nificantly potentiated (80%) hexobarbital (100 mg/kg ip) sleeping time in albino mice (5); it markedly diminished (88%) amphetamine (20 mg/kg ip) toxicity in aggregated mice (6) and antagonized amphetamine- (10 mg/kg sc) induced stereotypy (continuous sniffing, biting, and compulsive gnawing) in albino rats (7). The alkaloid completely inhibited lysergide- (3 mg/kg sc) induced piloerection and tremors in mice (8).

Using rats and mice, the effects on the rotarod test, conditioned avoidance response, and induced aggressive behavior were examined. Gentianine markedly inhibited (80%) the ability of trained mice to remain on a rotating rod for a maximum time trial of 180 sec (9). The alkaloid selectively blocked the avoidance response to the conditioned stimulus (buzzer), without affecting the escape response to the unconditioned stimulus (electric shock), when tested on trained rats (10). However, in higher doses (50 mg/kg ip), there appeared to be an appreciable motor deficit as characterized by suppresssion of the escape response (40%) to the unconditioned stimulus in these animals. Gentianine inhibited (60%) foot-shock-induced fighting behavior in paired mice (11).

The effects of gentianine on morphine analgesia, anticonvulsant action of diphenylhydantoin, electroshock seizure, and pentylenetetrazol convulsion were also determined. Gentianine markedly potentiated (150%) the analgesic activity (12) of subanalgesic doses (2 mg/kg ip) of morphine (13) but had no analgesic activity *per se* at this dose (20 mg/kg). It significantly potentiated (60%) the anticonvulsant activity of a subanticonvulsant dose (2.5 mg/kg ip) of diphenylhydantoin but had no anticonvulsant activity *per se*. With higher doses (50 mg/kg ip), however, it showed noteworthy anticonvulsant activity (40%) as tested by the electroshock seizure method (14). In higher doses (50–100 mg/kg ip), gentianine offered significant protection (70%) against pentylenetetrazol- (70 mg/kg sc) induced convulsion (15).

The toxicity (16) and the LD_{50} of gentianine after intraperitoneal administration in albino rats were studied; the LD_{50} was calculated as 276 mg/kg. The drug appears to possess only a moderate to low order of toxicity as evidenced from the lack of any obvious toxicity on prolonged intraperitoneal administration, 20 mg/kg daily for 3 weeks.

Gentianine exhibited significant antipsychotic activity in the battery of tests accepted for arriving at such a conclusion (17). It has the added advantage of its minimal toxicity. The alkaloid, bearing a skeleton (lactonic monoterpene) different from those of known antipsychotic agents, is thus of potential importance as an antipsychotic drug.

(1) S. Ghosal, P. V. Sharma, R. K. Chaudhuri, and S. K. Bhattacharya, *J. Pharm. Sci.*, **62**, 926(1973).

(2) E. Steinegger and T. Weibel, quoted by W. C. Wildman, J. Le Men, and K. Wiesner, in "Cyclopentanoid Terpene Derivatives," W. I. Taylor and A. R. Battersby, Eds., Marcel Dekker, New York, N.Y., 1969, p. 274.

(3) A. K. Singh, M. Pharm. thesis, Banaras Hindu University, Varanasi-5, India, 1973, p. 61.

(4) R. A. Turnerin, "Screening Methods in Pharmacology," vol. 1, Academic, New York, N.Y., 1965, p. 26.

(5) W. L. Kuhn and E. F. Van Maanen, *J. Pharmacol. Exp. Ther.*, **134**, 60(1961).

(6) J. H. Mennear and A. D. Rudzik, *Life Sci.*, **5**, 349(1966).

(7) Y. L. Shchelkunov, *Farmacol. Toksicol.*, **27**, 628(1964).

(8) G. Chen and B. Bohner, *Arch. Int. Pharmacodyn. Ther.*, **125**, 1(1960).

(9) W. J. Kinnard and C. J. Carr, *J. Pharmacol. Exp. Ther.*, **121**, 354(1957).

(10) L. Cook and E. Weidley, *Ann. N.Y. Acad. Sci.*, **66**, 740(1957).

(11) R. E. Tedeschi, D. H. Tedeschi, A. Mucha, L. Cook, P. A. Mattis, and E. J. Fellows, *J. Pharmacol. Exp. Ther.*, **125**, 28(1959).

(12) O. L. Davies, J. Raventos, and A. L. Walpole, *Brit. J. Pharmacol.*, **1**, 255(1946).

(13) S. K. Bhattacharya, M. K. Raina, D. Banerjee, and N. C. Neogi, *Indian J. Exp. Biol.*, **9**, 257(1971).

(14) E. A. Swinyard, W. C. Brown, and W. K. Young, *J. Pharmacol. Exp. Ther.*, **106**, 319(1952).

(15) L. S. Goodman, M. S. Grewal, W. C. Brown, and E. A. Swinyard, *ibid.*, **108**, 168(1953).

(16) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57**, 261(1944).

(17) C. L. Zirkle and C. Kaiser, in "Medicinal Chemistry," vol. II, A. Burger, Ed., Wiley-Interscience, New York, N.Y., 1970, p. 1410.

S. K. Bhattacharya

Department of Pharmacology
Institute of Medical Sciences
Banaras Hindu University
Varanasi-5, India

S. Ghosal[x]
R. K. Chaudhuri
A. K. Singh
P. V. Sharma

Pharmaceutical Chemistry Research Laboratory
Department of Pharmaceutics
Banaras Hindu University
Varanasi-5, India

Received October 9, 1973.
Accepted for publication January 30, 1974.
[x] To whom inquiries should be directed.

Renal Contribution to Drug Biotransformation

Keyphrases □ Renal metabolism—contribution to drug biotransformation, true renal clearance values □ Drug biotransformation—renal contribution, assessment of determination of true renal clearance values □ Metabolism, drug—renal contribution to drug biotransformation, assessment of determination of true renal clearance values

To the Editor:

Wan and coworkers (1–4) recently described an imaginative pharmacokinetic approach for assessing the contribution of the kidneys to the biotransforma-

tion of drugs in the body. Their method is based on the measurement of *(a)* the true renal clearance of a drug metabolite at the steady state during intravenous infusion of that metabolite and *(b)* the apparent renal clearance of this metabolite at the steady state during intravenous infusion of its precursor. An increase in apparent renal clearance over the true renal clearance is used as a measure of the rate of metabolite formation by the kidneys.

In discussing the theoretical basis of their method, Wan and coworkers emphasized the need to monitor the true renal clearance of the metabolite during the entire experiment since it may be affected by a number of factors, particularly by possible competitive effects of the precursor (1). They, therefore, infused radioactive metabolite during all phases of their apparent renal clearance measurements so that any changes in true renal clearance could be recognized readily. Unfortunately, they measured only the rate of excretion of the radioactive metabolite and not its concentration in the plasma during infusion of the precursor. This precluded determination of true renal clearance.

It is essential to determine not only the rate of excretion but also the concentration in the plasma of radioactive metabolite before and during precursor administration when using the method of Wan and Riegelman (1) for assessing the contribution of the kidneys to the biotransformation of drugs in the body. Failure to do so in the studies reported to date (1–4) makes it advisable to consider the results as tentative, pending further investigations according to a more appropriate protocol.

These researchers (4) stated that if the rate of excretion of radioactive metabolite stays constant during the experiment (*i.e.*, while precursor is infused at various rates), then the true renal clearance of the metabolite is in fact constant. This reasoning is incorrect. If a metabolite is eliminated entirely by renal excretion (for example, salicyluric acid in humans as in the study described in Ref. 4), then the rate of excretion during constant infusion of this metabolite is equal to the rate of infusion (*i.e.*, rate in = rate out) at the steady state by definition. This is so regardless of the true renal clearance value. What is affected by a change in the true renal clearance is the steady-state concentration of radioactive metabolite in the plasma since this concentration is inversely proportional to the true renal clearance. Failure to determine the plasma concentration of radioactive metabolite during all phases of the experiments makes it impossible to determine whether the true renal clearance did, in fact, remain constant.

Wan and coworkers stated that, under conditions when renal clearance is constant, the observed excretion ratio of cold to labeled compounds should be the same as the infusion ratio of cold and labeled compounds. If the two infused species are not metabolized, then their steady-state excretion rate ratio must be the same as the infusion ratio (rate in = rate out) irrespective of any changes in the true renal clearance of one, the other, or both compounds. If the cold compound is biotransformed partially while the labeled compound is eliminated only by excretion, then the ratio of excretion rates at the steady state equals the infusion rate of cold compound times f divided by the infusion rate of the labeled compound, where f is the fraction of cold compound excreted as such. A constancy in the steady-state excretion rate ratio of the compounds under these conditions indicates only constancy in f. This could (but need not) reflect constancy in the renal clearance of unchanged drug, since a change in the rate constant of one of several parallel elimination pathways changes the quantitative ratio of the products of elimination. It definitely does not reflect a possible change in the renal clearance of a metabolite.

If a precursor is eliminated solely by biotransformation (as is practically the case with benzoic acid in the study described in Ref. 1), then a change in true renal clearance would have no effect on the ratio of excretion rates of labeled and unlabeled metabolite at the steady state because the rate in equals the rate out at the steady state. In any event, a change in the rate of infusion of the precursor obviously causes a change in the ratio of excretion rates, so that it is impossible to detect a precursor concentration-dependent change in the true renal clearance of the metabolite. As Wan and Riegelman (1) pointed out, such a change, if unrecognized, can lead to erroneous values for the rate of metabolite formation by the kidneys.

We wish to emphasize that we consider the method of Wan and Riegelman (1) as a potentially valuable research tool and that our critical comments are limited entirely to an aspect of its execution in the studies reported until now (1–4).

(1) S. H. Wan and S. Riegelman, *J. Pharm. Sci.*, **61**, 1279(1972).
(2) *Ibid.*, **61**, 1284(1972).
(3) S. H. Wan, B. Von Lehmann, and S. Riegelman, *J. Pharm. Sci.*, **61**, 1288(1972).
(4) B. Von Lehmann, S. H. Wan, and S. Riegelman, *ibid.*, **62**, 1483(1973).

Burde L. Kamath
Gerhard Levy [x]

Department of Pharmaceutics
School of Pharmacy
State University of New York at Buffalo
Buffalo, NY 14214

Received February 21, 1974.
Accepted for publication May 7, 1974.
Supported in part by Grant GM 20852-01 from the *Institute of* General Medical Sciences, National Institutes of Health, Bethesda, MD 20014

[x] To whom inquiries should be directed.