viously described[4]. Rats were killed by decapitation at 10.00 h, i.e. 14 h after the initiation of the sucrose diet. Serum was obtained for determination of corticosterone by radioimmunoassay (Radioassay Systems Laboratories, Inc., Carson, CA).

The entire small intestine was removed. The duodenum was discarded and the jejunoileum was divided into 3 equal parts along its length. The proximal third of jejunoileum (jejunum) was flushed with ice cold saline and mucosa was scraped, using a microscope slide. Jejunal mucosa was homogenized with 4 volumes of 10 mM potassium phosphate buffer (pH 7.0). Sucrase and maltase activity was assayed according to Dahlqvist[9] and lactase activity according to Koldovský et al.[10]. Protein was determined by the method of Lowry et al.[11].

Results and discussion. All results are summarized in the table. Since starvation can influence the specific activity of lactase[12-14], we considered it important to control the food intake in sham-operated and adrenalectomized rats by force feeding. During the 5-day-period of force feeding, all animals gained body weight. It is noteworthy that in adrenalectomized rats, the body weight gain was significantly higher than in sham-operated rats. The protein content per intestinal segment was practically the same in all 4 groups.

Feeding of the sucrose diet for 14 h led to a marked increase of lactase, sucrase and maltase activity (specific, i.e. per protein and total, i.e. per segment). This result confirms our previous report[15] obtained on animals fed ad libitum.

The same experiment was performed on adrenalectomized rats. The success of adrenalectomy was confirmed by inspection and determinations of serum corticosterone levels. The serum levels of corticosterone of adrenalectomized rats correspond to those reported earlier[16,17]. Since no special precautions were taken to prevent a stress situation during the sacrificing of the animals (by decapitation), the values found in intact animals are approximately 2 times higher than those reported by others[16,17].

Force feeding of the sucrose diet to adrenalectomized rats led to an increase of lactase, sucrase and maltase activities to the levels that were observed in the sham-operated sucrose-fed animals. Therefore, we conclude that activity of the 3 disaccharidases is elevated by an increased intake of dietary carbohydrate without the involvement of adrenals.

Whereas the response of lactase activity to dietary changes has not been reported previously, our results concerning sucrase and maltase activities confirm and solidify the conclusions from published studies of Deren et al.[18]. They showed the increase of the activity of these 2 α-disaccharidases in adrenalectomized rats refed ad libitum sucrose diets after 3 days of starvation. However, Deren et al.[18] did not verify the success of adrenalectomy and did not measure the food intake.

From a developmental point of view, it is of note that whereas precocious increase of sucrase activity in suckling rats induced by dietary sugars is essentially dependent on adrenals[19,20], in adult rats dietary induced increase of sucrase activity is independent of adrenals.

1 Supported by the National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA (AM 27624).

2 McCarthy, D.M., Nicholson, J.A., and Kim, Y.S., Am. J. Physiol. *239* (1980) G445.
3 Bustamante, S., Gasparo, M., Kendall, K., Coates, P., Brown, S., Sonawane, B., and Koldovský, O., J. Nutr. *111* (1981) 943.
4 Yamada, K., Bustamante, S., and Koldovský, O., Biochim. biophys. Acta *676* (1981) 108.
5 Celano, P., Jumawan, J., and Koldovský, O., Gastroenterology *73* (1977) 425.
6 Boyle, J.T., Kelly, K., Krulich, L., and Koldovský, O., Am. J. Physiol. *243* (1982) G359.
7 Raul, F., Noriega, R., Nsi-Emvo, E., Doffoel, M., and Grenier, J.F., Gut *24* (1983) 648.
8 Hahn, P., and Koldovský, O., Utilization of nutrients during postnatal development. Pergamon Press, London 1967.
9 Dahlqvist, A., Analyt. Biochem. *7* (1964) 18.
10 Koldovský, O., Asp, N.G., and Dahlqvist, A., Analyt. Biochem. *27* (1969) 409.
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
12 Ecknauer, R., Biomedicine *29* (1978) 129.
13 Yamada, K., Goda, T., Bustamante, S., and Koldovský, O., Am. J. Physiol. *244* (1983) G449.
14 Raul, F., Noriega, R., Doffoel, M., Grenier, J.F., and Haffen, K., Enzyme *28* (1982) 328.
15 Yamada, K., Bustamante, S., and Koldovský, O., FEBS Lett. *129* (1981) 89.
16 Buckingham, J.C., and Hodges, J.R., J. Endocr. *63* (1974) 213.
17 Gwosdow-Cohen, A., Chen, C.L., and Besch, E.L., Proc. Soc. exp. Biol. Med. *170* (1982) 29.
18 Deren, J.J., Broitman, S.A., and Zamcheck, N., J. clin. Invest. *46* (1967) 186.
19 Lebenthal, E., Sunshine, P., and Kretchmer, N., J. clin. Invest. *51* (1972) 1244.
20 Goda, T., Yamada, K., Bustamante, S., Edmond, J., Grimes, J., and Koldovský, O., Ped. Res. *18* (1984) 203 abstr.

0014-4754/84/111287-02$1.50 + 0.20/0

Serotonin and 5-hydroxyindoleacetic acid concentrations in individual hypothalamic nuclei and other brain areas of rat

K. Oomagari, H. Uchimura, T. Matsumoto, H. Yokoo, M. Hirano, J.S. Kim and T. Nakahara

Department of Neuropsychiatry, Faculty of Medicine, Kyushu University, Maidashi 3-1-1, Fukuoka 812 (Japan), Laboratory of Neurochemistry, Hizen National Mental Hospital, Kanzaki, Saga 842-01 (Japan), and Department of Chemistry, Faculty of Science, Kyushu University, Hakozaki, Fukuoka 812 (Japan), 29 November 1983

Summary. Serotonin and 5-hydroxyindoleacetic acid (5-HIAA) were measured in individual nuclei of rat hypothalamus and other brain areas using HPLC with electrochemical detection. 5-HIAA levels were first demonstrated in hypothalamic and some discrete brain areas. The 5-HIAA/5-HT ratio was highest in the n. caudatus putamen, high in the n. ventromedialis and lowest in the n. suprachiasmaticus.

Key words. Rat, brain; brain, rat; hypothalamus, rat; serotonin; 5-hydroxyindoleacetic acid.

The major metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), provides very good information as to serotonin neuron activities[1]. For determination of serotonin and 5-HIAA in localized regions of rat brain, a method with extremely high sensitivity is required. Radioenzymatic assay is most sensitive for measurement of serotonin[2], but does not allow deter-

mination of 5-HIAA. On the other hand, the GC-MS method[3] for measurement of 5-HIAA is specific, but its sensitivity is insufficient for measurement of 5-HIAA in small discrete regions such as hypothalamic nuclei. Recently, highly selective and sensitive HPLC methods using electrochemical detection (ECD) for simultaneous measurement of serotonin and its metabolite in brain tissue have been developed[4-8]. We[9] modified Lacković's[7] method using 5-hydroxyindole as an internal standard, and improved the sensitivity. The detection limits of this system were about 2 picograms for each compound. In the present communication, we describe serotonin and 5-HIAA concentrations in the discrete areas of prefrontal cortex, limbic areas and hypothalamus using this method.

Adult male Wistar-King rats weighing 400–450 g were used. Animals were housed in group 5 with food and water available ad libitum and a 12-h day/night cycle. The animals were killed at 13.00 h by decapitation and the brains were immediately removed and frozen at −80 °C. Serial slices 300 µm thick were made in a cryostat at −12 °C and mounted on glass slides. The glass slides were placed on a metal plate that was cooled electrically to −25 °C, and 13 brain regions were carefully dissected freehand with a microknife under a stereomicroscope according to the atlases of König and Klippel[10] and Lindvall et al.[11]. Dissected hypothalamic nuclei and other brain areas are shown in the figure. The dissected frozen tissues were immediately placed in a microhomogenizer which was cooled at −45 °C to prevent them from thawing. The tissues were homogenized in 70–300 µl of ice-cold 10% (w/v) $ZnSO_4$ containing 0.002% (w/v) ascorbic acid. Following homogenization, 30 µl 0.5 N NaOH was added to 40 µl homogenate. After centrifugation (10,000 × g, 5 min), internal standard (5-hydroxyindole) was added and 20 µl of supernatant was injected into HPLC. Protein in the homogenate was determined by the method of Lowry et al.[12].

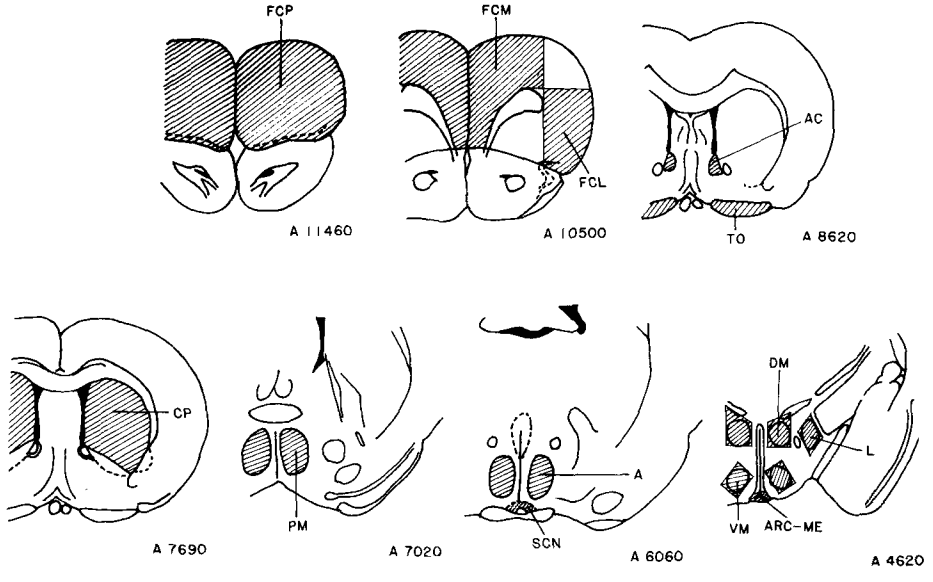
The HPLC system consisted of model 6000A (Waters) pump set to flow rate 2.0 ml/min, a reverse phase column (µ Bondapak C-18, 300 × 4 mm, Waters) and ECD (LC-4A, Bioanalytical System) with carbon paste/silicon oil composition (CPS) electrode set to a potential of 0.5 V versus a Ag/AgCl reference electrode. The mobile phase was prepared by mixing 0.1 M citric acid with 0.2 M sodium phosphate dibasic to pH 4.8. The buffer was diluted with an equal volume of water, degassed under vaccum, filtered and 8% methanol added. Standard curves for serotonin and 5-HIAA were obtained between 2 pg and 500 pg per 20 µl injection.

The concentrations of serotonin and 5-HIAA are shown in the table. There was a 3.6-fold difference between the lowest and highest serotonin levels measured. Serotonin levels in prefrontal cortex, n. preoptic medialis, n. hypothalamicus anterior, n. dorsomedialis and n. ventromedialis were in general agreement with those described by Saavedra et al.[13,14]. But they reported very high serotonin concentrations compared with our results in n. accumbence, tuberculum olfactorium, n. suprachiasmatics, n. hypothalamicus lateralis, n. arcuatus and median eminence. On the other hand, our results are similar to those obtained by other HPLC-ECD methods[5,8]. Therefore this discrepancy between our results and those of Saavedra et al. may due to the methodological difference between the HPLC method and radioenzymatic assay. In the present study, 5-HIAA levels in the nuclei of the hypothalamus, n. suprachiasmaticus and some limbic areas were first demonstrated. The distribution of 5-HIAA was relatively uneven throughout the discrete brain areas examined. A 2.5-fold difference was found between the nucleus with the lowest (n. suprachiasmaticus) and the highest (n. hypothalamicus lateralis). As to 5-HIAA/5-HT ra-

Distribution of 5-HT and 5-HIAA in the prefrontal cortex, limbic areas and hypothalamus

Nucleus and area	5-HT	5-HIAA	5-HIAA/5-HT
Prefrontal cortex			
polar field	4.52 ± 0.42 (8)	1.60 ± 0.14 (8)	0.35
medial field	5.18 ± 0.12 (9)	1.77 ± 0.11 (9)	0.34
lateral field	6.32 ± 0.23 (8)	1.76 ± 0.07 (7)	0.28
N. accumbens	5.19 ± 0.30 (8)	1.72 ± 0.07 (7)	0.33
Tuberculum olfactorium	9.88 ± 0.47 (9)	2.59 ± 0.16 (9)	0.26
N. caudatus putamen	3.34 ± 0.11 (9)	1.92 ± 0.14 (9)	0.57
N. preopticus medialis	8.23 ± 0.48 (5)	2.46 ± 0.06 (5)	0.30
N. hypothalamicus anterior	8.81 ± 0.63 (6)	2.68 ± 0.23 (6)	0.30
N. suprachiasmaticus	6.27 ± 0.26 (5)	1.48 ± 0.10 (5)	0.24
N. hypothalamicus lateralis	11.91 ± 0.27 (6)	4.05 ± 0.13 (6)	0.34
N. dorsomedialis	9.97 ± 0.32 (7)	3.18 ± 0.08 (7)	0.32
N. ventromedialis	6.21 ± 0.45 (8)	2.40 ± 0.11 (8)	0.42
N. arcuatus-median eminence	5.35 ± 0.51 (8)	1.98 ± 0.23 (7)	0.37

The results were expressed as mean ± SEM ng/mg protein (number of determinations).



Schematic representation of microdissected brain nuclei according to the atlases of König and Klippel[10] and Lindvall et al.[11]. Abbvreviations are as follows: FCP, Prefrontal cortex polar field; FCM, Prefrontal cortex medial field; FCL, Prefrontal cortex lateral field; AC, Nucleus accumbens; TO, Tuberculum olfactorium; CP, Nucleus caudatus putamen; PM, Nucleus preopticus medialis; A, Nucleus hypothalamicus anterior; SCN, Nucleus suprachiasmaticus; L, Nucleus hypothalamicus lateralis; DM, Nucleus dorsomedialis; VM, Nucleus ventromedialis; ARC-ME, Nucleus arcuatus-median eminence.

tio, however, 2 times difference between the highest and the lowest was found. The 5-HIAA/5-HT ratio was highest in n. caudatus putamen, higher in n. ventromedialis and lowest in n. suprachiasmaticus. These results suggest that the turnover rate of serotonin, reflected by the ratio 5-HIAA/5-HT, is different in each brain area examined. Gaudin-Chazal et al.[15] showed negative correlation between 5-HIAA/5-HT ratio and 5-HT level in different structures of cat brain. In discrete rat brain areas examined, however, such a correlation was not found. Our highly sensitive method for simultaneous measurement of serotonin and 5-HIAA by HPLC-ECD made it possible to study the serotonin neuron function even in discrete hypothalamic nuclei.

1 Reinhard, J.F. Jr, and Wurtman, R.J., Life Sci. *21* (1977) 1741.
2 Saavedra, J.M., Brownstein, M., and Axelrod, J., J. pharmac. exp. Ther. *186* (1973) 508.
3 Cattabeni, F., Koslow, S.H., and Costa, E., Science *178* (1972) 166.
4 Mefford, I.N., and Barchas, J.O., J. Chromat. *181* (1980) 187.
5 Koch, D.D., and Kissinger, P.T., Life Sci. *26* (1980) 1099.
6 Reinhard, J.F. Jr, Moskowits, M.A., Sved, A.F., and Fernsrom, J.D., Life Sci. *27* (1980) 905.
7 Lacković, Z., Parenti, M., and Neff, N.H., Eur. J. Pharmac. *69* (1981) 347.
8 Johnson, M.D., and Crowley, W.R., Life Sci. *31* (1982) 589.
9 Oomagari, K., Uchimura, H., Matsumoto, T., Yokoo, H., Hirano, M., Shimomura, M., and Nakahara, T., Neurosciences, Jap. *8* (1982) 64.
10 König, J.F.R., and Klippel, R.A., The Rat Brain. Williams and Wilkins, Baltimore 1963.
11 Lindvall, O., Björklund, A., and Divac, I., Brain Res. *142* (1978) 1.
12 Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
13 Saavedra, J.M., Palkovits, M., Brownstein, M., and Axelrod, J., Brain Res. *77* (1974) 157.
14 Saavedra, J.M., Fedn Proc. *8* (1977) 2134.
15 Gaudin-Chazal, G., Daszuta, A., Faudon, M., and Ternaux, J.P., Brain Res. *160* (1979) 281.

0014-4754/84/111288-03$1.50 + 0.20/0

Genome size variation of rhizobia

S.K. Chakrabarti, A.K. Mishra and P.K. Chakrabartty

Department of Microbiology, Bose Institute, Calcutta 700009 (India), 22 August 1983

Summary. Slow-growing species of *Rhizobium* have a genome size almost double that of the fast-growing species. This provides additional support in favor of their being placed in separate genera.
Key words. *Rhizobium* genus; genome size; rhizobia, slow-growing; rhizobia, fast-growing; taxonomic classification.

The genus *Rhizobium* (family Rhizobiaceae) comprises the bacteria responsible for infecting roots of members of the plant family Leguminosae and inciting the production of morphologically defined nodules in which they fix nitrogen in symbiosis. The existing classification of rhizobia recognizes 6 species, i.e., *Rhizobium leguminosarum, R. phaseoli, R. trifolii, R. meliloti, R. japonicum* and *R. lupini,* based on cross-inoculation between the bacteria and their legume hosts, in the single genus *Rhizobium*[1]. Of these 6 species, the first 4 mentioned are fast growers as judged by in vitro growth on yeast extract media. However, the existing classification is considered inadequate and can be treated as only tentative. This is mainly because of mutual symbiotic promiscuity and the entire exclusion of other groups, such as the 'Cowpea miscellany' group[2]; these are slow growers and also symbiotically promiscuous.

The distinction between the slow-growing and fast-growing rhizobia was recognized in the 8th edition of Bergey's Manual[1], where they are placed in 2 separate groups under the genus *Rhizobium*. Studies on DNA base composition[3], nucleic acid hybridization[4], internal antigens[5] and numerical taxonomy[6] provide additional support in favor of their taxonomic spearation. Based on these studies it has been further argued[7] that these 2 groups of rhizobia should be placed in separate genera, considering their differences in properties. The name *'Brachyrhizobium'* has been proposed by Jordan[8] for a new genus to include the slow growers.

Knowledge of bacterial genome size has taxonomic implications[9] and is known to be useful in assessing the evolutionary relationships[10]. In a previous study, the genome sizes of 3 slow-growing species of rhizobia were estimated[11]. In the present study the genome sizes of 5 species of fast growing and slow-growing rhizobia were measured for a comparison to probe the proposal for splitting the genus *Rhizobium* into 2. Our study further supports the justification of the proposal for the separation of the 2 groups of rhizobia into separate genera.

Materials and methods. The bacterial strains: *R. meliloti* (SU216), *R. trifolii* (T19), *R. japonicum* (SB16) obtained from Dr K.R. Dadarwal, Haryana Agricultural University; and *R. phaseoli* (CC365), *R. leguminosarum* (SU391) acquired from Dr J. Brockwell, CSIRO, Australia, were used in this study. The bacteria were grown in yeast extract mannitol medium at 28 °C and harvested during the stationary phase of growth. DNA was extracted and purified by a modification of Marmur's method[12]. The final DNA preparation had absorbance ratios 260 nm/280 nm and 260 nm/230 nm of about 2 and greater than 2, respectively. The preparation had an amount of RNA contamination of less than 4% as mesured by solubility in cold perchloric acid[13]. DNA was heared by partial depurination and alkaline cleavage[14]. Hybridization was performed at 60 °C in 0.12 M phosphate buffer, pH 6.8 ($Na^+ = 0.18$ M) to the desired C_0t values. C_0t was calculated as: optical density$_{260\,nm}$ × time of incubation in h/2. Hybrid DNA was fractionated by chromatography on hydroxyapatite columns[15-17]. The concentration of single stranded and reassociated DNA was estimated from the column eluates and the percentage of reassociation was calculated. The data were plotted on a log-C_0t plot to estimate the $C_0t_{½}$ values of hybridization.

Results and discussion. Our previous study[11] showed that strains of slow-growing *Rhizobium* had a 2.5 to 3.5 times bigger genome size than that of *Escherichia coli.* The genomes of fast-growing rhizobia may well be smaller than those of slow-growing rhizobia. For a mesure we compared the genome size of a few fast- and slow-growing strains of rhizobia, by comparing the $C_0t_{½}$ of hybridization of rhizobial DNA with that of *E. coli* B DNA. It has been shown that there is an apparent proportionality between $C_0t_{½}$ of hybridization and the genome size of DNA[18]. The figure shows the reassociation kinetic profiles of DNA isolated from *R. meliloti* (SU216), *R. phaseoli* (CC365), *R. trifolii* (T19) and *R. leguminosarum* (SU391), all of which are fast-growers, and of *R. japonicum* (SB16) which is a slow-grower. All the DNAs hybridized to the extent of 90% or more at a C_0t of 100. No significant hybridization was observed below a C_0t of 0.1. All the curves followed a second order kinetic pattern typical of bacterial DNA reasso-