of relative cortical-subcortical function are valid, they present far-reaching implications for the interpretation of cerebral function.

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4 April 1957

Differential Excretion of D-Phenylalanine in Man

In the course of an investigation into the extent of genetic control over certain aspects of phenylalanine metabolism in man, a major discrepancy was noted between the results of two methods used in determining urinary phenylalanine after ingestion of the L form of this amino acid. The two methods were one-dimensional paper chromatography, in which a butanol-acetic acid-water mixture was used, and a modified form of the decarboxylase method of Udenfriend and Cooper (*1*). The paper chromatographic results were markedly higher than the decarboxylase values and were well beyond any methodologic differences. Since the decarboxylase method is specific for L-phenylalanine, while chromatographic techniques do not distinguish between the L and D forms, the simplest explanation of these results was that the ingested phenylalanine was racemic. Consequently, tests were run to examine this hypothesis, and they clearly demonstrated that the original substrate was slightly racemic. The purpose of this report is to point out the possibility and implications of minor isomeric contamination in studies of amino acid metabolism and to consider the interesting variation in excretion rate of D-phenylalanine that was observed among individuals in these experiments (*2*).

Meister *et al.* (*3*) have discussed the problem of determining the degree of optical purity of various amino acids. They point out that techniques such as polarimetry cannot detect isomeric impurities of less than 1.00 mg percent, while the use of oxidases and decarboxylases in properly designed Warburg experiments can detect isomeric contamination of less than 0.10 mg percent. In this work, the presence of D-phenylalanine was demonstrated by measuring the oxygen uptake on incubation with D-amino acid oxidase, according to Burton (*4*). The original sample of phenylalanine was run in the Warburg apparatus with known amounts of D-phenylalanine as controls, and oxygen uptake occurred equivalent to 0.60 mg percent of D-phenylalanine. A second sample of L-phenylalanine from a different source, but not used in these metabolic experiments, was tested in a similar manner and was found to contain 0.17 mg percent of D-phenylalanine.

For most biochemical and physiological experiments, such minor impurities would probably be undetectable. However, in studies that involve recovery of ingested amino acids from the urine, isomeric contamination of the order of 0.10 mg percent could lead to serious error. This follows from the fact that the kidney acts as a highly selective filter, retaining most L-amino acids with an efficiency of more than 95 percent, while the D forms are excreted quite readily (*5, 6*).

In the present studies, 2 g of the L-phenylalanine, estimated to contain 0.60 mg percent of the D-isomer, was given orally, and urine was collected for the following hour. The phenylalanine concentration in the urine samples was determined by the decarboxylase and paper chromatographic methods, the difference between the two being taken as an estimate of the concentration of D-phenylalanine in the specimens. The percentage of the D-isomer in these specimens averaged more than 50 percent. With feedings of from 5 to 10 g of the substrate, isomeric contamination of as little as 0.10 mg percent could lead to an error of the same order of magnitude.

The simplest way to avoid the problem of minor isomeric contamination would be to utilize techniques that are specific for the L form of the amino acid, such as the enzymic, and most microbiological methods. Another approach would be to use the assays suggested by Meister *et al.* (*3*) to insure minimal isomeric contamination. In this connection we should like to suggest the possibility of using man as a concentrating mechanism for a suspected racemic amino acid mixture before assaying it for D contamination. Such a system could increase by 100-fold the sensitivity of detection of isomeric contamination.

In Table 1 are given the data on the excretion rate of D-phenylalanine after the ingestion of 2 g of the racemic phenylalanine, containing 0.60 mg percent of the D-isomer. The data are reported as milligrams of D-phenylalanine per milligrams of creatinine and represent the average concentration for the first hour's urine specimen following the feeding. The experimental subjects were normal monozygotic and like-sexed dizygotic twins.

As can be seen, there is considerable variation in the excretion rate of D-phenylalanine, the range being more than 15-fold (0.024–0.379). Variability in the excretion rate of D-isomers, and in particular of D-phenylalanine, has been observed before (*5, 7*). The physiologic ex-

Table 1. Urinary excretion rate of D-phenylalanine in monozygotic and dizygotic twins.

Twins	Sex	Ratio of D-phenylalanine to creatinine (mg/mg)	Intrapair differences	Mean intrapair difference
		Monozygotic		
1 A	♀	0.086	0.020	
B		0.106		
2 A	♀	0.091	0.026	0.025
B		0.117		
3 A	♂	0.052	0.028	
B		0.024		
		Dizygotic		
4 A	♀	0.074	0.036	
B		0.110		
5 A	♀	0.035	0.017	
B		0.052		
6 A	♀	0.144	0.060	
B		0.084		0.058
7 A	♀	0.205	0.174	
B		0.379		
8 A	♂	0.025	0.004	
B		0.029		
Intrapair variance ratio, dizygotics/monozygotics				11.7*

* Significant beyond the 0.05 level.

planation of this variation in excretion rate is far from clear, although several possibilities, such as variations in kidney D-amino acid oxidase activity and renal readsorption mechanisms, have been discussed (*5, 8*). Because of the low levels of D-phenylalanine in this work, it was not possible to examine any of these physiologic hypotheses.

What is of most interest to us is the genetic information that can be obtained from a comparison of the intrapair differences and variances in excretion rates between the pairs of monozygotic and dizygotic twins. As can be seen in Table 1, the average intrapair difference for the dizygotic twin pairs is more than twice as large as that for the monozygotic pairs. The intrapair variance ratio for the dizygotics/monozygotics is 11.7, which, with 5 and 3 degrees of freedom, is significant at higher than the 5 percent level. This would mean that at least part of the observed variation in excretion rate of D-phenylalanine is the result of genetic differences. The work of Goodman (*8*) on mice is particularly relevant here, since she also found evidence for genetic control of variation in excretion rates of the D-isomers of several amino acids, including phenylalanine. It would appear that further work on the physiology and genetics of differential excretion of the D-isomers would be very rewarding.

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11 April 1957

Glia/Nerve Cell Index for Cortex of the Whale

Since man occupies the top position in the phylogenetic scale and has attained the highest intellectual development, there has been a well-understood tendency to relate certain characteristics of the morphology of his brain to this development. Early attempts to parallel intellectual performance with brain weight, relationship of brain weight to body weight, and number of convolutions now have only historical interest. Certain aspects of the histology of the cerebral cortex were also thought to reflect phylogenetic development. The idea that the more highly developed cortex contains more "space" between nerve cells probably was expressed first by Nissl (*1*) and was later supported by such an authority on cerebral cytology as Economo (*2*). More recently, Friede (*3*) studied the glia index (number of glia cells per nerve cell) in the cortex of various animals and found that it increases from the frog to man. He concluded that the ascending phylogenetic development of the cortex is characterized by a relative increase in glia cells.

However, the human brain is not only the most highly developed but it is also the largest brain that is usually studied. It is possible, therefore, that certain histological characteristics of the cerebral cortex may reflect an increase in size and not in the phylogenetic development. Thus, Tower (*4*), studying the cell density of the cerebral cortex, included in his series the brain of the whale and elephant, which are the only two animals with a brain weight higher than that of man. He found that the cortical cell density was inversely correlated with brain weight, and not with the position of the animal on the phylogenetic scale.

It is the purpose of this report to show that the glia/nerve cell index reflects brain weight rather than phylogenetic development. Our histological material was a portion of that used by Tower (*4, 5*) and consisted of 20-μ paraffin sections of two whale (*Balaenoptera physalus* L.) brains which weighed 6500 g and 7150 g, respectively. Cortex from three sections, two from the frontal region and one from the occipital region, was studied. A 20-μ paraffin section of the temporal cortex of a 36-year-old woman was also examined for the purpose of the comparison of our results with those of Friede (*3*).

Photomicrographs at a magnification of 80 were made, and the glia cells and nerve cells were marked on them with the aid of direct microscopic observation of the slides. No attempt was made to differentiate between the types of glia cells, but care was taken to avoid marking endothelial nuclei. The marked cells were counted according to the following rules. In the whale cortex, the counts were made separately only for layer II; the deeper layers were counted together. Separate counts for nerve cells containing or not containing nucleoli were made. In the human cortex, layers II to IV were counted separately, layers V and VI together. All nerve cells were counted, irrespective of whether or not they contained nucleoli. Data are recorded in Table 1.

Table 1. Number of glia and nerve cells counted and the glia/nerve cell index for whale and man.

Layer	Cells counted		Glia/nerve cell index
	Glia	Nerve	
Whale, specimen No. Cst-1			
II	185	167	1.11
III to VI	2116	473	4.47
All	2301	640	3.59
Whale, specimen No. C-293			
II	277	150	1.84
III to VI	1717	264	6.50
All	1994	414	4.81
Whale, specimen No. C-293			
II	290	186	1.56
III to VI	2454	311	7.89
All	2744	497	5.52
Whale (total)			
	7039	1551	4.54
Man			
II	714	468	1.53
III	627	343	1.83
IV	870	424	2.06
V and VI	657	371	1.27
Man (total)			
	2868	1606	1.78

As can be seen from Table 1, the glia/nerve cell index of the cerebral cortex is much higher in the whale than in man. The value for the human cortex is 1.78, which is very close to the ratio of 1.68 established by Friede. Our index was obtained by investigation of only one region in the first temporal convolution. Because of the agreement of our value with that of Friede, we did not investigate other regions. In the whale, we studied three regions, two from one animal and one from the other. Though the ratio varied from 3.59 to 5.52, it was significantly higher than in man, the average for all three regions being 4.54. Statistical analysis of these results was made, applying the formula

$$t = \frac{x - y}{\sqrt{\frac{x(1-x)}{N_1} + \frac{y(1-y)}{N_2}}}$$

and the results were found to be highly significant.

These values for the whale were obtained when all nerve cells, whether they contained nucleoli or not, were counted. By applying such a method, we incurred an error resulting from the fact that when particles are counted in histological sections, the true number per volume is smaller than that counted (*6*). This error increases with the increase of the ratio between the size of the particles and the thickness of sections. Since the nerve cells of the whale are larger than those of man, and since all our sections were 20 μ thick, we overestimated the number of nerve cells for the whale cor-