II a more potent cytotoxic agent. As the release of I begins to occur (Fig. 4 and Table I), its protective effect *via* binding and inhibition of adenosine deaminase is initiated. This occurs within 48 hr with the concurrent potentiation of the cytotoxic property of II. At 72 hr, inhibition by II is almost complete with remaining cells at 30% viability. At 96 hr, when the silicone polymer–I is present initially, inhibition by II is total. Although it was not possible to achieve total cell death when I was added directly (at concentrations as high as 100 μg/ml) with II (Figs. 2 and 3), that end was reached when lower concentrations of I were released into the growth medium from the silicone polymeric delivery device in the presence of II. The few remaining cells at 144 hr were found to be nonviable (Fig. 4) when the combination of the I–silicone polymer plus II was used.

Although both I and II are separately stable when incubated in cell-free medium, analyses of the data using HPLC (Table I) suggest that L-1210 cells have the capacity to metabolize I and/or II. Concentrations of I and II gradually decrease at longer sample times. Previously it was demonstrated that cell-free enzyme extracts of L-1210 cells converted II to its nucleoside monophosphate (18). Similarly, recent studies by Venner and Glazer (19) have shown that L-1210 cells convert I to its mononucleotide to the extent of ≤16%, while most of the parent drug is excreted unchanged in the urine of tumor-bearing mice. Studies by two groups have sought to develop models and data for I alone to describe the pharmacokinetics in normal and leukemic (L-1210) mice (20, 21).

The present study has demonstrated that the adenosine deaminase inhibitor I is capable of greatly potentiating the antileukemic effects of II, particularly when the former is released at a controllable rate from a silicone monolithic polymeric matrix. The clinical application of protection labile oncologic agents in this manner will be explored in the near future.

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Time-Dependent Kinetics VII: Effect of Diurnal Oscillations on the Time Course of Carbamazepine Autoinduction in the Rhesus Monkey

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Abstract □ Extensive blood sampling and repeated long-term carbamazepine infusions were carried out in four rhesus monkeys to examine the time course of carbamazepine autoinduction in detail and assess the intraanimal variability in the rate constant of induction. Diurnal oscillations in carbamazepine blood levels were observed during all infusions and these prevented a good data fit for the biochemical model previously proposed for describing the decline in drug blood levels during induction by carbamazepine. An attempt at fitting only selected blood samples to the model resulted in variable (and perhaps questionable) induction rate constants, even in the same animal. Previous variability in calculated induction rate constants may be due to the presence of diurnal oscillations superimposed on the autoinduction phenomenon. It is proposed that the simultaneous expression of diurnal oscillations and autoinduction are the result of effects on drug metabolism at two independent levels.

Keyphrases □ Carbamazepine—autoinduction in the rhesus monkey, rate constant determination, effect of diurnal oscillations on drug metabolism □ Metabolism—of carbamazepine in the rhesus monkey, autoinduction rate constants, effect of diurnal oscillations □ Diurnal Oscillation—effect on metabolism of carbamazepine in the rhesus monkey

The ability of carbamazepine to induce self-elimination during chronic administration in the rat, dog, monkey, and human is well established (1–10). This increased elimination is reflected in a decline in carbamazepine steady-state blood levels. To describe this time-dependent decline, equations have been proposed based on a biochemical model of exponentially increasing levels of drug-metabolizing enzymes in the liver (11–14). These equations have

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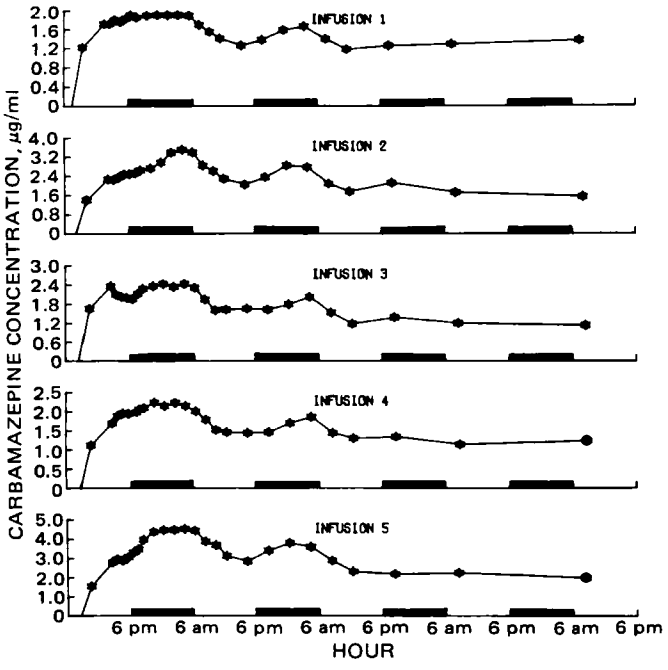

Figure 1—*Carbamazepine concentration* versus *time for five infusions in monkey 202.*

been used to fit the plasma concentration–time data during auto- and heteroinduction by carbamazepine and to calculate a first-order rate constant (induction rate constant) which characterizes the time course of the decline in plasma drug levels (15–18). Recently, further support for this model was provided by the finding of a close correlation between the change in liver enzymes responsible for drug metabolism (cytochrome P-450) and the change in metabolic clearance of carbamazepine in monkeys during long-term infusions (19).

An interesting result of the application of this model has been the remarkable interanimal variability in the induction rate constant (twofold or more) (15–18). Although

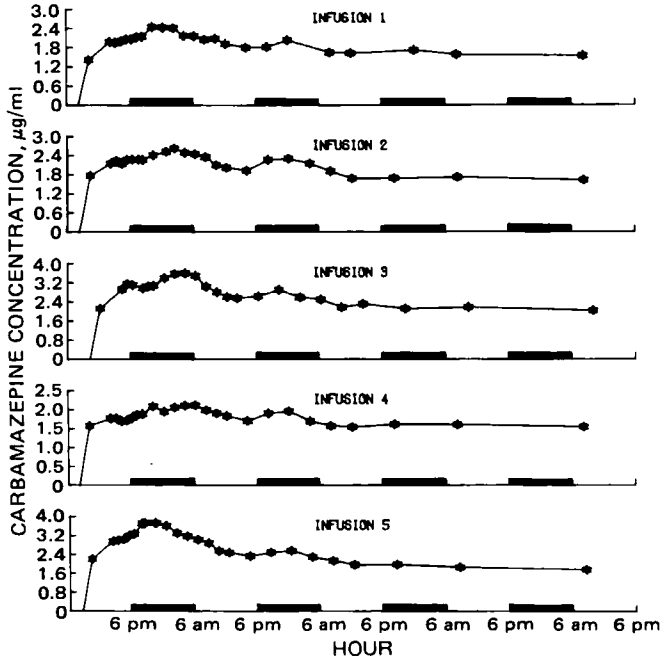

Figure 2—*Carbamazepine concentration* versus *time for five infusions in monkey 204.*

Table I—Calculated Induction Half-Life [a] During Carbamazepine Long-Term Infusions

Monkey	Infusion	Infusion Rate, ml/hr	r^b	Calculated Induction Half-Life, hr
202	1	1.00	0.866	4.0
202	2	1.41	1.000	10.4
202	3	1.19	0.965	13.8
202	4	1.07	0.941	6.9
202	5	1.69	0.988	16.5
203	1	1.45	0.946	13.4
203	2	1.54	0.994	13.9
203	3	1.76	0.964	9.3
203	4	1.54	0.957	7.8
203	5	1.58	0.995	20.1
204	1	1.40	0.892	6.6
204	2	1.39	0.926	7.2
204	3	1.23	0.995	10.5
204	4	1.41	0.962	11.4
204	5	1.54	0.944	11.0
60409	1	1.63	0.868	7.2
60409	2	1.56	0.947	10.1
60409	3	1.47	—[c]	—
60409	4	1.60	0.979	32.9
60409	5	1.49	0.931	302.0

[a] Calculated using only the blood samples collected at 18, 20, 22, 24, 26, 28, 32, 52, 72, and 96 hr, fitting the calculated intrinsic clearance at these times to a model that predicts a monoexponential increase in intrinsic clearance (12, 13). [b] Correlation coefficient from fitting intrinsic clearances to a monoexponential equation. [c] — Lost due to technical difficulties.

it is tempting to speculate about the meaning of this observation, such speculation should be limited until the reproducibility of the rate constant in a given animal can be determined. Following an assessment of intraanimal variability in this parameter, the interanimal variability in the induction rate constant and the possible contribution of genetic factors could be evaluated. The present study was undertaken: (*a*) to characterize in greater detail the time course of carbamazepine autoinduction in the rhesus monkey, (*b*) to quantitate the intraanimal variability in the rate constant of autoinduction, and (*c*) to examine factors that may affect the rate constant determination.

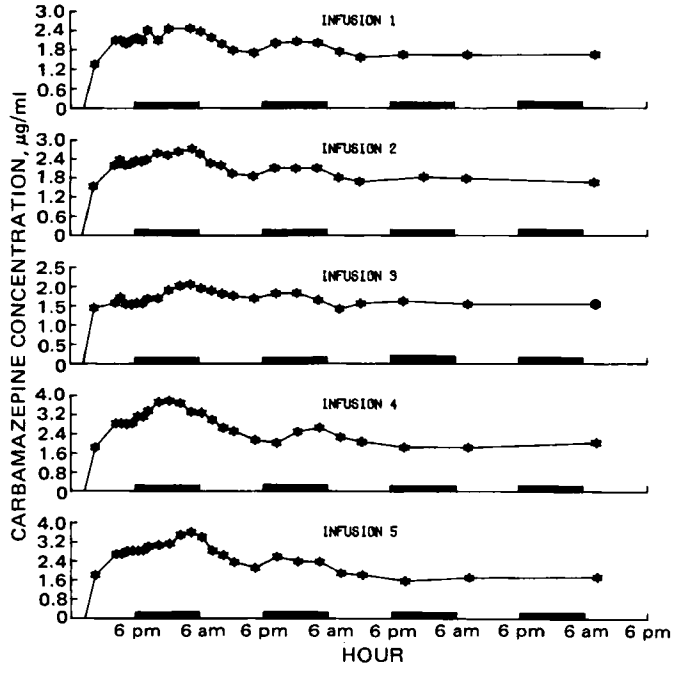

Figure 3—*Carbamazepine concentration* versus *time for five infusions in monkey 203.*

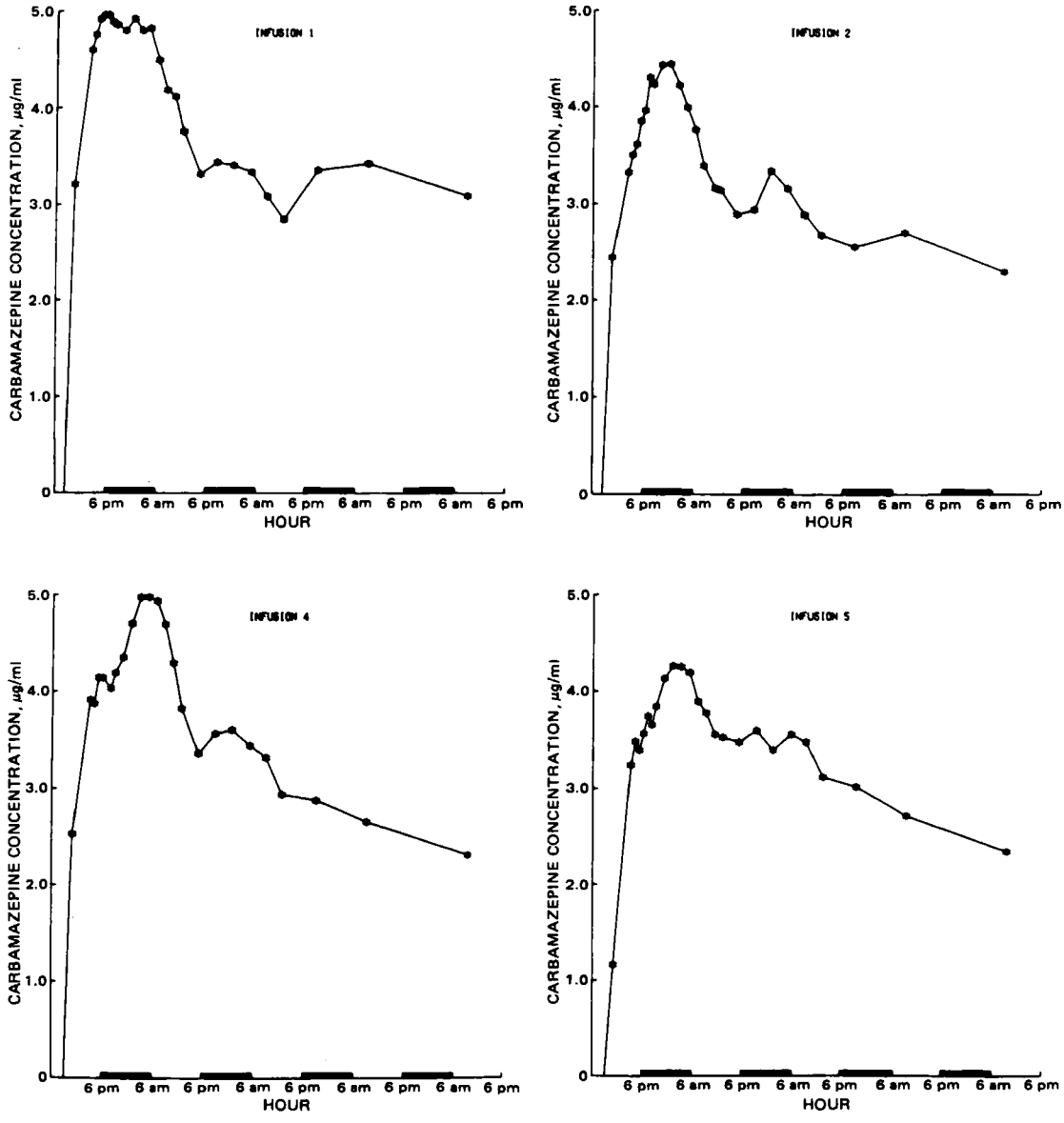

Figure 4—*Carbamazepine concentration* versus *time for four infusions in monkey 60409. (The third infusion was lost due to technical difficulties.)*

EXPERIMENTAL

Animals—This study was conducted in five restraint chair-adapted normal rhesus monkeys (*Macaca mulatta*). Each animal was equipped with two chronic indwelling catheters (femoral for drug infusions and jugular for blood sampling).

Dosing—Four animals received five 96-hr carbamazepine infusions, a fifth animal received a single 96-hr carbamazepine infusion. Infusions were separated by at least a 3-week period, during which time no drugs were administered. Sterile carbamazepine solutions (10 mg/ml) in 60% polyethylene glycol 400 were used for drug administration. Carbamazepine was infused at rates of 1.0–1.8 ml/hr with an infusion pump[1].

Blood Sampling—Blood samples (1.4 ml) were obtained at 2, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 22, 24, 26, 28, 32, 36, 40, 44, 48, 52, 60, 72, and 96 hr during the carbamazepine infusion. Each sample was divided and stored at −20° until assayed.

Liver Biopsies—To investigate the relationship between the cytochrome P_{450} level and the diurnal changes in carbamazepine clearance observed in this study, an additional but limited investigation of diurnal change in the cytochrome P_{450} level was undertaken. Liver biopsies were obtained in three of the five monkeys at 1:00 a.m. and 1:00 p.m. Monkeys received only saline (no carbamazepine) during this phase of the study, with a 3-week rest period between biopsy samples. The liver tissue was immediately placed on ice, blood clots were eased away from the tissue, and the biopsy was blotted dry and weighed. The liver tissue was homogenized in 20% glycerol phosphate buffer to give 10 mg of liver tissue/ml of buffer. The homogenate was centrifuged at 200×*g*, and the supernatant was removed and assayed for cytochrome P_{450} and protein.

Analytical Procedures—Whole blood samples were assayed in duplicate for carbamazepine by GC interfaced with a mass spectrometer in chemical ionization mode (20). Cytochrome P_{450} measurements were performed with a scanning spectrophotometer[2] by using the sodium dithionite difference technique reported by Estabrook *et al.* (21) and Joly *et al.* (22). Protein was determined with the sodium anazolene[3] assay reported by Spector (23).

RESULTS AND DISCUSSION

Plots of the time course of carbamazepine blood levels from replicate infusions in four monkeys are given in Figs. 1–4. These curves appear to show two types of phenomena: (*a*) an overall decrease in steady-state carbamazepine blood levels between the first and the fourth day and (*b*)

[1] Holter model 905; Extracorporeal Medical Specialties, Inc., King of Prussia, PA 19406.

[2] Aminco DW-2 UV-VIS spectrophotometer; American Instruments Co., Silver Spring, MD 20910.

[3] Comassie Blue; Pierce, Rockford, IL 61125.

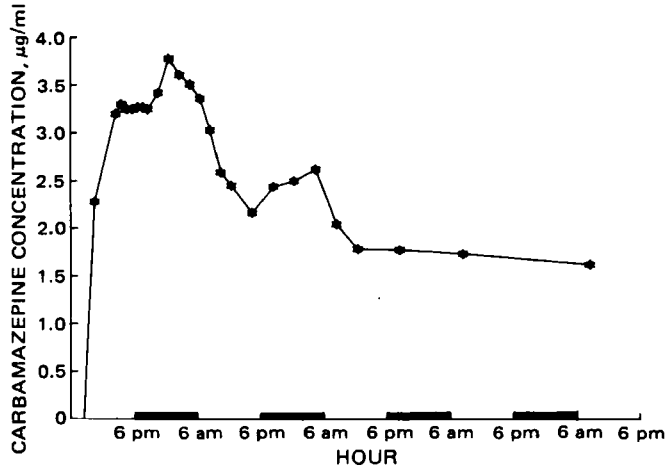

Figure 5—*Carbamazepine concentration* versus *time for a single infusion in monkey N436.*

oscillations in blood levels (increase followed by decrease) during the first two dark periods. This pattern in carbamazepine blood levels was reproducible in replicate studies in four monkeys and was also found in the single infusion of monkey N436 (Fig. 5).

The decrease in carbamazepine blood levels during the 4-day infusions involves a chemically induced, time-dependent increase in clearance (24) (autoinduction) which has been observed consistently during long-term infusions of carbamazepine in the rhesus monkey (16, 25). The oscillations in carbamazepine levels during the dark periods are probably the expression of a diurnal phenomenon. Diurnal oscillations in steady-state levels of several drugs (valproic acid, ethosuximide, and carbamazepine) have been observed in the rhesus monkey (26–28) and are most likely the result of an endogenous physiological process (24). This study shows that these two different types of time-dependent phenomena can occur simultaneously. As a result, the blood level data could not be fitted to previous equations describing the time course of induction (12, 13).

In an effort to separate the diurnal oscillations from the autoinduction phenomenon, carbamazepine blood samples taken at 18, 20, 22, 24, 26, 28, 32, 52, 72, and 96 hr were used to describe the time course of induction. The systemic clearance of carbamazepine at each of these times was computed by dividing the rate of infusion by the respective blood concentration. Each systemic clearance was then used in conjunction with an estimated liver blood flow rate [2.735 liters/hr/kg (29, 30)] to calculate the intrinsic clearance at these respective times. Both the intrinsic and systemic clearance values were fitted with the BMDX85 computer program to the equation (13, 14):

$$CL_t = CL_0 + P(1)\{1 - e^{-[P(2)(t-\theta)]}\} \quad \text{(Eq. 1)}$$

where CL_t represents the clearance at any time t, CL_0 represents the initial clearance calculated from the steady-state blood levels, P(1) represents the change between the initial and final clearance values, P(2) represents the rate constant of induction, and θ is the time lag before the start of the induction process. The induction half-lives obtained by dividing ln 2 by the rate constant of induction exhibited large variability (intra- and interanimal) whether intrinsic (Table I) or systemic clearance values were used. The poor reproducibility in induction half-lives probably reflects the inability to accurately separate the diurnal oscillations from the autoinduction process. The variability observed in the past may also be a reflection of this problem (15–18, 31). However, other biochemical factors which may also vary and influence the time course of induction cannot be ruled out.

The diurnal phenomenon observed in this study is consistent with what has been reported for other drugs in the rat and human (32–40). Factors that could contribute to this phenomenon include a decrease in liver blood flow, enterohepatic recycling, and/or a decrease in the carbamazepine free fraction during the night. However, there are a number of reasons to suspect that the oscillations are more likely due to changes in intrinsic drug clearance by the liver. First, carbamazepine is extensively metabolized and only a small amount is eliminated unchanged in the monkey (41). Second, the liver appears to be the predominant eliminating organ based on intravenous and portal vein catheter experiments in this laboratory[4]. Third, liver blood flow would have to decrease an average of 40% each night to explain the observed decrease in drug clearance and elevation of carbamazepine levels. Fourth, ethosuximide and valproic acid, which are extensively metabolized in the monkey and have clearances insensitive to changes in liver blood flow, also exhibit pronounced diurnal oscillations (26, 27). Fifth, the time course of change in carbamazepine blood levels overnight is too slow to be compatible with an enterohepatic recycling process. The absence of elevated carbamazepine levels following meals during the day also suggests that the oscillations are probably not due to an enterohepatic recycling process. Sixth, in view of the linear binding (free fraction 0.35 ± 0.02) of carbamazepine in monkey plasma between 1 and 10 μg/ml, a large decrease in this parameter during the night seems unlikely.

[4] Unpublished data.

Table II—Cytochrome P_{450} Determination in Liver Homogenates [a]

Monkey	Cytochrome P_{450} Levels, nmoles/g liver 1:00 a.m.	1:00 p.m.
204	32	36
N436	32	28
203	—[b]	33
Mean ± *SD*	32 ± 0	32 ± 4

[a] Monkeys 202 and 60409 were not available for P_{450} determinations. [b] — P_{450} could not be determined due to the small amount of liver tissue available.

If the diurnal oscillations observed in the present study are due to changes in hepatic metabolic activity, the lack of any apparent change in cytochrome P_{450} (Table II) suggests that the diurnal variations are not due to changes in this enzyme. This is consistent with other observations in rats, which also exhibit pronounced diurnal oscillations in drug metabolism but little diurnal change in cytochrome P_{450} levels (42, 43). This hypothesis is further supported by the consistent observation in this study that diurnal oscillations are unaffected by the process of induction which is known to involve an increase in liver cytochrome P_{450} enzymes.

An alternative mechanism for the diurnal oscillations in this study may be concurrent oscillations in cytochrome P_{450} reductase activity. The levels of the reductase are one-tenth to one-twentieth those of cytochrome P_{450} levels (44, 45), and reductase levels have been found to be a rate-limiting component in microsomal monoxygenase systems (44, 46). Furthermore, several studies in rats have shown diurnal oscillations in cytochrome P_{450} reductase activity (42, 43, 47). In addition, the formation of at least one major metabolite of carbamazepine (carbamazepine-10,11-epoxide) in the human has been shown to be dependent on the activity of this enzyme (48). If the reductase activity undergoes diurnal oscillations in the monkey and is also a rate-limiting component in some of the pathways of carbamazepine elimination, the two time-dependent phenomena observed in this study could be the result of effects on separate components of the drug metabolizing system (diurnal oscillations related to oscillations in cytochrome P_{450} reductase activity and autoinduction related to increase in cytochrome P_{450} enzymes).

It should be noted that endogenous steroids are also known to exhibit diurnal variations in both the rat and human. Whether these steroids are responsible for changes in drug metabolism or just spuriously related is unknown. Whatever the relationship between endogenous steroids and the diurnal phenomena noted for drug metabolism, it is probable that a key to the mechanism lies in understanding the factors responsible for changes in drug metabolism at the cellular level.

This study provides the first evidence that two types of time-dependent phenomena, diurnal oscillations and autoinduction, can occur simultaneously. As a result, blood level data cannot be fitted to previous equations for describing the time course of induction. Use of selected blood samples resulted in variable induction rate constants, even in the same animal. In view of the observed intraanimal variability, the first-order rate constants of induction computed in the past should be interpreted with some caution. We propose that the autoinduction and diurnal phenomena seen in this study may be the result of effects on drug metabolism at two independent levels.

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