Table III—Recovery of Nitrofurantoin and Furazolidone Added to Rat Urine

Concentration in Urine, mcg./ml.		Number of Determinations	Recovery			
			Nitrofurantoin, mcg./ml.		Furazolidone, mcg./ml.	
Nitrofurantoin	Furazolidone		Mean ± *SD*	Mean %	Mean ± *SD*	Mean %
2.5	2.5	6	2.38 ± 0.39	95.2	2.53 ± 0.070	101.2
5.0	5.0	6	5.01 ± 0.18	100.2	5.11 ± 0.177	102.2
10.0	10.0	6	10.03 ± 0.37	100.3	10.05 ± 0.18	100.5
15.0	15.0	7	15.16 ± 0.60	101.0	15.06 ± 0.157	101.0

served points were a better fit to the theoretical regression and linearity.

The size of inoculum affected the growth, the form of regression, and the slope of regression in the assays with serum and urine. The optimal levels of inoculum for the assay of nitrofurantoin and furazolidone in rat and human serum and urine were 0.03 and 0.06 ml., respectively, of the standardized culture/100 ml. of the assay medium. Furthermore, the assays were influenced by the quantity of serum and urine added to the standard and the test sample tubes. Satisfactory assays were achieved when the same amount of serum and urine was present in the test and the standard. When the test samples are to be assayed at more than one level, the standard set for each level of serum or urine is to be preferred.

In turbidimetric assay, conventionally 10 ml. of the assay medium per tube is used. However, the low level of drug in serum *in vivo* and the activity of nitrofuran compounds often demand the use of larger amounts of biological fluid to fall in the linear range of the graph. In this assay, 5 ml. of the medium per tube was used, because it permits the use of a lesser volume of biological fluid for the estimation and improves sensitivity.

SUMMARY

A turbidimetric assay procedure for nitrofurantoin and its active congeners was developed using *S. faecalis*. Optimal conditions for the estimation were established using two nitrofurans (nitrofurantoin and furazolidone) in serum and urine. The method was found to be rapid and reliable for the estimation of small amounts of drug in biological fluids. The method can be equally useful and sensitive for other active congeners once the optimal conditions are determined.

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▲ To whom inquiries should be directed.

Effect of Benzene on Rat Liver Ribonucleic Acid

G. P. TRYFIATES* and N. H. CHOULIS†▲

Abstract □ The intraperitoneal administration of benzene to rats does not alter the sucrose density sedimentation behavior of liver ribonucleic acid but causes an increase of soluble ribonucleic acid species. Treatment of ribonucleic acid *in vitro* with benzene results in an irregular sucrose density pattern.

Keyphrases □ Benzene, intraperitoneal administration—effect on rat liver ribonucleic acid □ Ribonucleic acid, rat liver—effect of intraperitoneal administration of benzene on sucrose density sedimentation and soluble species □ Sucrose density patterns—effect of benzene on ribonucleic acid *in vitro*

Studies in this laboratory (1–3) showed that the intraperitoneal administration of benzene to rats: (*a*) causes disaggregation of liver polyribosomes which results in accumulation of ribosomal monomer-dimers and appearance of an intermediate, previously absent, peak; (*b*) affects significantly the capacity of liver polyribosomes to synthesize protein *in vitro* (>50% inhibition); and (*c*) inhibits considerably the incorporation of labeled ribonucleic acid precursors into liver polyribosomes but does not interfere with their incorporation into total liver ribonucleic acid and does not alter the size of the acid-soluble fraction radioactive label pool.

These observations suggested an action of benzene at the messenger ribonucleic acid–ribosome level which causes disorganization of the endoplasmic reticulum and breakdown of polyribosomes as is the case with liver poisons, in general (4–6). The possibility that benzene may also affect the ribonucleic acid of the ribosomes (ribosomal ribonucleic acid and r-ribonucleic

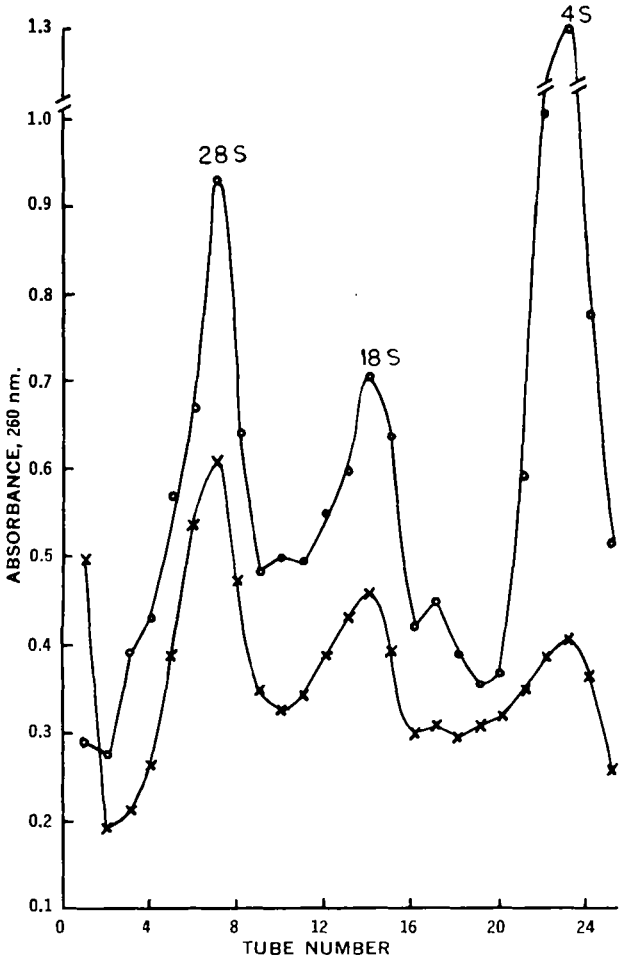

Figure 1—*Sucrose density gradient analysis of liver cytoplasmic ribonucleic acid of benzene-treated and untreated rats. Key: O--O, ribonucleic acid from benzene-treated animals; and ×—×, ribonucleic acid from untreated animals. All experiments were performed in triplicate.*

acid) became apparent from the results of preliminary tests which showed that when ^{14}C-labeled benzene was given to rats, radioactivity was found associated with ribonucleic acid isolated from purified liver cytoplasm. The effect of the aromatic on liver cytoplasmic ribonucleic acid was subsequently studied both *in vivo* and *in vitro*. The results are reported here.

METHODS

Ribonucleic acid was isolated from liver cytoplasm of Wistar strain male rats by phenol–sodium dodecyl sulfate extraction before or after treatment with benzene. Benzene[1] was given intraperitoneally to animals weighing approximately 250 g. at a dose of 3.37 mmoles/100 g. body weight. In brief, the liver homogenate from untreated rats or from animals treated with benzene for 20 min. was made up to 0.5% with sodium dodecyl sulfate, shaken, and extracted twice with an equal volume of water-saturated redistilled phenol. Following removal of phenol (by ether) and ether (by bubbling nitrogen), the aqueous phase was adjusted to 2% with potassium acetate, and the ribonucleic acid was precipitated by the addition of 2.5 volumes of cold 95% ethanol. After standing at −20° overnight, the precipitate was washed twice with 75% cold ethanol and dissolved in a minimum volume of 0.01 *M* acetate buffer, pH 5.1, containing 0.1 *M* NaCl and 1.5×10^{-3} *M* $MgCl_2$. Ribonucleic acid

[1] Fisher.

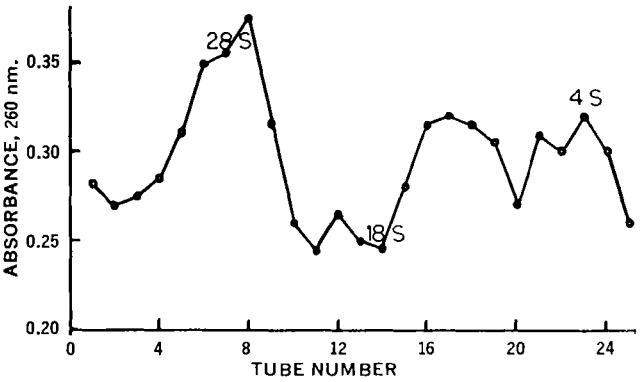

Figure 2—*Effect of benzene on cytoplasmic ribonucleic acid* in vitro. *All experiments were performed in triplicate.*

obtained in this manner had a 260/280 absorbance ratio of 2.05. A detailed account was made previously (7, 8). Cytoplasmic ribonucleic acid was subsequently analyzed by sedimentation on linear 5–20% sucrose density gradients at 4°. The gradients were made in 0.01 *M* acetate buffer, pH 5.1, containing 0.1 *M* NaCl, as described previously (7, 8).

To study the effect of benzene on ribonucleic acid *in vitro*, 1 mmole benzene was added to a solution containing 71 absorbance ribonucleic acid units (at 260 nm.), and the same sample was immediately subjected to centrifugation on linear sucrose gradients. The total volume of the sample containing the benzene was approximately 0.65 ml.

RESULTS AND DISCUSSION

The absorbance sedimentation pattern of liver cytoplasmic ribonucleic acid following centrifugation on sucrose density gradients is shown in Fig. 1. The figure shows representative results obtained from repeated experiments (three) which gave practically identical data. Ribonucleic acid prepared from untreated and/or benzene-treated animals was resolved into distinct 28 S, 18 S, and 4 S species. A high concentration of soluble ribonucleic acid species (4 S peak) accumulated following treatment with benzene.

Figure 2 shows the sucrose density sedimentation pattern of liver cytoplasmic ribonucleic acid following treatment with benzene *in vitro*. The figure shows a highly irregular and altered absorbance pattern (see Fig. 1). Treatment with benzene altered these macromolecules and perhaps degraded them to an extent as seen by the actual displacement of the absorbance to the lighter regions of the gradient.

The results presented here show that the intraperitoneal administration of benzene to rats did not alter the sucrose density gradient sedimentation behavior of cytoplasmic liver ribonucleic acid. Treatment, however, with the aromatic *in vivo* resulted in an increase of soluble (4 S) ribonucleic acid species. Moreover, an irregular and highly altered sedimentation pattern of ribonucleic acid was obtained following treatment of the sample with benzene *in vitro*. The absorbance appeared shifted toward the right of the sucrose gradient (light regions).

A precise interpretation of the effects of benzene on ribonucleic acid *in vivo* and *in vitro* is difficult to offer. A possible explanation may be that benzene interacts with helical ribonucleic acid regions in a hydrophobic fashion, causing conformational changes which affect the size of the ribonucleic acid species. Conceivably, this could cause the observed irregular ribonucleic acid sedimentation pattern. How the increase in the soluble ribonucleic acid species following the intraperitoneal administration of benzene is brought about is presently under investigation.

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▲ To whom inquiries should be directed.

Relationship between Dose, Effect, Time, and Biophasic Drug Levels

VICTOR F. SMOLEN*▲, RONALD G. BARILE†, and THEOPHANIS G. THEOPHANOUS†

Abstract □ A detailed basis for the use of experimentally determined dose–effect curves, in the manner of calibration curves, to transform observed intensities of pharmacological response into biophasic drug levels at all times following dosing by any route is presented. The principles are graphically exemplified through the construction of dose–response–time surfaces. The conditions of applicability of the described approach are also discussed.

Keyphrases □ Drug levels (biophasic), dose, effect, and time—relationship, equations □ Pharmacological response, intensity—basis for transforming into biophasic drug levels at any time by any route, equations □ Dose effect curves—used to transform pharmacological response intensity into biophasic drug levels, equations

The use of biophasic drug level–time profiles, obtained from the transformation of observed pharmacological data, for the purposes of biokinetic systems analysis (1, 2), drug absorption analysis (1–5), and determination of the time course of pharmacological effects from the results of optimized *in vitro* drug release testing (3), was described in previous reports. The validity of implementing the pharmacological method to a particular drug system can be confirmed from observed pharmacological data alone; therefore, this approach can be implemented with drugs for which assays for their detection in biological media are difficult or nonexistent. In contrast to the use of data derived from direct assays for the drug, another advantage of the pharmacological approach is that its applicability to the performance of drug absorption analysis is not limited to systemic routes of administration alone. Provided biophasic drug levels sufficient to induce detectable magnitudes of pharmacological effects are achieved, both the systemic and biophasic (1, 2) drug availability can be computed for any route by which the drug is administered.

The use of pharmacological data is based upon the implementation of an experimentally observed dose–effect curve to provide a relationship between experimentally observed intensities of pharmacological response, I (at any time following dosing by any route of administration), and the corresponding values of the quantities of drug in the biophase, Q_B. The justification for this procedure involves mathematical steps which were not made explicitly apparent in earlier reports. The purpose of the present article is to outline a more rigorous and detailed explication of this approach.

THEORETICAL

Experimental Data Required to Construct I–Q_B Relationship—The necessary data are constituted by observed I time, t, profiles for varying doses, D, of drug administered either intravenously or by any route by which the drug is known, *a priori*, to be absorbed either directly to the site(s) of action in the biophase or to the systemic circulation by apparent first-order processes[1].

Assumptions Implicit in Treatment and Confirmation—For the pharmacological method of biokinetic analysis to be applicable in its simplest, unmodified form, as described earlier (1–3), the pharmacokinetic processes of drug absorption[1], distribution, and elimination should be nonsaturable (*i.e.*, dose independent) and, therefore, describable by a linear compartment model. Therefore, following dosing, Q_B can be expressed in terms of a sum of exponential terms multiplied by D (as given by Eq. 1, where n is the number of terms, and A_i and m_i are equation parameters):

$$Q_B = D \sum_{i=1}^{n} A_i e^{-m_i t} \qquad \text{(Eq. 1)}$$

The confirmation of this condition is embodied in the congruency test and β-ratio test as previously described (1, 2).

The second condition which must be met for the accurate implementation of the unmodified approach requires that the intensity of pharmacological effect is instantaneously responsive to the quantity of drug in the biophase and is a nonhysteretic (single-valued) monotonic function of Q_B. The verity of the occurrence of this condition is indicated by a satisfactory conclusion to the $f(I)$ ratio test (1, 2).

Construction of Dose–Effect Curve—The dose–effect curve consists of a plot of the intensity of effect, I_r, (recorded consistently at any arbitrarily chosen time, t_r, following dosing), as a function of the dose. For both practical and theoretical reasons (1, 2), t_r is best chosen as the time, $t_{max.}$, corresponding to the maximum observed response. If the discussed conditions are realized, the value of $t_{max.}$ is constant and dose independent.

Relationship between I and Q_B at Any Time—The basis for the relationship of Q_B to I and its inverse becomes apparent from a consideration of the following equations. Consider:

$$I = g(Q_B) \qquad \text{(Eq. 2)}$$

[1] The necessity of this condition applies only to the route of administration employed for the construction of the dose effect curve. The pharmacological method of drug absorption analysis (1, 2) is otherwise independent of the kinetics and mechanisms of drug absorption and can be applied to their elucidation.