ferences in the mean percentages of correct answers, we found the F ratios at chance level for tasks (i) and (ii): F_{i} (2, 23) = 2.6; F_{ii} (2, 23) = 3.0. A t-test for percentage correct differences between the two workloads of task (iii) was at chance level: t (24) = 0.7. Similarly, neither the F-test for task (ii) response times, nor the t-test for task (iii) response times was significant: F (2, 23) = 1.1; t (24) = 0.2. Differences in average solution time between the three workloads of task (i) were marginally significant: F (2, 23) = 3.5; $P < .05$. Similar calculations for differences in mean accuracy and mean response time for practice items were significant for all tasks. The lack of significant differences between workloads of the tasks for both performance measures [except as noted for task (i)] suggests that the procedures used to equalize performance-related factors across subjects and tasks were effective.

8. Recorded with a Grass model 6 electroencephalograph. Two EEG technologists independently edited and discarded data contaminated by extracortical artifacts. Reference was ipsilateral ear in experiment 1, linked ears in experiment 2. Forehead was at ground potential. The effect of reference electrode on measurements of asymmetry are discussed by A. Amochaev and A. Salamy (*Psychophysiology*, in press) who found slightly larger task-related EEG asymmetries when electrode reference was to ipsilateral ears as compared with C_z.
9. Features used were spectral intensity averaged over the band; ratios of banded spectral intensity for the following pairs: F3-F4, C3-C4, P3-P4, O1-O2, C3-P3, P3-O1, C4-P4, P4-O2. Ratios were of form $(a - b)/(a + b)$. In each study, approximately 30 minutes of EEG from each task was used in the multivariate analysis. For technical details, see A. S. Gevins, C. L. Yeager, S. L. Diamond, J. P. Spire, G. M. Zeitlin, A. H. Gevins, *IEEE Proc.* **63**, 1382 (1975); A. S. Gevins, C. L. Yeager, G. M. Zeitlin, S. Ancoli, M. S. Dedon, *Electroencephalogr. Clin. Neurophysiol.* **42**, 267 (1977); A. S. Gevins, G. M. Zeitlin, S. Ancoli, C. L. Yeager, *ibid.* **43**, 31 (1977); A. S. Gevins, G. M. Zeitlin, C. D. Yingling, J. C. Doyle, M. S. Dedon, J. H. Henderson, R. E. Schaffer, J. J. Roumasset, C. L. Yeager, *ibid.*, in press; A. S. Gevins *et al.*, *ibid.*, in press.
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11. In experiment 1, a randomly selected trial of the two or three 1-minute trials of each task by each subject was placed in the test data set for validating the decision rule derived from the remaining trials (method 1). In another series of classifications, the test data set was composed of the observations from eight subjects randomly selected from the total group of 23, with similar results (method 2). Both methods were also used in experiment 2. For method 1, a randomly selected one-third of all trials was saved for the test set. Method 2 was used with seven of the 21 subjects comprising the test set. This was repeated for several different groups of seven subjects comprising the test set. Except for minor differences, results and conclusions were the same. Results of experiments 1 and 2 reported in Tables 1 and 2 were obtained with methods 1 and 2, respectively.
12. Estimates of the significance of these pairwise classifications obtained from the binomial probability distribution may be positively biased by intrasubject correlation of multiple observations of a single type of task. In experiment 1, even under the most extreme assumption of intrasubject correlation, that is, that the number of independent observations of each task is equal to the number of subjects (23), each of the pairwise classifications of the tasks listed in Table 1 achieves better than $P = .01$. None of the classifications in experiment 2 reach this level of significance under the same stringent assumption. (Since there were 21 subjects in experiment 2, 77 percent classification accuracy was required for a $P < .01$ significance. Based on 23 subjects, classification accuracy for experiment 1 of 74 percent or more was significant at $P < .01$.) Under the most lenient assumption, that is, that each trial of a task constitutes an independent observation, all of the classifications in experiment 2 reach this level of significance. If one assumes moderate intrasubject correlation such that the effective number of observations of a task is half the number of trials of a task, the active task versus the visual fixation task classifications are highly significant ($P < 10^{-5}$), whereas the active task versus the active task classifications fail to achieve the same level of significance ($P = .02$ for rotation of blocks versus addition; $P = .03$ for rotation of blocks versus letter substitution; $P = .06$ for letter substitution versus addition).
13. For display purposes, intensities measured for each task were grouped with those for visual fixation and transformed to standard scores. This was performed separately for each subject. The mean and standard deviation across subjects of these standard scores are shown in Fig. 1. To maximize classification performance, standardization was over the two tasks involved in each pattern recognition analysis. Descriptive statistics were computed for the unstandardized EEG feature values as well as for those standardized as described. Since only minor differences were found between the three sets of data, our conclusions are not attributable to the standardization method used.
14. An analysis of variance by task and scalp location for θ intensity (active tasks standardized against visual fixation) yielded significant F ratios for task [F (1, 352) = 126.02; $P < .001$] and scalp location [F (7, 352) = 4.23; $P < .001$], yet no significant interaction [F (7, 352) = 0.9]. A similar analysis of β intensities yielded a marginally significant F ratio for task [F (1, 352) = 4.5; $P < .05$], a significant F ratio for scalp placement [F (7, 352) = 5.9; $P < .001$], and no significant interaction [F (7, 352) = 0.6]. When this analysis was performed for α intensities, only the scalp location was marginally significant [F (7, 352) = 2.58; $P < .05$], with task [F (1, 352) = 3.6] and task by scalp location [F (7, 352) = 0.4[not being significant. Correlated t-tests comparing α intensities between the two tasks were significant for F3 [t (22) = 6.0; $P < .001$], and not significant for F4 [t (22) = 2.0]. The data for Koh's block design and writing from memory were also analyzed with statistical methods identical to those applied to data collected from the same tasks from the same electrodes (P3 and P4) (*2*). Application of Wilcoxon matched pairs signed ranks test to the difference in right-left ratios of parietal α-band spectral intensity between Koh's block design and writing confirmed previous results, that is, a positive sign for the difference was found in 18 out of 23 subjects ($P < .01$). However, the results in Fig. 1, left center, show that this difference between ratio measurements is attributable to a reduction in α-band intensity over the left parietal (P3) electrode, during writing.
15. No significant differences were found in parietal α ratios between block rotation and addition when Wilcoxon's test was applied as described in (*14*). To test the effect of having shortened the time per task in experiment 2, the Koh's blocks versus writing discrimination of experiment 1 was repeated with the first 15-second segment of data from each task. This segment was even more strongly differentiated than was the full minute (test set classification of 98 percent versus 85 percent respectively); the topographic distribution of features was essentially the same.
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Human Muscular Dystrophy: Elevation of Urinary Dimethylarginines

Abstract. *The amounts of the dimethylarginines* N^G,N^G*-dimethylarginine (DMA) and* N^G,N'^G*-dimethylarginine (DM'A) excreted in the urine of muscular dystrophic patients were examined and compared with the amounts excreted by normal controls, patients with other types of neuromuscular diseases, and patients with disuse muscle atrophy resulting from traumatic paralysis. The patients with muscular dystrophy excreted high concentrations of DMA and this urine showed high ratios of DMA to DM'A. This finding indicates a relation between protein methylation processes and muscular dystrophy.*

Although the urinary amino acids and peptides in patients with muscular dystrophies have been examined (*1*-*3*), no consistent finding has been reported, presumably because of the lack of quantitative precision of the analytical techniques employed. Our laboratory has focused on studying the ninhydrin-positive constituents in normal human urine by means of a one-column, ion-exchange chromatographic system developed by Hamilton (*4*). This system, with its high sensitivity (1×10^{-9} mole) and high resolution, has enabled us to establish a clear chromatographic pattern for the complex urinary amino acids and their derivatives (*5*). Recently, we have used the system to examine the urine of patients with muscular dystrophies and have observed that, compared to normal controls, these patients excrete larger amounts of N^G,N^G-diamethylarginine (DMA), and their urine shows a characteristic high ratio of DMA to N^G,N'^G-dimethylarginine (DM'A). The results of our study suggest that this disease may be associated with the altered metabolism of the arginine methylated proteins.

We have studied 12 patients with muscular dystrophy (ten with the Duchenne type, one with the Becker type, and one female with possible Duchenne type); five patients with other neuromuscular diseases (one with spinal muscular atrophy resulting from traumatic paralysis; and ten normal control subjects of comparable age (5 to 16 years). Urine was collected from each individual early in the morning and was stored without preservatives at −80°C pending analysis. Standard DMA, DM'A, and monomethylarginine were purchased from Calbiochem.

The ion-exchange chromatographic

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system for amino acid analysis has been described (*4*). Urine (500 μl) was treated by adding 15 percent sulfosalicylic acid (100 μl). Fifty microliters of the protein-free supernatant were applied on the column and eluted with citrate buffer (five concentrations in sequence) as reported (*4*) except that the *p*H of sodium citrate buffer (3.8; V_3) was lowered to *p*H 3.74 and the temperature of the column was programmed as T_1 (36°C) from 0 to 280 minutes and 880 to 1160 minutes; T_2 (63.5°C) from 280 to 880 minutes and 1160 minutes until the end of the run.

By using the modified procedure, the resolution of the basic amino acid region has been greatly improved. Both DMA and DM'A, as well as carnosine, homocarnosine, and tryptophan, could be completely resolved from the neighboring ninhydrin-positive peaks.

Our results are summarized in Table 1. Because of the characteristic low excretion value of creatinine in patients with muscular dystrophies we express the concentration of amino acids as micromoles per gram of creatine plus creatinine. The Jaffe reaction was used for creatinine determination according to the technique of DiGiorgio (*6*). Creatine was converted to creatinine and measured by the Jaffe reaction. The urine of the muscular dystrophic patients contained more than twice the amount of DMA and slightly more DM'A than the urine of the normal subjects. The amount of arginine excreted remained the same in both groups. 3-Methylhistidine, which is an end-product of muscle proteins (*7*), was not significantly increased in the muscular dystrophic patients.

Patients with other types of neuromuscular diseases and patients with disuse muscular atrophy excreted DMA and DM'A in quantities similar to normal control values (Fig. 1).

Figure 2 depicts a comparison of the ratios of DMA to DM'A among all four groups studied. Patients with muscular dystrophies showed distinctively higher ratios (mean value 2.59), independent of their disease state whereas patients with the other neuromuscular diseases or with disuse muscular atrophy showed ratios within the range of the normal children (mean value 1.38).

The presence of dimethylarginines in normal human urine was first reported by Kakimoto and Akazawa (*8*). Subsequently, these substances were established to be endogenous in origin (*8*, *9*). Kakimoto *et al.* (*10*) investigated the distribution of dimethylarginines in various mammalian organs, and found that DM'A is predominantly located in the myelin whereas DMA is widely distributed in tissues such as the lung, brain, liver, and intestine. Investigations to date have shown that DMA is mainly localized in the cell nuclei as the structural unit of nonhistone nuclear proteins (*10*, *11*). Neither DMA nor DM'A is found in muscle proteins except in the developing leg muscle of chickens and rabbits (*12*). It has been established that only the arginines in nascent proteins could be methylated selectively by a specific enzyme, protein methylase I (adenosylmethionine: protein arginine methyltransferase; E.C. 2.1.1.23) (*13*). The dimethylarginines are the degradation products of such proteins and were found to have a high threshold of clearance. These amino acid derivatives are considered to be a useful index of the turnover rate of the arginine methylated proteins (*8*, *9*).

Table 1. Urinary excretion of dimethylarginines and 3-methylhistidine in normal and muscular dystrophic children. The amino acids were measured in micromoles per gram of creatine plus creatinine.

	Ratio of DMA to DM'A	Amino acids			
		DMA	DM'A	Arginine	3-Methylhistidine
		Normal children (N = *10*)			
Range	1.11 to 1.95	35.1 to 71.4	30 to 64	10 to 40	120 to 385.2
Mean ± S.D.	1.38 ± 0.26	57.2 ± 10.6	42.3 ± 10.1	22.6 ± 9.8	216.4 ± 81.9
		Patients with muscular dystrophy (N = *12*)			
Range	1.3 to 4.24	75.8 to 211.9	22.8 to 101	11.4 to 57.7	195.5 to 369.1
Mean ± S.D.	2.59 ± 0.9	125.6 ± 38.6	53.1 ± 20.7	25.4 ± 11.9	276.4 ± 67.2

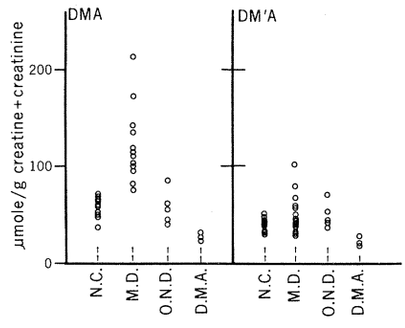

Fig. 1. The excretion levels of DMA and DM'A in the urines of normal children (*N.C.*; $N = 10$), muscular dystrophic patients (*M.D.*; $N = 12$), patients with other neuromuscular diseases (*O.N.D.*; $N = 5$), and patients with disuse muscle atrophy (*D.M.A.*; $N = 3$).

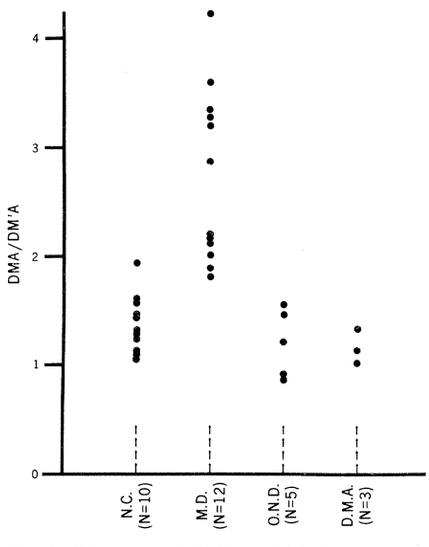

Fig. 2. The ratio of DMA to DM'A in normal children (*N.C.*) and patients with muscular dystrophies (*M.D.*), other neuromuscular diseases (*O.N.D.*), and disuse muscle atrophy (*D.M.A.*).

On the basis of our observations it is reasonable to speculate that a fast turnover rate of the arginine methylated proteins occurs in certain tissues of patients with muscular dystrophy. Since DMA is found in the myosin of developing leg muscle in animals, it is conceivable that the high excretion rate of DMA in the Duchenne and Becker patients may reflect myosin turnover in muscle regenerating from satellite cells. It is also possible that some of the methylarginines may originate from tissues other than muscle because we found that the concentration of 3-methylhistidine in the urine of muscular dystrophic patients was not as significantly increased as that of DMA (Table 1). Kakimoto and associates (*8*) found that brain tissue is a rich source of DMA-containing proteins. Paik *et al.* (*14*) reported that brain contains high protein methylase I activity. In addition, muscular dystrophic patients have lower average intelligence quotients (*15*, *16*). Recently, cerebral atrophy was observed in patients with Duchenne muscular dystrophy (*17*). Brain tissue, therefore, could be one of the sites for the alteration of the metabolism of the arginine methylated proteins.

Carnegie *et al.* (*9*) have studied the excretion of DMA and DM'A in relation to liver diseases. They observed a slight increase in the ratio of DMA to DM'A in patients with liver disease (1.31) as compared to normal adults (1.08). We also

observed a one-to-one ratio of DMA to DM′A in normal adults, in agreement with the findings of Kakimoto and Akazawa (*8*) and Carnegie *et al.* (*9*). The ratio of DMA to DM′A changes much more in muscular dystrophies than in liver diseases (*9*). This change, which may relate directly to the etiology of this disease, could, perhaps, be used as a marker for muscular dystrophies and thus provide a means for earlier or preclinical diagnosis of this disease.

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Nitrogenous Nutrition of Marine Phytoplankton in Nutrient-Depleted Waters

Abstract. *Variability in the small-scale temporal and spatial patterns in nitrogenous nutrient supply, coupled with an enhanced uptake capability for nitrogenous nutrients induced by nitrogen limitation, make it possible for phytoplankton to maintain nearly maximum rates of growth at media nutrient concentrations that cannot be quantified with existing analytical techniques.*

Several investigators have described the role that nitrogen plays in regulating the growth of natural populations and laboratory cultures of marine phytoplankton. The rates by which phytoplankton assimilate nitrogen and grow can reasonably be approximated by rectangular hyperbolic functions of either the aqueous (external) nitrogenous nutrient concentration (*1*–*3*) or the cellular nitrogen content (cell quota) (*4*, *5*), or both, depending upon growth conditions. Similar results have been demonstrated for other nutrients under limiting conditions (*6*). Goldman (*7*) recently showed that under steady-state conditions in continuous cultures phytoplankton growth rates and nutrient uptake rates were coupled and could be described simultaneously by external and cellular nutrient concentrations.

In natural marine environments, particularly in impoverished oceanic waters, steady-state conditions for bacterial or phytoplankton nutrition are seldom, if ever, established (*8*), partly because the temporal and spatial patterns in the fluxes of required nutrients into and within the euphotic zone are highly episodic. In several recent studies nitrogenous nutrients in the near-surface waters of the North Atlantic and adjacent seas have been below the limit of detectability, even though considerable photosynthetic activity was noted (*9*, *10*). The absence of measurable concentrations of nitrogenous nutrients in the environment of physiologically active phytoplankton raises the perplexing but crucial question of how nitrogen is made available and utilized by marine phytoplankton in these oceanic waters.

From recent laboratory studies (*11*, *12*) it is evident that, under conditions of nitrogen starvation and during time intervals that are short relative to cell generation periods, the nitrogen uptake rates of marine phytoplankton can exceed the nitrogen uptake rates required for population growth. The major implication of these findings is that a phytoplankton cell need only be exposed to intermittent pulses of nitrogen in order to acquire its daily ration of this nutrient. Such situations conceivably could exist when cells randomly and perhaps frequently pass into microenvironments in which nitrogenous nutrient concentrations are elevated as a result of either metabolic waste excretion by animals or the degradation of organic matter by bacteria. This phenomenon has been addressed conceptually (*13*), but we believe that its significance in the open ocean has not been fully realized because the critical response period of the phytoplankton to nutrient pulses is on the order of minutes rather than tens to hundreds of minutes (*12*). This hypothesis would be consistent with our recent observations that, in oceanic waters where dissolved nitrogenous nutrients were undetectable, rates of primary productivity were measurable.

We explored this question by assessing the short-term nitrogen uptake capabilities of two clones of the marine diatom *Thalassiosira pseudonana* in NH_4^+-limited continuous cultures. Clone 3H was originally isolated from Long Island Sound, and clone 13-1 was from the Sargasso Sea (*14*). Steady-state levels of biomass were first established at different growth rates, and then short-term uptake experiments with added $^{15}NH_4^+$ were carried out immediately, 20 minutes after, and 1 hour after the nutrient supply pump was stopped. Complete details of the continuous culture design and function, culturing protocols, and

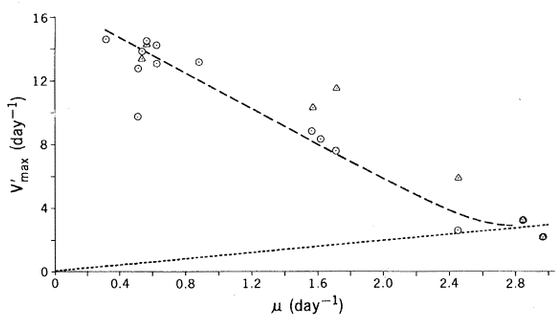

Fig. 1. Maximum rate of NH_4^+ uptake per unit of plant nitrogen (V'_{max}) at specific growth rates (μ) for *Thalassiosira pseudonana* (clone 3H) grown in a NH_4^+-limited chemostat. The circles represent rates determined immediately and 20 minutes after the nutrient supply ceased. The triangles represent rates determined 120 minutes after the nutrient supply ceased. Rates of NH_4^+ uptake adequate to meet the requirements of growth are depicted by the dotted line.

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