was not used in these experiments. For formation of protoplasts, cells were resuspended in ice-cold medium containing 1.0 mole of NaCl (*9*), instead of the 0.4 mole of sucrose (which proved less effective in stabilizing these protoplasts), 0.02 mole of sodium phosphate at *p*H 6.8, and 0.01 mole of $MgSO_4$ per liter. Lysozyme was added to a final concentration of 1.2 mg/ml. After protoplast formation, as judged by microscopic examination (usually about 5 minutes), the action of lysozyme was arrested by the addition of 10 volumes of the aforementioned medium. The protoplasts were centrifuged and lysed as before, and they were analyzed on a sucrose density gradient (Fig. 4). The polysomal pattern showed less than 10 percent single 70*S* ribosomes, and the polysomes were sensitive to small amounts of ribonuclease.

In all the strains of bacteria examined, we find that the quantity of polyribosomal material is considerably larger than that found by previous methods. In the strains of *E. coli* approximately 85 to 90 percent of the ribosomal material sediments as polysomes, suggesting that there may be few free single ribosomes in rapidly proliferating cells. This view has been supported by Mangiarotti and Schlessinger (*3*), who have also used chloramphenicol in the preparation of bacterial polysomes. However, their results are qualitatively different from ours. We find 85 to 90 percent of the ribosomal material on polyribosomes and only 10 to 15 percent as single ribosomes and ribosomal subunits; Mangiarotti and Schlessinger find only about half of the ribosomes on polysomes and the remainder as 50*S* and 30*S* ribosomal subunits.

The question of what chloramphenicol does to the equilibrium of ribosomes and polysomes in the cell must be taken into account in evaluating our results. Chloramphenicol apparently blocks protein synthesis by preventing the movement of the ribosome along the messenger (*8, 10*). It might therefore tend to freeze the polysomal distribution and preserve it during the lysis and extraction procedure. However, since chloramphenicol apparently does not block attachment of the ribosome to messenger RNA, it is possible that the dearth of free ribosomes which we observed may be caused by an attachment, at 0°C, of the free ribosomes normally found in cells to existing polysomes. Alternatively, the polysome profile obtained in the absence of the drug may reflect the differential release of ribosomes due to residual protein synthesis at 0°C (*11*).

The advantages of our method are that it is gentle, easy to apply, and apparently of wide application. The cells are maintained metabolically inactive during preparation of the polysomes; thus this method is likely to yield a better representation of the true intracellular state of bacterial ribosomes. It does permit the extraction of essentially all the polysomes in largely undegraded state from exponentially growing bacteria and thus makes possible rapid kinetic studies of normal cell polysomes.

Note added in proof: Recently Hotham-Iglewski and Franklin have described a method for preparing polysomes from *Escherichia coli* which is very similar to that described above. Their polysomal distributions are close to those reported here, with more than 80 percent of the ribosomes found in polysomes.

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7. The lysing medium contains in final concentration 0.5 percent Brij 58 (Atlas Chem. Co.), 0.5 percent sodium deoxycholate, 4 μg of deoxyribonuclease per milliliter (Mann Research-DNase I "DPFF"), 100 μg of chloramphenicol per milliliter, 0.05*M* NH_4Cl, 0.01*M* $MgSO_4$, 0.01*M* Tris-HCl, *p*H 7.4. Deoxycholate is added just before use.
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Daily Rhythm in Tyrosine Concentration in Human Plasma: Persistence on Low-Protein Diets

Abstract. *The concentration of tyrosine in the plasma of normal males varies diurnally. It is lowest (9.5 ± 0.35 micrograms per milliliter) between 0130 and 0230 hours and rises to a peak of 16.2 ± 0.82 micrograms per milliliter by 1030 hours. This rhythm persists when subjects are maintained for 2 weeks on a diet that is very low in protein.*

Circulating tyrosine can undergo at least three kinds of metabolic transformation (*1*): the amino acid can be taken up in the tissues and incorporated into peptides and proteins; small amounts can be converted to low-molecular-weight compounds such as thyroxine, melanin, and the catecholamines; or it can be deaminated to form *p*-hydroxy phenylpyruvic acid, which is a substrate for gluconeogenesis.

Recently (*2*) it was demonstrated that the activity of tyrosine transaminase, the enzyme that catalyzes this last transformation, shows marked diurnal fluctuation in the livers of untreated rats. When animals are kept under light for 12 hours each day, enzyme activity is greatest several hours after the onset of darkness and falls to basal levels by the beginning of the light period. Although tyrosine transaminase activity is known to be induced by certain adrenocortical steroids (*3*), this enzyme rhythm is not generated by the adrenal secretory cycle inasmuch as it persists in the adrenalectomized or hypophysectomized rat (*2*).

Since transamination may account for a major fraction of the tyrosine that leaves the blood stream, we have examined the concentration of this amino acid in plasma, from normal

human volunteers, to determine whether tyrosine levels also vary diurnally. We now show that the concentration of tyrosine in plasma varies markedly each day in the normal human, and that this fluctuation does not simply result from cyclic ingestion of protein or from exercise.

Six healthy male volunteers, students at Massachusetts Institute of Technology and ranging in age from 18 to 24, were admitted to the M.I.T. Clinical Center at least 24 hours before blood was sampled. They were fed a diet containing 0.71 g of egg protein and about 30 calories per kilogram of body weight in equal portions at 0800, 1230, 1730, and 2200 hours. The subjects were kept in bed from midnight to 0700 hours, but their activities were not otherwise restricted. Starting at 0730 hours, eight 5-ml samples of blood were taken during a 24-hour period at intervals of 3 to 5 hours (Fig. 1). The blood was mixed with heparin, and the plasma was separated by centrifugation and assayed for tyrosine (*4*).

A characteristic temporal variation in concentration of tyrosine in the plasma was observed in all subjects tested (Fig. 1). Tyrosine levels rose to peaks at about 1030 hours; over the next few hours they declined to intermediate concentrations, where they remained until about 2130 hours when they fell sharply, reaching a nadir at 0130 to 0230 hours. After 0430 hours they rose again so that the concentration at 0730 hours, on the second morning of the study, did not differ significantly from that observed 24 hours earlier. The average peak concentration of the amino acid (at 1030 hours) was 16.2 ± 0.82 μg/ml; the lowest (at 0130 hours) concentration was 9.5 ± 0.35 μg/ml.

Two of the subjects on the standard diet underwent vigorous physical exercise from 1600 to 1645 hours: they walked at 6 km/hour on a treadmill inclined at 10 deg. Blood was sampled before and after the exercise; in the plasma of both subjects the concentration of tyrosine rose by 8 percent (Fig. 1).

The rhythm of tyrosine in plasma may have been generated by the cyclic ingestion of dietary protein. Dietary tyrosine [or phenylalanine, which is converted to tyrosine in the human liver (*1*)] may have influenced the amino acid in the plasma in several ways: The overflow from the material delivered to the liver by the portal circulation may have contributed directly to systemic blood levels. Alternatively, dietary tyrosine may have induced the formation of tyrosine transaminase in the liver (*3*) and caused an increase in the rate at which the circulating amino acid was metabolized.

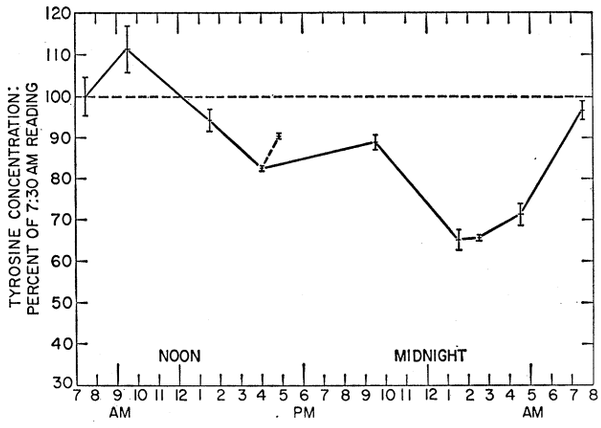

Fig. 1. Changes in tyrosine concentration in plasma with time. Blood was sampled from six volunteers at eight times: four were sampled at 0130 and 0430 hours; two, at 1645 hours after 45 minutes of vigorous exercise (dotted line) and again at 0230 hours. All were bled at all other times indicated: 0730, 1030, 1330, 1600, and 2130 hours. Horizontal bars represent standard errors of the means.

To examine the dependence of the tyrosine rhythm in plasma on the cyclic ingestion of protein, eight volunteers were placed for 14 days on a diet that was extremely low in protein [2.7 g/day (*5*)]. On the last day blood was sampled periodically between 2200 hours and noon (Fig. 2) and examined for tyrosine content (*4*). Two of these subjects were among the six used for the first study, so that we could examine the effect of protein deprivation on the tyrosine rhythm in plasma in a single individual (Fig. 2). Sufficient calories were provided during the period of deprivation of protein to maintain body weight.

The characteristic rhythm in tyrosine concentration persisted in all the subjects kept on the low-protein diet (Figs. 2 and 3). Peak tyrosine levels occurred at the same time in all individuals: 0830 hours. Because the concentration of the amino acid was low at both the start and the end of the blood-drawing period (2200 hours and noon), the time of lowest tyrosine levels could not be identified. The peak concentration of the amino acid was 12.7 ± 0.44 μg/ml; the lowest mean concentration (at 2200 hours) was 7.8 ± 0.31 μg/ml. Both these concentrations were significantly lower than the highest and lowest in subjects fed adequate amounts of protein.

These data indicate that the concentration of tyrosine in the plasma of healthy males varies almost twofold during each 24-hour period. Although tyrosine levels are influenced by the amount of protein in the diet, their diurnal fluctuation is not generated by man's tendency to ingest protein cyclically, since they persist in subjects fed only trace amounts of protein. It also appears likely that the amino acid rhythm is not generated by physical-activity rhythms, inasmuch as the greatest rise in tyrosine concentration occurs between 0230 and 0730 hours—when our subjects were sleeping.

The biochemical mechanism responsible for the tyrosine rhythm in plasma could act on all of the amino acids, or

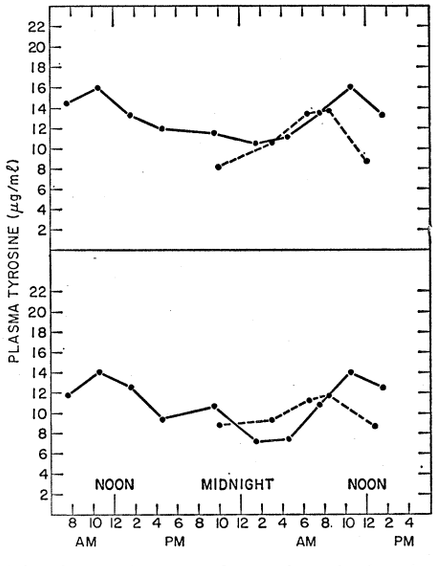

Fig. 2. Persistence of tyrosine rhythm in plasma during deprivation of protein. Blood was taken from two volunteers before (solid line) and after (broken line) 2 weeks on a low-protein diet (2.7 g of protein daily).

it could be peculiar to tyrosine. Feigin *et al.* (*6*) recently demonstrated that the total concentration of amino acids in human serum also varies diurnally with a rhythm greatly resembling our results with tyrosine (Fig. 1). They found the total amino acid concentration lowest (252 μg/ml) at 0400 hours; by noon it had risen to a peak value of 279 μg/ml.

Our studies indicate that the daily rise in tyrosine alone can account for at least one-fourth of the amplitude of the "total amino acid" rhythm, so one cannot yet say whether all amino acids or only three or four key compounds show diurnal rhythms in their concentrations in plasma. The plasma levels of some amino acids (such as tyrosine and tryptophan) may be uniquely susceptible to rhythmic changes, since their metabolic transformations are catalyzed by enzymes (tyrosine transaminase and tryptophan pyrrolase) whose activities vary diurnally (see *2* and *7*).

Preliminary studies with rats indicate that tyrosine levels in plasma do fall soon after the daily rise in activity of hepatic tyrosine transaminase. This finding suggests that one basis for daily rhythms in the amino acid concentrations in plasma may be inverse cycles in the activities of their catabolizing enzymes, in which case the tyrosine rhythm, at least, would be independent of the adrenal cortex (*2*).

An alternative hypothesis for the genesis of the tyrosine rhythm in plasma may be that it is produced by the adrenocortical secretory cycle. In normal human subjects, the concentration of 17-hydroxycorticoids in plasma is lowest between midnight and 0400 hours, when it rises sharply to peak levels at 0600 to 0800 hours (*8*). Adrenocortical steroids depress tyrosine levels in plasma (*9*), possibly by stimulating uptake of the amino acid into liver and other tissues. Hence the nocturnal rise in tyrosine levels in plasma may result from a decline in adrenal steroid secretion several hours earlier. Similarly, tyrosine levels in plasma may begin to fall at 1030 hours because the concentration of 17-hydroxycorticoids in the blood has risen 2 or 3 hours earlier. Ultimate evaluation of this hypothesis awaits studies on subjects whose adrenocortical secretory rhythm has been abolished by pituitary or adrenal disease. However, preliminary experiments with rats suggest that the tyrosine rhythm in plasma does persist after hypophysectomy.

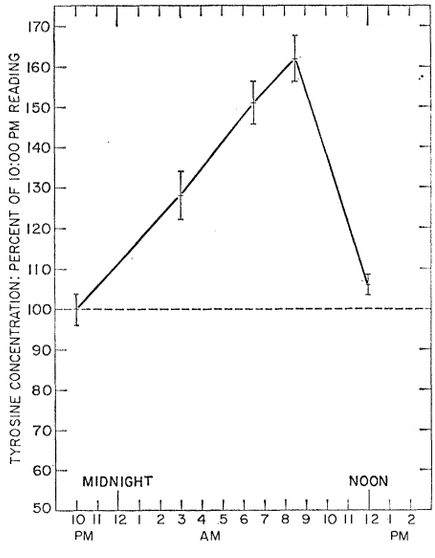

Fig. 3. Persistence of tyrosine rhythm in plasma during deprivation of protein. Blood was sampled at 2200, 0300, 0630, 0830, and 1200 hours from eight volunteers who had been fed 2.7 g of protein daily for 2 weeks. Horizontal bars represent standard errors of the means.

Among subjects maintained on a low-protein diet, the tyrosine concentration in plasma was very low at 2200 hours and rose significantly between then and 0300 hours (Fig. 3). However, subjects fed a diet containing normal amounts of protein had intermediate tyrosine levels in plasma at 2200 hours, and lowest levels 4 hours later. This difference in the phasing of the tyrosine rhythm may have resulted from the contribution of dietary tyrosine to levels of amino acids in the bloods of the group fed the normal diet.

It seems likely that the tyrosine rhythm is generated by an unknown endogenous "signal" which acts to raise tyrosine levels in plasma early each morning. Various oscillating and nonoscillating exogenous inputs, including food intake and physical exercise, may probably produce "noise" that modifies the expression of this signal. Several physiologic processes, in addition to tyrosine transamination and adrenocortical secretion, may provide the endogenous signal: these include changes in the biosynthesis of tyrosine from phenylalanine, changes in the secretion of growth hormone or thyroxine, with secondary alterations in the rate of incorporation of circulating tyrosine into tissue protein, and changes in the size of the body-fluid compartment in which tyrosine is distributed.

The observation that the tyrosine concentration in plasma varies diurnally in the normal human necessitates reevaluation of the relations between certain disease states and "normal tyrosine levels." The concentration of tyrosine in plasma taken at 0900 hours from fasting subjects is reportedly greater in hyperthyroid than in euthyroid individuals (*9*). This difference may result from some fundamental alteration in the manner in which the hyperthyroid person metabolizes tyrosine; but it may also represent desynchronization of the normal tyrosine rhythm. It is possible that the tyrosine concentration in the plasma of hyperthyroid subjects is normal or even subnormal later in the day. The physiological significance of the tyrosine rhythm in plasma is not yet clear.

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