with one parent heterozygous at both
the soluble GOT and GPT loci, there
were no recombinants out of a possi-
bable five. Further studies are required
to determine whether or not these two
loci are genetically linked.

Table I shows the incidence of GPT
phenotypes among Seattle blood donors
of three ethnic groups and their cal-
culated gene frequencies. The frequen-
cy of Gpt¹ is highest in the Afro-Amere-
cans, lowest in the Caucasians, and
intermediate in the Orientals, mainly
of Japanese origin.

We also determined the GPT pheno-
types of several hundred blood speci-
mens obtained from natives of New
Guinea, the Philippines, the Congo,
Mozambique, North America (Indian),
and Peru (Indian). In all populations
tested, GPT was polymorphic, the fre-
quency of Gpt¹ ranging from 0.87 in
Mozambique to 0.29 in the Philippines.
Thus, GPT can be added to the rela-
tively short list of polymorphic sys-
tems with gene frequencies highly
favorable to their use as genetic mark-
ers in man (21).

SHI-HAN CHEN
ELIOISE R. GIBLETT
King County Central Blood Bank, Inc.,
Terry-at-Madison
Seattle, Washington 98104

References and Notes
1. J. W. Boyd, Biochem. J., 81, 434 (1961); P.
Borg and E. M. Posters, Biochim. Biophys.
Acta 54, 188 (1961); N. S. Henderson, Exp.
Zool. 158, 263 (1965); S. Hopper and H. L.
Segal, Arch. Biochem. Biophys. 165, 501
Genet., in press; R. G. Davidson and J. A.
Cortner, Nature 215, 765 (1967); Science 157,
1509 (1967); R. G. Davidson, J. A. Cortner,
M. C. Rattazzi, P. H. Rudlue, H. A. Lubs,
Chem. 181, 259 (1950); S. Hopper and H. L.
Segal, Arch. Biochem. Biophys. 165, 501
(1964); A. Meister, Adv. Enzymol. 16, 165
(1955).
4. A. Karmen, P. Wrobleski, J. S. LeDue,
J. Clin. Invest. 34, 126 (1955); G. W. Loh
and E. D. Waller, Folia Haematol. 78, 385
(1962); R. Radhakrishnamury and Z. L.
Sahy, Curr. J. Biochem. 46, 1081 (1968); S.
5. P. W. Gasbrowne, S. Hopper, L. S. Gila,
ibid. 243, 5929 (1966); M. H. Sailer Jr., and W. T.
16, 118 (1970); C. A. Nichell and F. Rosen,
Adv. Enzyme Reg. 1, 343 (1963); F. Rosen,
224, 476 (1953); H. L. Segal, D. S. Beantin,
S. Hopper, ibid. 237, 1914 (1962); H. L. Segal,
Y. S. Kim, S. Hopper, Adv. Enzymol. 29 (1965);
H. M. Wegman, H. Brummer, R. C. Klett,
7. M. Van Rysenat and H. J. Tappe, ibid. 229
(1962); H. M. Wegman, H. Brummer, R. C.
10. The staining mixture consists of 0.1M tro-
HCl buffer, pH 7.6, 200 mM L-alanine, 8.7 mM
α-ketoglutarate, 1 mM NADH, and approxi-
mately 8 units of LIDH per milliliter of solu-
tion. A filter paper (Whitman No. 1) satu-
rated with the staining solution was laid on
the bottom of the sliced gel, which was then
incubated at 37°C for 3 to 4 hours. Then the
gel was photographed under a long-
wave ultraviolet light with a Polaroid camera,
with a yellow filter.
11. E. R. Giblett, Genetic Markers in Human
12. Supported by PHS grant AM 09745. We
thank Jeanne E. Anderson and Bente L.
Grundvig Poum for technical assistance.
24 March 1971; revised 3 May 1971

Brain Serotonin Content: Physiological
Dependence on Plasma Tryptophan Levels

Abstract. Brain serotonin concentrations at 1 p.m. were significantly elevated
1 hour after rats received a dose of L-tryptophan (12.5 milligrams per kilogram,
intrapertioneally) smaller than one-twelfth the normal daily dietary intake of the
subject. Plasma and brain tryptophan levels were elevated 10 to 60 minutes after the
injection, but they never exceeded the concentrations that occur nocturnally in un-
treated animals as a result of their normal 24-hour rhythms. These data suggest
that physiological changes in plasma tryptophan concentration influence brain
serotonin levels.

The initial step in the biosynthesis of
brain serotonin involves the 5-hydrox-
ylation of its precursor amino acid, L-
tryptophan (1). The activity in brain
fractions of tryptophan hydroxylase, the
enzyme that catalyzes this reaction,
is relatively low (2); hence, this enzyme
could limit the rate at which the in-
doleamine is formed in vivo (2-4). How-
ever, the affinity of tryptophan hydroxylase for its substrate is also low (2), and the concentration of tryp-
tophan usually present in neurons that
produce serotonin may not be sufficient
to saturate the hydroxylase (2, 3, 5). Thus, brain serotonin synthesis may
normally be limited by the availability of tryptophan.

Experimental manipulations that
markedly alter the tryptophan available
to the body can raise or lower brain
serotonin levels (3). For example, the
concentration of serotonin is depressed
in brains of animals given diets with.
little or no tryptophan (6), and is elevated in rats receiving diets with large amounts of tryptophan (7) or in rats receiving intraperitoneal injections of tryptophan (50 to 1600 mg/kg) (8). A variety of drugs and treatments thought to accelerate brain serotonin synthesis have been shown to induce parallel changes in brain tryptophan content (9). The concentrations of tryptophan in the plasmas and brains of untreated animals exhibit a characteristic rise of 50 to 150 percent between the daily nadir and peak (10). We show that very low doses of tryptophan, which do not raise plasma and brain tryptophan concentrations above their normal nocturnal peaks and which do not modify concentrations of other amino acids in the plasma, cause significant increases in brain serotonin content. These observations indicate that the physiological control of brain serotonin synthesis may normally be coupled to plasma tryptophan levels.

Male Sprague-Dawley rats (Charles River Laboratories) weighing 150 to 200 g were housed five per cage and exposed to light (11) from 9 a.m. to 9 p.m. daily; they had free access to food (Big Red Laboratory Animal Chow) and water. The animals received either L-tryptophan (12.5 to 125 mg/kg, intraperitoneally) at noon or at midnight and were killed 1 hour later. Data are presented as mean concentrations of tryptophan (micrograms per milliliter of plasma) ± standard error of the mean. Light was on from 9 a.m. to 9 p.m.

Table 1. Plasma tryptophan levels after tryptophan injection. Groups of 16 rats received L-tryptophan (12.5 mg/kg, intraperitoneally) or its diluent at noon or at midnight and were killed 1 hour later. Data are presented as mean concentrations of tryptophan (micrograms per milliliter of plasma) ± standard error of the mean. Light was on from 9 a.m. to 9 p.m.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (µg/ml)</th>
<th>Tryptophan (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noon</td>
<td>21.34 ± 0.96</td>
<td>25.76 ± 1.37*</td>
</tr>
<tr>
<td>Midnight</td>
<td>31.51 ± 0.991</td>
<td>32.80 ± 0.772</td>
</tr>
</tbody>
</table>

* P < .02 when compared with noon control.  
† P < .001 when compared with noon control but not significantly elevated when compared with midnight control.

Blood was collected from the cervical wound in tubes containing heparin, and then centrifuged; the plasma was frozen and later assayed for tryptophan (12). Brains were quickly removed, bisected midsagittally (11), and frozen on Dry Ice. One half of each brain was assayed for tryptophan (13) and the other half for serotonin (25).

In initial experiments, groups of ten rats received L-tryptophan (12.5, 25, 50, or 125 mg/kg) and were killed 1 hour later. All doses tested caused brain concentrations of both tryptophan and serotonin to rise (Fig. 1). At certain doses of tryptophan (25 mg or less per kilogram), the increment in brain serotonin content was proportional to the rise in brain tryptophan.

To determine whether the elevated plasma tryptophan concentrations produced by administering tryptophan (12.5 mg/kg) at noon were still within the normal daily range, groups of 16 animals were given tryptophan or its diluent at noon or at midnight (that is, at times of day when plasma tryptophan levels in rodents were expected to be low and high, respectively (10)), and were killed 1 hour later. One hour after the noon administration of the amino acid, plasma tryptophan levels were significantly higher (P < .02), (Table 1) than those of control rats killed at the same time. However, they were also significantly lower (P < .02) than those of control animals killed 12 hours later at 1 a.m. Brain tryptophan levels were almost twice as high in control animals killed at 1 a.m. as in control rats killed at 1 p.m. (9.36 ± 1.00 µg/g as compared to 5.18 ± 0.57 µg/g. P < .01). The increase in brain tryptophan produced by the administration of amino acid (12.5 mg/kg) at noon was also smaller than the nocturnal rise observed in brains of untreated animals (Fig. 2).

To examine the possibility that the intraperitoneal administration of L-tryptophan (12.5 mg/kg) caused plasma or brain tryptophan levels to rise to a peak that exceeded the normal daily range but was of too short a duration to be detected after 1 hour, an experiment was performed in which groups of ten animals were killed 10, 30, or 60 minutes after receiving the amino acid. The concentrations of tryptophan in plasma and brain rose steeply, attained within 10 minutes values that were 120 percent and 56 percent, respectively, of those observed in control animals killed at the same time (Fig. 2), and remained elevated during the next 50 minutes. At no time after tryptophan administration did plasma or brain tryptophan levels increase beyond their characteristic nocturnal peaks in normal animals. Brain serotonin levels began to rise 30 minutes after tryptophan injection and were 23 percent greater (P < .01) than those present in control animals after 60 minutes.

The administration of a large dose of tryptophan might modify plasma concentrations of other amino acids; such fluctuations could, in turn, affect the activity of neurons that contain serotonin. To determine whether concentrations of other amino acids were altered in animals receiving tryptophan (12.5 mg/kg), plasma samples taken from rats killed after 10, 30, or 60 minutes were analyzed for 11 amino
acids with an amino acid analyzer (16). Tryptophan administration caused no changes in their concentrations.

These data indicate that small doses of tryptophan, which do not cause plasma or brain tryptophan concentrations to exceed their normal daily peaks and which do not influence plasma concentrations of other amino acids, produce significant elevations in brain serotonin. The lowest dose of tryptophan (12.5 mg/kg, or 1.9 to 2.5 mg per rat) administered comprised less than one-twentieth of the amount of tryptophan that rats (200 g) normally consume daily in dietary protein (17), and thus probably does not cause an unusual metabolic stress. Our findings do not allow us to state whether the rise in brain serotonin induced by tryptophan reflects an acceleration in the synthesis of the indoleamine, or a decrease in its turnover, or both. However, previous studies using larger doses of tryptophan have demonstrated that the rise in brain serotonin is accompanied by an even greater increase in the concentrations of 5-hydroxyindoleacetic acid in brain and cerebrospinal fluid (3, 8). These observations are most economically interpreted as reflecting increases in both the synthesis and turnover of the amine.

If, as seems likely, physiological changes in plasma tryptophan concentrations can cause parallel changes in brain serotonin synthesis, the factors that control plasma tryptophan levels must be considered, for they represent potentially important determinants of the activity of a population of central nervous system neurons. Tryptophan enters the systemic circulation from two main sources: as the overflow from the portal circulation following protein ingestion and as the efflux from the bound and free tryptophan pools in the tissues (3). Tryptophan leaves the plasma by uptake into tissues, by metabolism in the liver (catalyzed by tryptophan pyrrolase), and to a minor extent, by excretion into the urine (3).

The ingestion of dietary protein elevates plasma tryptophan concentrations (15). The magnitude of this elevation apparently depends on the time of day that the protein is consumed, perhaps reflecting variations in the activity of the main enzyme that catabolizes tryptophan, that is, hepatic tryptophan pyrrolase. In humans, who eat what and when they choose, the tryptophan concentration of the plasma is 60 to 80 percent higher in midafternoon than it is 12 hours earlier (10). The shape of the curve describing the daily plasma tryptophan rhythm is similar to that of tyrosine, and that of the other essential amino acids; however, plasma tryptophan levels seem to be more influenced by meals than the plasma levels of other amino acids (10).

The flux of amino acids into and out of the tissues is influenced by a variety of hormones (19). The response of tryptophan to insulin appears to differ from that of all other amino acids thus far examined. Insulin administration to rats causes a sizable increase in plasma tryptophan levels (20). This increase may underlie the elevation in dienecholic serotonin and 5-hydroxyindoleacetic acid levels observed in rats receiving insulin (21).

Tryptophan injection into the rodent brain is known to vary diurnally (22). Our data raise the possibility that this rhythm may result partly from the daily rhythm in plasma tryptophan concentration. Neurons that contain serotonin in the brain have been implicated in the control of sleep, body temperature, and other rhythmic physiological processes (23). If changes in brain serotonin concentration do, in fact, reflect alterations in the functional activity of these neurons (24) (that is, if the increment represents serotonin that can be released into the synapse), it seems possible that a variety of neural and behavioral functions might be influenced by physiological changes in plasma tryptophan levels. Given the multiplicity of factors known to influence plasma tryptophan concentrations, this hypothesis seems at odds with the generally held view that critical brain functions are isolated from general metabolic activity.

JOHN D. FERNSTROM
R. J. WURTMAN
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References and Notes
11. "Duo-Tone" (Chase-Hall Corporation, North Bergen, N.J.), 40 to 60 sec/cm.
12. Tryptophan solutions were prepared in water and the pH was adjusted by adding 1N HCl until a clear solution was obtained (at pH 7). To determine whether or not the injection of dilute HCI by itself affected any of the variables studied, groups of six rats received water or the acid diluent at above; plasma and brain tryptophan and brain serotonin were measured. No differences were noted between the two groups.
14. To ensure that no error was introduced by the process of halving the brains, both halves of brains from 12 untreated animals were assayed for tryptophan and serotonin. No significant differences were observed between the tryptophan or serotonin concentrations of the right and left brain halves from individual animals.
16. A Beckman model 120C amino acid analyzer was employed to determine the plasma concentrations of threonine, serine, cystine, arginine, glutamic acid, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, glycine, and alanine.
Cyclic Urinary Leukopoietic Activity in Gray Collie Dogs

Abstract. The urinary activities for bone marrow colony formation were measured on consecutive 24-hour urine samples from two gray collie dogs with cyclic neutropenia and from two normal collies. The activity varied cyclically in the gray collies with a peak activity developing during the neutropenic phase, which anteceded the return of blood neutrophils. The activity fell to undetectable levels after the blood neutrophil counts returned to the normal range. The urine of normal dogs showed no activity. Since the dogs with cyclic neutropenia have been shown to have periodic hematopoiesis, these data suggest a regulatory hormonal role for the substance measured by this assay.

The recently developed methods of short-term marrow culture in soft agar (1) or methylcellulose (2) have permitted the identification and partial characterization of a factor present in animal and human serum and urine which stimulates single bone marrow cells to form granulocytic and mononuclear colonies (3-5). This factor (referred to as colony-stimulating activity [CSA]) has been characterized in human urine to be a heat-stable glycoprotein having a molecular weight of about 190,000 (6). Its physicochemical properties closely resemble erythropoietin but its biological properties are distinctive (7). The role of this factor in the control of leukopoiesis is still unclear.

An animal model of naturally occurring periodic myelopoiesis occurs in gray collie dogs (8). In these dogs, neutrophils are virtually absent from the peripheral blood for 2 or 3 days at 12-day intervals. This defect is related primarily to intermittent neutrophil production. The cyclic neutropenia is a component of a more general marrow defect involving cyclic production of erythrocytes, monocytes, and platelets (9).

A central question in evaluating the physiologic role of the colony-stimulating substance measured in the in vitro assay of colony formation has been whether the serum or urine levels vary with the peripheral blood neutrophil count or with marrow myelopoietic activity. Since gray collie dogs have regularly recurring cycles of myelopoiesis without the complicating influences of cytotoxic drugs or malignancy, it was thought that their pattern of urinary CSA, if found, might be of general physiologic importance.

All urine specimens were obtained from two unrelated gray collies (one male, one female) and two normal collies. Consecutive 24-hour urine samples were collected while the dogs were housed in stainless steel metabolic cages. Urine was collected and frozen as it drained from the bottom of the cages into polyethylene plastic bottles supported beneath the cages in Styrofoam boxes filled with Dry Ice. A modest contamination of the urine with feces was unavoidable.

The urine specimens were then centrifuged at 3500 rev/min at 4°C for 20 minutes and the sediment was discarded. The supernatant was filtered through Millipore filters (pore size, 0.45 nm) and stored at −10°C until CSA was measured.

Colonystimulating activity in each urine specimen was assayed with C57Bl/6N mouse bone marrow as target cells (5). A modification of the methylcellulose technique for bone marrow culture, reported by Worton et al. (2), was employed. Femoral bone marrow plugs from three mice were obtained and pooled in 3 ml of McCoy 5A (modified) medium. A nucleated cell count was performed on the pooled specimens and a sample was diluted to a concentration of 750,000 nucleated cells per milliliter. Three-fourths milliliter of each dialyzed urine sample was added to separate plastic test tubes (17 by 100 mm; Falcon Plastics, Los Angeles) containing 0.5 ml of the diluted mouse marrow, 2.5 ml of 1.6 percent methylcellulose, 0.5 ml of fetal calf serum, and 1.25 ml McCoy medium supplemented with additional sodium bicarbonate, amino acids, vitamins, calf serum, and 6 percent (weight/volume) bovine serum albumin (10). For purposes of assuring that proper conditions for mouse marrow colonization existed, appropriate controls with a supernatant from an L-cells culture of known stimulatory activity were run in parallel with the experimental assays. After the addition of all ingredients, the test tubes were vigorously shaken to produce a homogeneous single-cell suspension. From each test tube, four 1.1-ml samples were removed and plated in separate plastic petri dishes (3.5 by 10 mm). Thus, 75,000 nucleated cells and 0.15 ml of dialyzed urine were contained in each dish. The dishes were placed in a humidified incubator containing 10 percent CO2 in air at 37°C. Seven days later, each dish was examined with an inverted microscope at a magnification of ×50, and all colonies with 20 or more cells were counted. The colony-stimulating activity was expressed as the mean number of colonies from four replicate dishes per 0.15 ml of a standardized daily urine output of 300 ml, with adjustment of the CSA being made for dilution or concentration of the urine sample during dialysis. White blood cell and differential counts were performed by