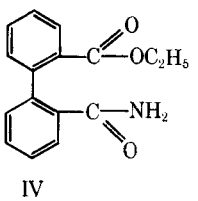
In 1963, Jenkins *et al.* (3) reported that during the attempted synthesis of a series of dialkylaminoalkyl diphenimide hydrochloride salts, using a similar procedure to that reported by Demers and Jenkins (1), the corresponding hydrochloride salts of ethyl diphenamate were formed rather than the diphenimide derivatives. The similar chemical nature of the alkylating species in these reactions prompted us to repeat



Scheme I

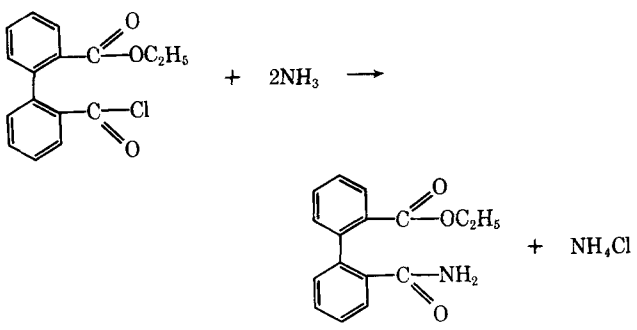
the work of Demers and Jenkins to determine if Compound III had actually been obtained.

The results of our work, which we report at this time, shows that Demers and Jenkins did not prepare *N*-(2-hydroxyethyl)-diphenimide (III) as reported but instead obtained ethyl diphenamate (IV).



IV

Identification of Compound IV was established as follows: (*a*) an NMR spectrum showed peaks at 6.83–8.00 δ (m, 8, Ar), 6.08 δ (b.s., NH_2), 4.09 δ (q, CH_2),



Scheme II

and 1.02 δ (t, CH_3); and (*b*) a mixed melting-point determination of the product prepared by the method of Demers and Jenkins (1) with a sample of ethyl diphenamate prepared by reacting ethyl diphenoyl chloride with ammonia showed no depression, and single melting-point determinations and NMR spectra were identical within experimental error.

Ethyl Diphenamate (IV)—(*a*) This was prepared following the procedure of Demers and Jenkins (1), m.p. 93° (lit 93°).

Anal.—Calcd. for N[1]: 5.20. Found: 5.19.

(*b*) Items prepared by reacting ethyl diphenoyl chloride with ammonia as follows. Approximately 74 mmoles of ethyl diphenoyl chloride was prepared in a manner reported by Demers and Jenkins (1), except that the final product was not isolated following the 8-hr. reflux period because of its hygroscopic nature. Instead the solvent was first removed *in vacuo*, and three successive 10-ml. portions of benzene were then added to the residue and each stripped off in a like manner to remove unreacted thionyl chloride, solvent, and HCl. The residue was dissolved in 200 ml. of anhydrous ether and cooled in a dry ice bath. Ammonia was then bubbled slowly into the mixture for several minutes (Scheme II). A white precipitate (NH_4Cl and ethyl diphenamate) formed immediately and was collected. The solid was triturated with water to remove the NH_4Cl. The remaining solid (ethyl diphenamate) was collected and recrystallized from alcohol and water. Yield 95%, m.p. 89–90°, NMR spectrum identical to that obtained in (*a*).

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[1] Nitrogen determination by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Evidence for Capacity-Limited Biotransformation of Sulfanilamide

Keyphrases ☐ Sulfanilamide—capacity-limited biotransformation ☐ Biotransformation, sulfanilamide—rate limited ☐ Dose dependence—sulfanilamide half-life

Sir:

Blood as well as lymph concentrations of various agents were determined following intravenous administration in recent studies concerned with the influence of plasma protein binding on rate of drug distribution to the body (1). Among these agents were several sulfonamides. The data obtained with sulfanilamide appeared to indicate that the fraction of unmetabolized drug increased with increasing dose. Subsequent to the recently presented evidence that the biotransformation of salicylate, in the analgesic dose range usually used in man, is rate limited (2), a significant number of other agents were shown also to be metabolized in man and animals by various enzyme systems which are capacity limited (3). Since clear evidence for the capacity-limited nature of acetylation has been obtained only for *p*-aminobenzoic acid (4), we carried out additional studies to determine whether the biotransformation of low doses of sulfanilamide was, indeed, a rate-limited phenomenon.

These studies were carried out using nonfasted male rats of the Sprague-Dawley strain, weighing between 250 and 300 g. Sulfanilamide was injected into the femoral vein in doses of 2.5, 5.0, or 10.0 mg. Blood was sampled by cardiac puncture at 12, 18, 30, 42, and, in two instances, 5 min. after administration. Since lymph was also collected in some of the experiments, the animals were kept under light ether anesthesia. How-

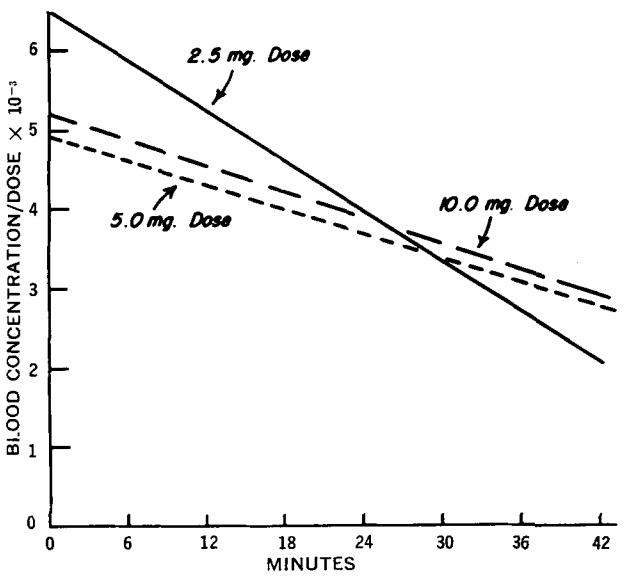

Figure 1—*Comparison of blood levels of unchanged drug after intravenous administration of several doses of sulfanilamide. Straight lines are regression lines calculated from the individual values determined at each time level (same number of animals as indicated in Table I).*

Table I—Effect of Dose on Percent of Total Sulfanilamide Present in Blood as Unmetabolized Drug

Minutes	Percent Unmetabolized[a] Dose, mg. 2.5	5.0	10.0
5	72.7 (2)	—	—
12	61.5 ± 12.3 (4)	81.3 ± 4.3 (4)	85.6 ± 3.8 (3)
18	65.8 ± 15.6 (4)	78.4 ± 2.8 (4)	87.1 ± 2.4 (3)
30	55.6 ± 16.9 (6)	72.6 ± 12.4 (4)	85.3 ± 4.5 (3)
42	56.5 ± 11.6 (7)	68.9 ± 11.1 (4)	79.0 ± 4.3 (5)

[a] Mean ± *SD*. Numbers in parentheses indicate number of animals.

ever, several experiments carried out in unanesthetized animals verified the blood level data collected in the anesthetized rats. The volume of all but the final sample of blood was 0.2 ml. The final sample, taken at 42 min., was sufficiently large to permit the determination of the hematocrit as well as the drug content in whole blood, plasma, and red cells. These data were then used to calculate the plasma concentrations for the earlier blood samples from the same animal. The distribution of sulfanilamide between red blood cells and plasma was also determined in another group of animals at 12, 18, and 30 min. after intravenous administration. For all samples the amounts of free (unmetabolized) and total (unmetabolized plus metabolized) sulfanilamide were determined by the method of Bratton and Marshall (5).

The results of the studies of distribution between plasma and red blood cells indicated that the interpretation of the data would be the same whether plasma concentrations or whole blood levels were used. The analyses of the data, therefore, were carried out using the actual values obtained for blood rather than the calculated plasma values. These analyses clearly indicate that the biotransformation of sulfanilamide has the characteristics of a capacity-limited process (3).

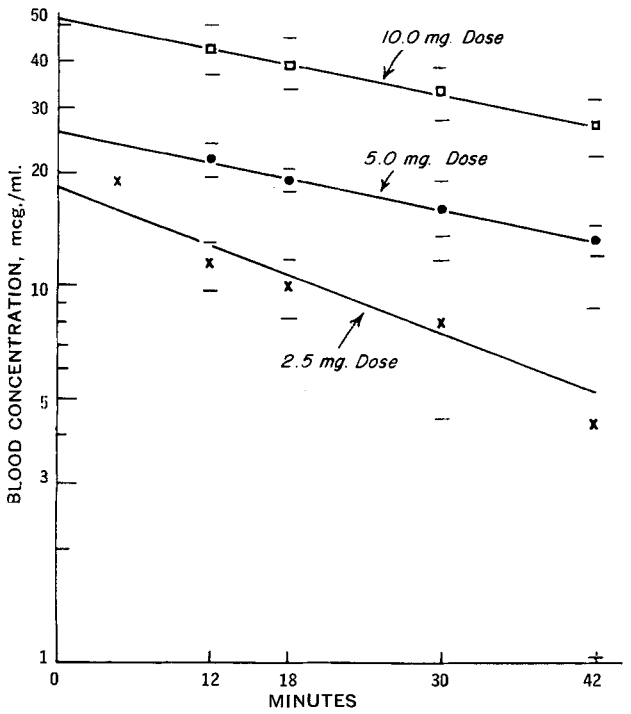

Figure 2—*Semilogarithmic plots of blood levels of unchanged drug after intravenous administration of several doses of sulfanilamide. Straight lines are regression lines calculated from the individual values;* ×, ●, *and* □ *are means at each time for doses of 2.5, 5.0, and 10.0 mg., respectively. Bars surrounding points represent standard deviations (same number of animals at each point as indicated in Table I).*

First of all, the percentage of the unchanged drug in the blood increases with dose (Table I). When the regression functions of the individual values of percent free sulfanilamide on time were estimated and tested for the three doses, it was found that the regressions were linear only for the 5.0 and 10.0-mg. doses and that the negative slopes of only these two regression lines were significantly different from zero. This indicates that above a dose of 2.5 mg., the rate of metabolism increases relatively with time. Thus, the fractional composition of sulfanilamide in blood is dependent on dose—a characteristic that is a good index of deviation from apparent first-order kinetics.

Second, deviation from first-order kinetics also can be demonstrated when the data are treated as in Fig. 1. For first-order kinetics the principle of superimposition should apply when blood concentration/dose is plotted against time (3). However, it can be readily seen that the regression line for the 2.5-mg. dose is not superimposable on the other two lines, and statistical analysis verified this conclusion. The slope of the line for the 2.5-mg. dose was found to be significantly different from those of 5.0 and 10.0 mg., and the slopes of the latter two lines were found to be insignificantly different from each other.

Finally, deviation from apparent first-order kinetics can be shown by the lack of linearity of semilogarithmic plots of blood concentration as a function of time (Fig. 2). Although each of the regression lines is insignificantly different from a straight line and the corresponding slopes are significantly different from zero, the slope of the line for the 2.5-mg. dose is again significantly different from those for the other two doses. Therefore, the drug half-life becomes dose dependent at a dose between 2.5 and 5.0 mg. Alinearity of the plots for the 5.0 and 10.0-mg. doses would be demonstrable only if the time of sampling was extended beyond 42 min.

The demonstration that a capacity-limited system for acetylation of sulfanilamide exists in the rat is important for drug metabolism studies in this species but may not be necessarily relevant to man. However, the saturation phenomenon occurred at doses in the rat that, when extrapolated to man, lie well within the therapeutic dose range. Moreover, Levy (3) pointed out that several capacity-limited drug-metabolizing systems observed in man have been demonstrated also in rats. These include a limited capacity for the conjugation of salicylate (6) and benzoate (3) with glycine and for sulfate and glucuronide conjugation with *N*-acetyl-*p*-aminophenol (7). Whether capacity-limited acetylation of sulfanilamide can be added to the list of similarities between man and the rat has to await additional evidence. Certainly it becomes increasingly apparent that drug metabolism studies must be carried out at multiple-dose levels and that linearity of the semilogarithmic plots of drug elimination as a function of time is frequently insufficient to discriminate between dose-independent or dose-dependent biotransformations.

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Apparent Dose-Dependent Elimination Kinetics as an Experimental Artifact

Keyphrases ☐ Dose-dependent elimination kinetics, apparent—experimental artifact consideration ☐ Plasma concentration—experimental blank errors

Sir:

It is now known that the kinetics of elimination of several drugs are dose dependent. The decline in the plasma concentrations of some drugs is exponential throughout, but the apparent first-order rate constant for this process descreases with increasing dose (1). The elimination of other drugs involves one or more saturable processes, and semilogarithmic plots of plasma concentrations as a function of time curve downward until they attain an exponential phase which is reached at the same concentration irrespective of the dose (2). In view of the great interest and investigative activity in the area of dose-dependent pharmacokinetics, it is appropriate to point out that errors in blank corrections can artifactually lead to the conclusion that an entirely linear, dose-independent system is in fact dose dependent and nonlinear.

Figure 1 shows hypothetical plasma concentrations obtained after intravenous injection of 1, 3, and 10 weight units of a drug which is actually eliminated by apparent first-order kinetics ($t_{1/2}$ = 2.0 hr.), with the plasma concentration data describable by means of a one-compartment open model. However, the data points in the figure are, in each case, 2 mg./l. lower than the "real" concentrations. This would be so if, for a number of possible reasons, a 2 mg./l. error in the blank value determination would have occurred. The data points thus obtained can be fitted readily to straight lines which yield a decreasing half-life with increasing dose. In the example shown, there is an almost 50% change in the apparent half-life.

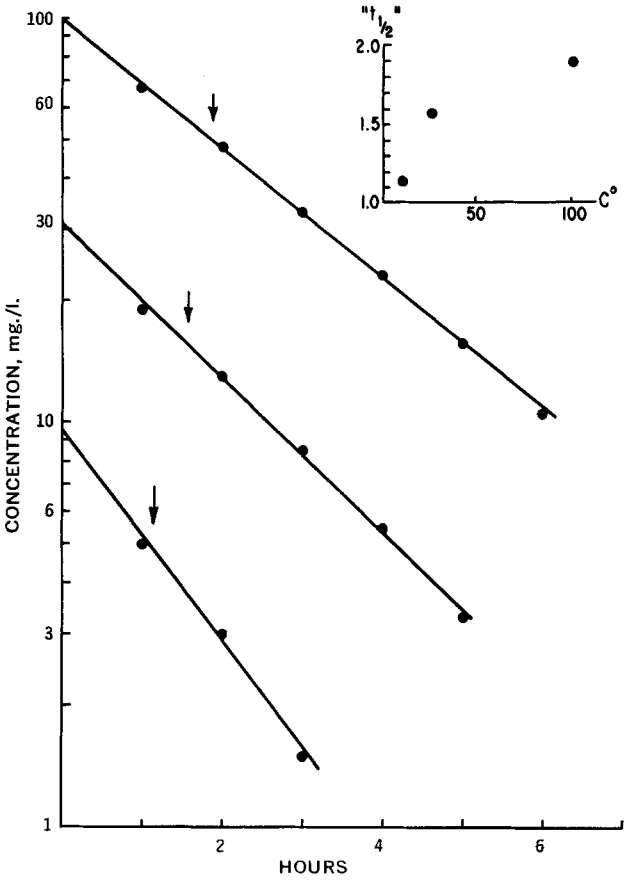

Figure 1—*Hypothetical plasma concentration data obtained after intravenous injection of 1, 3, and 10 weight units of a drug which is eliminated by apparent first-order kinetics* ($t_{1/2}$ = 2 hr.), *if the blank correction is 2 mg./l. too large. Arrows indicate apparent half-life. Inset: Relationship between initial plasma concentration* (C_0) *and apparent half-life* ("$t_{1/2}$").

If plasma concentrations are determined over a wide concentration range, an error in the blank correction can lead to the erroneous conclusion that elimination involves a combination of parallel linear and saturable (*i.e.*, capacity-limited) processes. Such systems may show an initial exponential concentration decline phase at high concentrations, a subsequent downward curvature, and finally another exponential phase which is steeper than the initial exponential phase (2). This pattern is evident in Fig. 2; the figure shows hypothetical plasma concentration data at two doses (differing 10-fold) of a drug, which is actually eliminated by apparent first-order kinetics but where an error of 3 mg./l. in the blank correction causes appreciable deviations from linearity. In this example, parallel straight lines may be fitted erroneously to the two sets of terminal data points, suggesting that the elimination kinetics above a plasma concentration of about 10 mg./l. are capacity limited.

The potential artifacts outlined in this article necessitate that considerable attention be directed to the correct determination of blank values. The magnitude