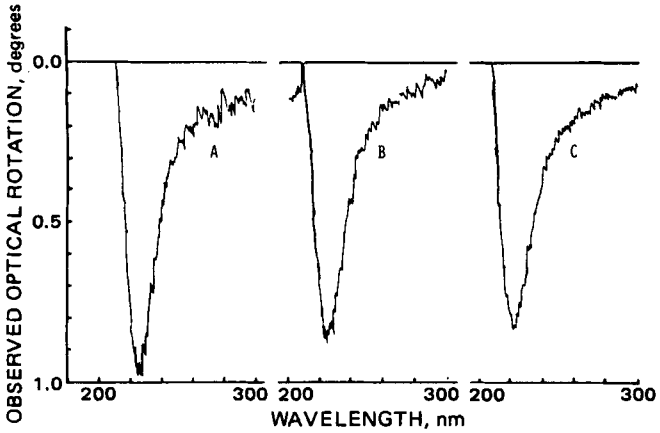

Figure 1—*Optical rotary dispersion curves for authentic D-leucine (A), authentic D-leucine recovered from cation exchange column (B), and enzymatically produced D-leucine after purification by cation exchange column chromatography (C).*

the radioactivity detected by autoradiography. The total resolution time including the ion exchange purification step was 40 min.

A nonradioactive resolution was analysed by optical rotary dispersion polarimetry in the range of 300–200 nm (Fig. 1). The optical rotary dispersion curve (Curve C) of the resolved D-leucine gave an optical rotation at 224 nm practically identical to that (Curve B) obtained by starting with half the amount of authentic D-leucine relative to the weight of DL-leucine used in the resolution and submitting it to the same ion exchange and reconstitution treatment applied to the resolved product.

The oxidative deamination of L-leucine in DL-leucine to the corresponding 4-methyl-2-oxopentanoic acid also was confirmed in the developmental work using nonradioactive DL-leucine by reacting aliquots of the reaction mixture with 2,4-dinitrophenylhydrazine yielding the 2,4-dinitrophenylhydrazone derivative which was monitored colorimetrically (440 nm) against standard 2,4-dinitrophenylhydrazone solutions. The keto acid yield was 84%. It was shown in this keto acid assay that the amino acid does not interfere with the analysis for the keto acid, making it unnecessary to separate the keto acid from the D-leucine.

Advantages of the oxidative deamination method for resolution are that it requires simple materials, is easily adaptable to hot cell conditions, and produces each of the enantiomers depending on the amino acid oxidase used.

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ACKNOWLEDGMENTS

Financial support for this research was supplied by Grant No. 1 ROM GM 27962-01 from the National Institute of General Medical Sciences.

The authors thank Dr. Earl Rees of the Department of Medicine, College of Medicine for the use of and assistance in the operation of the JASCO-ORD/UV-5 spectropolarimeter used in the optical activity determinations.

Correction of Perfusate Concentration for Sample Removal

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Received August 17, 1981, from the *College of Pharmacy, Washington State University, Pullman, WA 99164-6510.* Accepted for publication October 13, 1981.

Abstract □ Repeated sampling of a drug solution that is recirculated through a perfused body increases the rate of drug disappearance from the perfusate. When the volume of the drug solution (V_T) is maintained constant by addition of drug-free perfusate after sampling, the measured drug concentration (C_i) can be corrected for drug removed in previous samples by using the equation $C_i' = C_i V_T C_{i-1}'/(V_T - V_S)C_{i-1}$, where C_i' is the corrected drug concentration in the ith sample, V_S is the volume of the sample, and $C_1' = C_1$. An error in any particular C_i is not transmitted to a subsequent C_i' value. The method can be used when the time interval between samples and when V_S vary from sample to sample, but return of the drug from the perfused body to the perfusate after sampling may cause C_i' to be overestimated.

Keyphrases □ Perfusates—correction of perfusate concentration for sample removal □ Drug concentration—correction of perfusate concentration for sample removal □ Correction methods—perfusate concentrations for sample removal

Several experimental techniques involve the perfusion of a tissue, an organ, or an entire organism with a drug solution (perfusate); *e.g.*, muscle (1), kidney (2), placenta (3), liver (4), intestine (5), and fish (6). Samples of the perfusate are periodically removed for determination of drug concentration. One experimental approach involves sampling the perfusate after it is passed once through the perfused body. An alternative approach is to recirculate the perfusate, usually by pumping it from a reservoir, through the perfused body, and back to the reservoir. Using the once-through approach, correction for sampling is unnecessary. When samples are removed repeatedly from recirculated perfusate, however, the concentration of drug is reduced, as a result of sample removal, in all samples but the first. Sample removal thereby biases the concentration–time relationship and confounds a kinetic analysis of the data.

If the ratio of the total sample volume to the perfusate volume is small, the bias is small and may be ignored. This ratio can be reduced by reducing the sample volume, reducing the number of samples, or increasing the volume

0022-3549/82/0700-0820$01.00/0

of the perfusate. Reduction of the sample volume is limited by the sensitivity of the drug assay. Reducing the number of samples and increasing the perfusate volume are not good strategies as either one tends to obscure the kinetic behavior of the drug. In most cases the optimum approach is to remove a relatively large number of samples and to maintain the volume of the perfusate in the reservoir as small as is conveniently possible. To do this, the sampling-induced bias in the concentration–time data must be removed.

Two techniques for correction of perfusate concentration for sample removal have been described (4, 7). Both techniques require the assumption of a particular kinetic model before the correction can be made. The present report describes a relatively simple technique for correction of measured concentrations for previously removed samples. The correction is exact if no back transfer of drug occurs from the body to the perfusate after sampling. In many cases when back transfer does occur, the method introduces an acceptable error and may still be used.

EXPERIMENTAL

The perfusion system was the perfused body, an external reservoir, and a pump that recirculated perfusate between the reservoir and perfused body. Samples of perfusate were removed periodically from the reservoir and the concentration of drug in each perfusate sample was determined. The volume of the perfusate was maintained constant by addition of drug-free perfusate to the reservoir after a sample was removed. Because the sampling process removes drug from the perfusate, the concentration of drug in the sampled perfusate declined more rapidly than it would have if sampling had not occurred. If the perfused body eliminated drug from the perfusate by a zero-order process, the measured drug concentration would be corrected by adding the total amount of drug removed in previous samples divided by the volume of the perfusate.

Usually, however, the drug elimination rate is proportional to the drug concentration in the perfusate. In this case it is not correct to add the amount removed in samples to subsequently determined concentrations, since part of the drug removed as a sample would have been consumed if the sample had not been removed. The problem then is to determine how much of the drug that was removed in each sample to add to each subsequently measured concentration.

Assume that the drug concentration in the perfusate declines exponentially, and let C_i represent the measured drug concentration in the ith sample. The concentration of drug in the reservoir after removal of the first sample and addition of drug-free perfusate would be $C_1(V_T - V_S)/V_T$, where V_T and V_S are the volumes of the perfusate and the sample, respectively. The measured concentration of drug in the perfusate at the time that the second sample was removed can be corrected for drug removed in the first sample as follows:

$$C'_2 = C_2 V_T/(V_T - V_S) \qquad \text{(Eq. 1)}$$

C'_2 is the corrected drug concentration, *i.e.*, the concentration that would have been measured if the first sample had not been removed. In making the correction, it was assumed that the concentration of drug in the sample declined at the same rate as did the concentration of drug in the perfusate between the first and second samples. The measured concentration in the third sample can be corrected for drug removed in the first two samples:

$$C'_3 = C_3\left(\frac{V_T}{V_T - V_S}\right)\left(\frac{C'_2}{C_2}\right) \qquad \text{(Eq. 2)}$$

The term $V_T/(V_T - V_S)$ corrects for drug removed in the second sample, and the term C'_2/C_2 corrects for drug removed in the first sample. Correction of the fourth and subsequent samples is similar to the correction for the third sample:

$$C'_n = C_n\left(\frac{V_T}{V_T - V_S}\right)\left(\frac{C'_{n-1}}{C_{n-1}}\right) \qquad \text{(Eq. 3)}$$

RESULTS AND DISCUSSION

The validity of the method was explored by using it to correct calculated drug concentrations, assuming some probable cases for drug disappearance kinetics. The proposed method was exact when drug was eliminated monoexponentially from the perfusate by apparent first-order kinetics. The method permits flexibility in that the time interval between samples and the volume of each sample can be varied from sample to sample. In addition, an error in the concentration determined for a particular sample is not transmitted by the method to subsequent samples. When the drug concentration was assumed to decline according to a biexponential equation, the correction was also exact. However, a biexponential concentration–time relationship usually indicates that drug distributes between the perfused body and the perfusate. Simulations using the model described previously (7), a two-compartment model with drug distribution between perfusate and perfused body followed by drug elimination from the perfused body, showed that the method is not exact when drug returns from the perfused body as a result of an instantaneous decrease in the drug concentration in the perfusate. The corrected concentration in the sampled perfusate was higher than the corresponding concentration in unsampled perfusate from the third to the final sample. The percentage error increased with the sample number, and it depended on the amount of drug that returned to the perfusate after sampling, compared to the amount of drug in the perfusate. The error in the final corrected concentration increased with the number of samples, the sample volume, the apparent volume of distribution of the perfused body relative to the volume of perfusate, and as drug clearance from the perfused body increased relative to the reversible clearance between the perfusate and the perfused body. If the method is used when a distributive phase in the perfusate concentration–time data is apparent, simulation with the model described previously (7) is recommended to select a sampling regimen that does not introduce unacceptable error. In some cases the correction method described previously (7) may be required.

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ACKNOWLEDGMENTS

Supported in part by Grant GM 27924 from the National Institute of General Medical Sciences.