percentage of cells in division was calculated. The overall mitotic activity was determined, and no attempt was made to separate mitotic figures in the myeloid or erythroid series. All counts were performed blindly. Four hours after colchicine injection has been found to be an optimal time for study of mitotic activity in bone marrow of mice (*6*).

To determine whether a circadian variation in reticulocytes in peripheral blood occurred, tail vein blood was obtained from groups of five rats every 4 hours beginning at 8:00 a.m., each animal being used only once. Samples were obtained from 10 to 12 mice by exsanguination at the same times (except 4:00 a.m.). Reticulocyte counts were performed by utilizing supravital fluorescent staining (*7*). The results with this technique compare well with the results obtained with standard wet preparation Cresyl Blue staining, except that the values have been found to be somewhat higher and more easily reproducible. Blood was drawn to the 1 mark of a white blood cell pipette and diluted with a 0.1 percent solution of acridine orange, a fluorochrome which combines with the RNA of reticulum to produce an orange fluorescence under ultraviolet light. This was placed beneath a cover slip and sealed to prevent drying. Counts were first performed by using dark-field light to determine the number of erythrocytes in each field, then the same area was viewed under ultraviolet light to determine the percentage of reticulocytes. Student's *t*-test was used to determine the significance of experimental differences.

A marked circadian periodicity occurred in the frequency of mitoses of the bone marrow of both rats and mice (Fig. 1). The proliferation of the marrow was highest during the period from 6:00 a.m. to 2:00 p.m. and then dropped and remained low throughout the rest of the day. In the rat, the peak activity at 10:00 a.m. was significantly higher than those at other observed times except at 2:00 p.m. and 10:00 p.m. In the mouse, the peak activity at 12:00 noon was significantly higher than that at any other observed time. The total 24-hour mitotic rate in the rat was 46.5 percent (calculated from the sum of the percentages at each of the six times of day, thus representing an entire 24-hour period). If this same figure were calculated only on the basis of the value at 10:00 a.m., it would appear to be 78.6 percent over a 24-hour period, or if calculated from the 2:00 a.m. figure it would be only 27.0 percent. Thus, overall daily mitotic activity cannot be extrapolated from the mitotic activity determined at any one time.

This variation of cell division in the bone marrow of rats and mice, with the peak mitotic activity occurring during the morning hours, is very similar to mitotic rhythms found in other rodent tissues such as gastric mucosa (*5*), epidermis (*8*), and liver (*9*). In a study of human bone marrow, the largest number of mitoses was seen in the evening and the smallest in the morning (*1*). Opposite patterns are expected in rodents and humans, of course, as rodents are nocturnally active while humans have primary daytime activity.

A circadian variation was present in reticulocytes in both rats and mice, with the highest percentages occurring at 8:00 a.m. and the lowest from 4:00 p.m. to 8:00 p.m. (Fig. 1). The 8:00 a.m. percentage in the rat was statistically significantly higher than that at any other time of day except 4:00 a.m., and in the mouse the percentage at 8 a.m. was significantly higher than that at any other time except 12:00 noon.

Thus, there is a circadian periodicity present in both the bone marrow mitotic activity and peripheral blood reticulocyte levels in both rats and mice. Experimental results involving measures of hematologic function may be difficult or impossible to assess without considering these circadian variations. Studies done at one standard time of day may not be sufficient to eliminate these problems. The result of drug administration, for example, may be dependent upon the time of day if the drug's activity is primarily directed toward one portion of the cell cycle.

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Lysergic Acid Diethylamide: Role in Conversion of Plasma Tryptophan to Brain Serotonin (5-Hydroxytryptamine)

Abstract. *Injections of* D-*lysergic acid diethylamide decrease the turnover rate of 5-hydroxytryptamine of rat brain, as measured from the conversion of* ^{14}C*–tryptophan into* ^{14}C*–5-hydroxytryptamine. The 2-bromolysergic acid diethylamide given in doses fivefold greater than those of lysergic acid diethylamide fails to change the rate of* ^{14}C*–tryptophan conversion into* ^{14}C*–5-hydroxytryptamine. The effect of* D-*lysergic acid diethylamide is discussed with regard to its action on brain serotonergic neurons and its psychotomimetic effects.*

From indirect information available, we now can infer that D-lysergic acid diethylamide (LSD) may reduce the turnover rate of brain 5-hydroxytryptamine (HT) in rats (*1*, *2*). The validity of this inference is somewhat doubtful because it is based on results obtained with nonisotopic methods (*3*) derived from the application of steady-state kinetics to the dynamic equilibrium between the rates of HT formation and its metabolism and efflux from the brain. When a drug like LSD changes the "steady-state" concentrations of brain HT and 5-hydroxyindoleacetic acid (HIAA) in opposite directions (*1*, *4*), it is difficult to assume that the dynamic relation between the rates of formation of HT and HIAA remain comparable to that at the steady-state. In fact, the data on the turnover rate of brain HT in rats injected with LSD (*1*, *2*) might also be interpreted to indicate that this drug changes the route of metabolism of brain HT. One might infer that, as a result of LSD action, HT may be diverted toward a novel metabolic pathway which normally plays an insignificant role in the metabolic degradation of this brain amine. For instance, LSD

Table 1. Plasma A_{TP} and brain A_{HT} after intravenous infusion of ^{14}C-tryptophan. The rate of infusion of ^{14}C-tryptophan (22.7 mc/mmole) was 1 μc/kg per minute. Plasma was mixed with ten volumes of 0.4*N* perchloric acid; the supernatant (2 ml) was purified by passing through an ion-exchange column [Rexyn 102 (NA^+)]. Tryptophan was eluted with 4 ml of water and assayed (*8*, *11*). Tryptophan was converted to 9*H*-pyridoindole by the addition of formaldehyde (0.56 mmole), ferric chloride (1 μmole), and trichloroacetic acid (0.43 mmole) in 0.2 ml (1 hour in a boiling bath). The pyridoindole was extracted with 10 ml of ethyl acetate, after addition of 0.2 ml of 10*N* NaOH, and eluted into 3 ml of 0.1*N* HCl. Its fluorescence was measured (Aminco Bowman) (at excitation 370 nm and emission 450 nm), and its radioactivity was assayed by liquid scintillation (*12*). This procedure eliminates the following radioactive contaminants: HT, 5-hydroxytryptophan, HIAA, tryptamine, and indoleacetic acid. The specific activity of HT was assayed by a similar method. The Rexyn column was washed to remove tryptophan, 5-hydroxytryptophan, HIAA, and IAA. Then HT was eluted with 0.5*M* borate (*p*H 11) and assayed according to a method modified from Bogdanski *et al.* (*13*), Snyder *et al.* (*14*), and Vanadable (*15*). Radioactivity of HT was measured in Bray's mixture. Each value is the average of samples from at least three rats.

Time of infusion (min)	Plasma A_{TP} [(dpm/nmole) ± S.E.]	Brain A_{HT} [(dpm/nmole) ± S.E.]
10	179 ± 13	8 ± 3
20	361 ± 19	40 ± 4
30	640 ± 55	81 ± 16
40	553 ± 19	136 ± 8
50	581 ± 70	196 ± 7
60	720 ± 18	230 ± 38

could, perhaps, decrease the rate of HIAA accumulation elicited by probenecid (*1*) because this drug facilitates the formation of 5-hydroxytryptophol, which normally is a minor metabolite of brain HT (*5*). The report that treatment with small doses (10 μg/kg, intramuscularly) of LSD reduces the rate of discharge of raphe nuclei neurons [an area of the reticular formation containing most of the serotonergic cell bodies to be found in rat brain (*6*)] agrees with the proposal that LSD decreases the turnover rates of HT in the brain. Since stimulation of these nuclei increases brain HIAA (*7*) it seems logical to assume that a decreased neuronal activity may reduce the turnover rate of the amine. It appeared to us that the effects of LSD on the turnover rate of HT could be elucidated by determining directly the action of LSD on biosynthesis of HT in brain. In that tryptophan is the substrate of the rate-limiting enzyme for HT biosynthesis (*7*), we have investigated the problem of whether LSD changes the conversion rate of plasma ^{14}C-tryptophan into ^{14}C-HT.

We have developed a method to estimate the HT turnover rate in brain of unanesthetized rats during infusion of small doses of ^{14}C-tryptophan. The method (*8*) is based on the finding that these doses of ^{14}C-labeled tryptophan do not change its steady-state concentration in plasma, and that when stored in neurons HT behaves kinetically as if it were kept in a uniform kinetic compartment. During ^{41}C-tryptophan infusion the specific activity of plasma tryptophan increases with time (Table 1). The equation describing this increase is

$$\frac{dA_{TP}}{dt} = R - k_{TP}A_{TP} \qquad (1)$$

where A_{TP} is the specific activity of plasma tryptophan, R is the initial apparent rate of change of A_{TP} and k_{TP} is the fractional rate constant of plasma tryptophan. On integration and if $A_{TP} = 0$ at $t = 0$, Eq. 1 becomes

$$A_{TP} = \frac{R}{k_{TP}}(1 - e^{-k_{TP}t}) \qquad (2)$$

where t is the duration of ^{14}C-tryptophan infusion. During steady-state conditions

$$\frac{dA_{HT}}{dt} = k_{HT}(A_{TP} - A_{HT}) \qquad (3)$$

where A_{HT} is the specific activity of brain HT and k_{HT} is its fractional rate constant, Eq. 2 can now be substituted in Eq. 3.

$$\frac{dA_{HT}}{dt} = k_{HT}\left[\frac{R}{k_{TP}}(1 - e^{-k_{TP}t}) - A_{HT}\right] \qquad (4)$$

Since $A_{HT} = 0$ at $t = 0$, Eq. 4 on integration is

$$A_{HT} = \frac{R}{k_{TP}} \times \left[1 + \frac{1}{k_{HT} - k_{TP}}(k_{TP}e^{-k_{HT}t} - k_{HT}e^{-k_{TP}t})\right] \qquad (5)$$

Data of Table 1 show that the values of specific activity of HT in brain after various durations of ^{14}C-tryptophan infusions change with time, as predicted by Eq. 5. By substituting in Eq. 5 the experimental values of R and k_{TP} and solving for each value of a continual series of theoretical values of k_{HT}, one can obtain the corresponding values for the specific activity of HT. Thus we have constructed a graph depicting the relation between the value of brain k_{HT} and any possible experimental value of brain A_{HT}. By infusing ^{14}C-tryptophan and measuring brain A_{HT} and plasma A_{TP} at the end of infusion, one can obtain from the appropriate graph the value of k_{HT} corresponding to that set of data. This value (which has the dimension of reciprocal time) multiplied by the concentration of brain HT gives an estimate of the turnover rate of brain HT.

Table 2. Effects of LSD and BOL (the 2-bromo derivative of LSD) on the turnover rate of brain HT. ^{14}C-tryptophan was infused at a rate of 60 μc/kg per hour for 40 minutes. The total dose of ^{14}C-tryptophan administered was 1.76 μmole. The 2-bromo derivative of LSD (1 mg/kg) and LSD (1 mg/kg) were injected intraperitoneally 30 minutes before ^{14}C-tryptophan infusion. Number of animals is indicated by parentheses. Values are means ± S.E.

Treatment	Plasma TP (nmole/ml)	A_{TP} (dpm/nmole)	HT (nmole/g)	A_{HT} (dpm/nmole)	k_{HT} (hr^{-1})	HT Turnover rate per gram of brain (nmole/hr)
			Experiment 1			
None (5)	76 ± 6	927 ± 44	2.9 ± 0.1	133 ± 18	0.77 ± 0.13	1.9 ± 0.3
BOL (6)	77 ± 5	895 ± 52	2.7 ± 0.1	109 ± 9	0.66 ± 0.05	1.7 ± 0.2
			Experiment 2			
None (8)	100 ± 7	895 ± 52	3.1 ± 0.2	128 ± 6	0.71 ± 0.04	2.4 ± 0.3
LSD (8)	93 ± 6	902 ± 22	3.4 ± 0.1	63 ± 2*	0.31 ± 0.01	1.1 ± 0.1*

* $P < .05$ as compared to the control.

Table 3. Effect of LSD and its 2-bromo derivative infusion on brain HT turnover rate (nanomoles per hour per gram of brain). The ^{14}C-TP was infused at a rate of 60 μc/kg per hour for 40 minutes. The bromo derivative was mixed with ^{14}C-tryptophan and infused at a rate of 3 mg/kg per hour for 40 minutes. The LSD was mixed with ^{14}C-tryptophan and infused at a rate of 0.6 mg/kg per hour for 40 minutes. Values are means ± S.E. Number of animals is indicated by parentheses.

Treatment	HT (nmole/g)	A_{5HT} (dpm/nmole)	k_{HT} (hr^{-1})	Turnover rate per gram of brain (nmole/hr)
None (5)	2.8 ± 0.1	130 ± 9	0.73 ± 0.06	2.1 ± 0.2
BOL (5)	2.7 ± 0.2	123 ± 9	0.68 ± 0.06	1.8 ± 0.2
LSD (5)	3.2 ± 0.1*	89 ± 8*		

* $P < .01$ as compared to the control.

The validity of this estimation depends on the following assumptions. (i) Plasma tryptophan is in rapid equilibrium with the compartment of brain tryptophan involved in the biosynthesis of HT. This assumption is supported by the relatively short average lifetime of a tryptophan molecule (calculated to be about 40 minutes from the data of Table 1) in the plasma compartment. (ii) Tryptophan hydroxylase is rate-limiting for HT biosynthesis (*7*); therefore, the rate constant for decarboxylation of 5-hydroxytryptophan must be much greater than that for tryptophan hydroxylation, and tryptophan concentration in neurons saturates the specific hydroxylase. (iii) HT stored in the brain behaves kinetically as a single compartment where newly synthesized amine rapidly mixes with already stored HT, a uniform metabolic pool being formed.

The effects of LSD and its 2-bromo derivative on the turnover rate of brain HT were measured as described. Table 2 shows that 2-bromo derivative and LSD in the doses administered neither changed the steady-state of plasma tryptophan nor altered the fractional rate constant of plasma tryptophan, as revealed indirectly by the specific activity of plasma tryptophan. In addition, Table 2 shows steady-state concentrations of brain HT, its specific activity, k_{HT}, and turnover rate of brain HT after a single injection (intraperitoneally) of LSD or the 2-bromo derivative. These data demonstrate that LSD but not its 2-bromo derivative decreased the turnover rate of brain HT. It should be mentioned that under these experimental conditions, LSD did not alter the steady-state concentration of brain HT as reported (*1*, *4*). ^{14}C-Tryptophan infusion was started 30 minutes after the injection of LSD. However, when LSD was infused intravenously together with ^{14}C-tryptophan, the brain HT concentration increased (Table 3). Hence, equations based on steady-state kinetics cannot be applied to derive k_{HT} or brain HT turnover rate under this condition. Nevertheless, the data show that LSD reduced the specific activity of HT in the brain. The conversion of ^{14}C-tryptophan to ^{14}C-HT was affected, perhaps at the rate-limiting step of tryptophan hydroxylation. The results in Table 3 also show that the 2-bromo derivative of LSD, infused in doses five-fold greater than the LSD dose, did not change the specific activity of brain HT.

Although these data support a relation between firing rate of serotonergic neurons and turnover rate of brain HT as suggested by Aghajanian *et al.* (*6*), they fail to prove this point because we have infused doses of LSD greater than those given by Aghajanian *et al.* Several problems prevent establishing a direct relation between the biochemical results and the physiological report on LSD mentioned above (*6*). It must be kept in mind that in rats the half-life of LSD is short, and our technique of measuring turnover rate of brain HT requires an infusion of ^{14}C-tryptophan lasting 40 minutes. Therefore, to measure the LSD effects on brain HT turnover rate the dose of LSD had to be increased to assure a persisting effect of this drug. In relating these data to the pharmacological profile of the two drugs tested, we note that the 2-bromo derivative of LSD, which is a much less potent hallucinogenic drug than LSD, failed to alter brain HT turnover rate.

If our results reflect an action of LSD on brain serotonergic neurons, then we propose that in the central nervous system, as well as in the periphery, LSD interacts with HT receptors (*9*). When LSD is applied to brain slices or to neurons of various brain regions in vivo through a micropipette, it facilitates or antagonizes the action of HT (*10*). The report of Aghajanian *et al.* (*6*) indicates that concentrations of LSD in tissues resulting from the doses he used may compare with the concentration of LSD in vitro that facilitates HT action (*9*, *10*). Consequently we might suggest that if LSD interacts with brain HT receptors, it may facilitate the effect of the amine on neurons as it does on the isolated uterus of rats (*9*). Regardless of the mode of action of LSD on brain HT turnover rate, the hallucinogenic effect of this drug in man may be related to the effect of LSD on HT turnover. However, no data are available suggesting that this possibility can be explored successfully. In conclusion, our results showed that LSD decreased brain HT turnover rate. The doses used were higher than those reported to decrease neuronal activity of raphe neurons (*6*).

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Allergenic Component of a Liverwort: A Sesquiterpene Lactone

Abstract. Frullania *spp.* (*Hepaticae, Jungermanniales*) *are agents of allergies. Extraction and fractionation of* Frullania tamarisci *has given, as the only allergenic component isolated, a levorotatory crystalline substance, the structure of which is demonstrated. It is a sesquiterpene lactone. The racemic* (±) *form is isolated from a mixed sample of* Frullania tamarisci *and* Frullania dilatata; *the dextrorotatory form, from* Frullania dilatata. *The observed allergenic properties are shared by some other sesquiterpene lactones.*

Some occupational allergies (contact dermatitis) associated with the handling of European woods are caused by epiphytic Bryophytes, namely Hepaticae such as *Radula* and the ubiquitous *Frullania.* An active extract had been obtained with ether (*1*). We have now shown that it contains, besides inactive components, an active eudesmanolide (structure **1**).

Frullania tamarisci (L.) Dum., growing on oak, was collected in the Vosges in October 1968 (*2*), and it was dried at room temperature. It was extracted with ether in a soxhlet, after it was milled. Fractionation of the extract (about 3 percent of the dry weight) was monitored by patch tests applied to a cooperating patient. Chromatography (silica gel) gave a major