Table 1. Lactate production by resting leukocytes and by phagocytosing leukocytes after 30 minutes of phagocytosis at 37°C. Values expressed as micromoles of lactate produced per ½ hour per 1.0 × 10^8 cells.

Leukocytes from patient with	Resting	Phagocytosing
Asthma	3.15	4.48
Pneumonia	3.65	5.60
Peritonitis	4.27	5.29
Congestive heart failure	2.07	4.79
Pneumonia	1.73	2.90
Chronic granulomatous disease, Patient 1	2.80 3.71	4.03 4.57
Chronic granulomatous disease, Patient 2	1.67	3.15

tected after the addition of 0.2 percent oxidized nitroblue tetrazolium and 1m*M* KCN to the reaction mixture. Reduction of the dye produces a deep blue color.

The results observed in leukocytes derived from other infected and healthy individuals confirmed some of the observations of Karnovsky and his coworkers (*4*). We refer to these as "normal" leukocytes. During phagocytosis, normal leukocytes reduced nitroblue tetrazolium to a deep blue color within 5 minutes, whereas in the resting state they required up to 30 minutes to bring about an equal degree of dye reduction. There was increased lactate production during phagocytosis by normal cells (Table 1). In addition, there was a marked increase in the activity of the hexose monophosphate shunt, as manifested by the production of $C^{14}O_2$ from glucose-1-C^{14} (Table 2). There was only slight increase in production of $C^{14}O_2$ from glucose-6-C^{14}. The metabolic response of the intact leukocytes of the two patients with chronic granulomatous disease during phagocytosis was abnormal. As expected from the data of Holmes and co-workers (*2*), these cells ingested polystyrene spheres. On the other hand, they did not reduce nitroblue tetrazolium at a normal rate whether resting or phagocytosing. During phagocytosis they produced lactate at an increased rate (Table 1) but failed to generate an increase in the hexose monophosphate shunt (Table 2). When these leukocytes were exposed to methylene blue in the absence of polystyrene spheres, their hexose monophosphate shunt activity increased even more markedly than did that of normal cells (Table 3). In contrast to normal cells, oxygen consumption of the leukocytes, measured only in the second patient, did not increase during phagocytosis (*7*).

Table 2. Effect of phagocytosis on stimulation of the leukocyte hexose monophosphate shunt in resting (A) and phogocytosing (B) cells.

Leukocytes from patient with*	$C^{14}O_2$ (count/min per 10^8 leukocytes)		
	A	B	B/A
Pneumonia	377	2099	5.6
Pulmonary infarction	304	1270	4.2
Pneumonia	65	1056	16.3
Pneumonia	155	1640	10.6
Pyelonephritis	81	1226	15.1
Wilson's disease	192	7232	37.7
Chronic granulomatous disease, Patient 1	43	84	1.9
	155	160	1.0
	29	22	0.7
Chronic granulomatous disease, Patient 2	321	381	1.1
	409	125	0.3

* The leukocytes were incubated at 37°C for 30 minutes in Krebs Hensleit buffer at *p*H 7.4 with 11 m*M* glucose and glucose-1-C^{14} (2.5 × 10^5 count/min).

Table 3. Effect of methylene blue on stimulation of the hexose monophosphate shunt. Without methylene blue, A; with methylene blue, B.

Leukocytes from patient with*	$C^{14}O_2$ (count/min per 10^8 leukocytes)		
	A	B	B/A
Pneumonia	297	2427	8.2
Asthma	459	2511	5.5
Chronic granulomatous disease, Patient 1	85	3245	38.1
Chronic granulomatous disease, Patient 2	148	3462	23.3

* The leukocytes were incubated at 37°C for 30 minutes in Krebs Hensleit buffer at *p*H 7.4 with 11 m*M* glucose, 2.0 m*M* methylene blue, and glucose-1C^{14} (2.5 × 10^5 count/min).

Lysis by freezing and thawing of the granulocytes of both patients following the addition of either NADH or NADPH caused greater reduction of nitroblue tetrazolium than occurred when the cells were intact. This reaction was not inhibited by cyanide and was potentiated to a greater extent by NADH than by NADPH.

Our studies indicate that the intact phagocytosing leukocytes of the patients with chronic granulomatous disease fail to evince a cyanide-insensitive NADH oxidase which is apparently not essential to the ingestion process. The results of studies with methylene blue suggest that the failure of these cells to exhibit stimulation of the hexose monophosphate shunt during phagocytosis is not due to any loss of one or more of the enzyme activities usually associated with the operation of the hexose monophosphate shunt. Rather, it may be due to the lack of the NADH oxidase activity which stimulates the shunt in normal phagocytosing cells. Exactly how the lack of oxidase activity in intact leukocytes is related to the inability of these cells to destroy ingested bacteria is not established at this time, nor is the cellular site of the enzyme released during lysis by freezing and thawing. Of clinical significance is the fact that the addition of nitroblue tetrazolium to a suspension of leukocytes in a glucose-rich buffer containing cyanide appears to provide a sensitive, diagnostic, screening test for this condition.

ROBERT L. BAEHNER
DAVID G. NATHAN
Department of Medicine, Children's Hospital Medical Center, and *Harvard Medical School, Boston, Massachusetts*

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Relative Brain Size: A New Measure

Abstract. *The relation of the volume of the endocranial cavity to the area of the foramen magnum is a measure of relative brain size in mammals. The outstanding advantage of this method is that only a skull is required for a set of measurements.*

The method most commonly used to investigate relative brain size in mammals involves a comparison of brain weight and body weight (*1*). However, that method is limited because body weight varies greatly within a species. Furthermore, it is difficult to obtain statistically significant data for common species and any data at all for rare mammals. The ratio of brain weight to spinal cord weight has been proposed as an index of relative brain size (*2*), but that approach, while alleviating the first problem, makes it

even more difficult to obtain data. I propose a more convenient measure of relative brain size—the relationship between endocranial volume and the area of the foramen magnum.

The volume of the braincase is very close to that of the brain in most mammals, as is indicated by the details of brain topography reproduced on most mammalian endocranial casts. The area of the foramen magnum is larger than, but highly correlated with, the cross-section area of the caudal end of the medulla, and the latter, it is suggested, is directly related to body size (excluding fat). (The number of motor and proprioceptive neurons per muscle fiber is relatively constant (*3*) and the same presumably holds, in general, for exteroceptive neurons for a given area of body surface.) Exceptions would be expected in mammals with an unusually high degree of muscular control or postcranial tactile sensitivity, in which case there would be relatively more neurons per muscle fiber or unit surface area, and thus a relatively larger foramen magnum for a given body size.

To test this proposed method of investigating relative brain size, I made a preliminary survey of five orders of mammals, with measurements taken on one specimen from each of 117 species, most of which represented different genera. Species were selected to represent the size range of the order. In addition, mean values of brain size of apes and man (*4*) were plotted against mean values of the area of the foramen magnum. Cranial capacities were measured in most cases by water displacement of endocranial casts. For skulls with cranial capacities over 50 cm^3, equally accurate measurements of endocranial capacity were obtained with No. 9 (2 mm in diameter) shotgun pellets. The area of the foramen magnum was estimated from width and height measurements ($A = \frac{1}{4}\pi wh$).

The results of this initial survey are presented in Fig. 1 and Table 1. The relationship between endocranial volume and foramen mangum area for each group of points is best described by the standard allometric equation:

$$\log y = \log b + k \log x \qquad (1)$$

where $\log b$ is the y-intercept and k the slope (*5*). The lines were fitted to each set of points by the method of least squares, with errors assumed in both dimensions. Increase in relative brain size is indicated in the sequence

Table 1. Statistics on data presented in Fig. 1. *N*, number of specimens; *r*, product-moment correlation coefficient.

Animal	*N*	*r*	log *b*	*k*
Monkeys	19	0.96	1.61	1.29
Carnivores	32	.94	1.47	1.60
Artiodactyls	30	.97	1.46	1.34
Prosimians	17	.97	1.43	1.31
Rodents	7	.98	1.11	1.24
Insectivores	12	.90	0.93	1.60

insectivore < rodent < prosimian < monkey < man, which is the expected order. One possible explanation of the extensive overlap of monkeys with carnivores and of apes with monkeys (Fig. 1) is the increased muscular coordination and tactile sensitivity (in hands and feet) of the higher primates, which would result in relatively larger foramen magnum areas. The difference in slope between the insectivore and carnivore axes, on the one hand, and

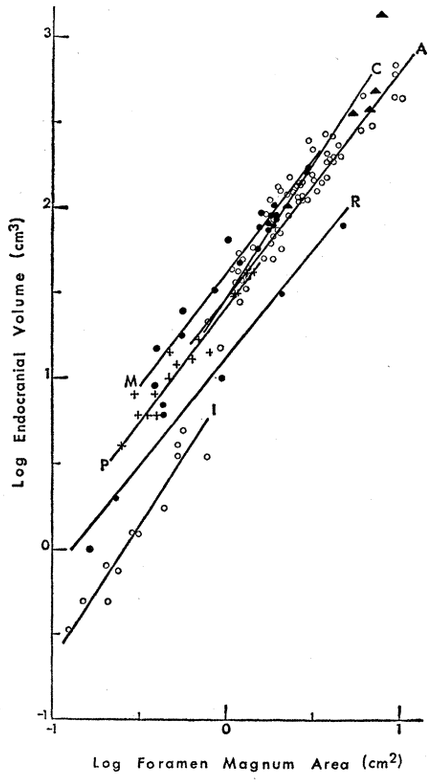

Fig. 1. Relation between endocranial volume and foramen magnum area in five orders of mammals. Letters refer to reduced major axes fitted to the points for each group. *I*, insectivores; *R*, rodents; *P*, prosimian primates; *M*, New and Old World monkeys combined; *C*, fissiped carnivores; *A*, artiodactyls. Open circles, insectivores, artiodactyls, and carnivores; filled circles, rodents and monkeys; crosses, prosimians; triangles, apes and man. Additional information is in Table 1.

those of the rodents, primates, and artiodactyls (Table 1) may be a sampling artifact. From this preliminary survey it appears that log *b* may differ in different families of carnivores; thus different combinations of genera taken to represent the order could have different slopes. For example, if the monkey and prosimian samples, which have different values of log *b*, are combined, *k* rises to 1.50. Not enough carnivore species were examined in this initial survey to allow analysis of subordinal groups, as was done with the primates. No interfamilial differences in log *b* were noted in the artiodactyl sample, which included representatives of all living families. The insectivore and rodent samples were too small to allow confirmation, from a biological viewpoint, of the slopes of their curves. Nevertheless, the demonstration of the expected sequence of groups in order of increasing relative brain size, the high coefficient of correlation for each group, and the relatively close agreement of slopes indicates that this is a useful method for measuring relative brain size.

One advantage in using foramen magnum area instead of body weight in investigations of relative brain size is that the former is less variable than the latter. In a sample of 24 adult female skunks of the species *Conepatus suffocans* from Chile (*6*), the coefficients of variation for foramen magnum area and body weight were 9.3 and 18.0, respectively. Coefficients of variation of foramen magnum area for samples of three primate species, *Galago senegalensis*, *Hylobates hoolocki*, and *Pan troglodytes*, were 9.2, 9.2, and 10.3, respectively. These are considerably lower than coefficients of variation of body weight (*7*).

The outstanding advantage of comparing endocranial volume to foramen magnum area, as opposed to all previously proposed measures of relative brain size, is that it requires only a skull for a set of measurements. Thus it is possible to investigate relative brain size in all genera of recent mammals and in many fossil forms as well (*8*). Data for large samples can be easily obtained for many species, data which enable us to study ontogenetic and intraspecific variation, as well as to make statistical comparisons between species.

LEONARD RADINSKY
Biology Department, Brooklyn College, Brooklyn, New York 11210

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Sporulation Mutations Induced by Heat in Bacillus subtilis

Abstract. *When spores of* Bacillus subtilis *strain Marburg are heated (90° to 100°C) in a vacuum for 9 to 12 hours and then plated, numerous mutants are obtained, and very few spores are killed. Disproportionately large numbers of these mutants exhibit abnormal sporulation.*

When spores of *Bacillus subtilis* strain Marburg were heated in a vacuum, large numbers and wide varieties of mutants were obtained. In contrast to most phenomena of mutagenesis, where mutation is often accompanied by extensive killing of cells or spores, we observed little or no killing under conditions which yielded significant numbers of mutants. At 100°C for 9 hours, more than 1 percent of the original spores were consistently recovered as viable mutants; in some cases as many as 5 percent were obtained. Although there was a variety of mutants, almost all showed some type of abnormal sporulation. There was no apparent correlation between sporulation and auxotrophic mutation.

Table 1. Viable and mutant counts of spore pellets heated at 100°C (series 1) and at 90°C (series 2) for 9 to 12 hours.

Average count per pellet ($\times 10^6$)			Total mutants (No.)	Population recovered as mutants (% of control)
Not heated viable	Heated			
	Viable	Mutant		
		Series 1		
163	34.3	4.6	55	2.82
237	92.7	12.0	72	5.08
136	46.8	3.3	13	2.43
239	37.0	8.3	50	3.47
114	43.1	4.7	57	4.12
207	53.3	11.5	69	5.55
		Series 2		
180	86	3.17	19	1.76
151	105	1.63	13	1.08
137	128	1.33	16	0.97
156	102	0.80	24	0.51

Spores were prepared from cells cultured at 30°C on modified SMM (*1*) containing 0.1 percent glucose and 0.1 percent glutamate. The cultures were shaken during the growth period. After 4 days, samples (1 ml) were collected in conical, glass centrifuge tubes, and the spores were centrifuged at 2600*g* for 15 minutes. The supernatant was decanted, and the pellets were dried for 2 hours at room temperature. Pellets were heated in a vacuum for specified temperatures and times, usually 90° to 100°C for 9 to 12 hours. As a control, some pellets were left at room temperature for the same length of time. The pellets were then cooled, suspended, diluted in the growth medium (omitting all carbon sources), and plated on potato dextrose agar (Difco) fortified with $MnSO_4$ (to 0.01 percent) and adjusted to *p*H 7. After 4 days at 37°C, wild-type colonies displayed a characteristic morphology and brown pigmentation on this agar, whereas mutants defective in sporulation showed morphological differences and variation or lack of pigmentation. All colonies were examined for auxotrophy by plating on glucose-salts agar, and suspected colonies were further checked individually. Absence of growth after a 2-day incubation period was the criterion for auxotrophy.

In Fig. 1, the logarithms of the numbers of survivors and mutants are plotted as functions of heating temperatures for 9 hours. In Fig. 2, the logarithms of the numbers of survivors and mutants are graphed as functions of heating time at 100°C. Colony formation is exponentially inactivated. The sharp increase in the number of mutants between 4 and 6 hours was unexpected because of the constant rate of killing observed.

When pellets were heated to 100°C or less for 9 hours, there were large numbers of mutants with relatively little killing. In some experiments, 1 to 5 percent of the original spores were recovered as viable mutants (Table 1); this was the maximum survival and mutation observed. Variations in the age of the spores or their growth temperature can yield less survival and mutation. Several of the experiments at 90° and 100°C were analyzed further. The mutants were originally identified by auxotrophy or differences in morphology or pigmentation, but after extensive microscopic examination almost all exhibited abnormal sporulation. In the experiments at both 90° and 100°C, 23 to 30 percent of all mutants had less than 1 percent wild-type sporulation, of which 78 to 80 percent (or 18 to 23 percent of total mutants) were asporogenic by examination under phase

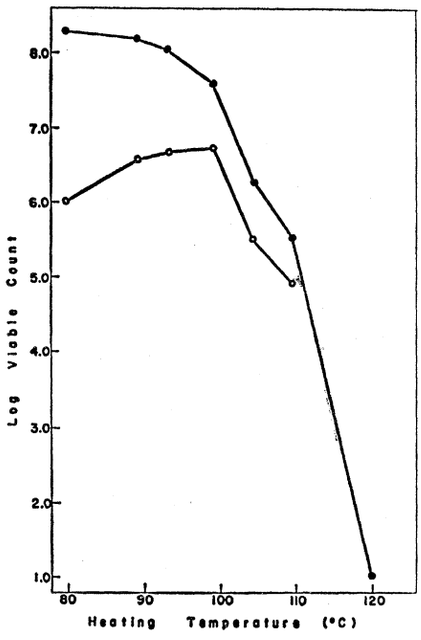

Fig. 1. Logarithm of the viable count of survivors (●———●) and mutants (○———○) as heating temperature increases. All heating periods are 9 hours.

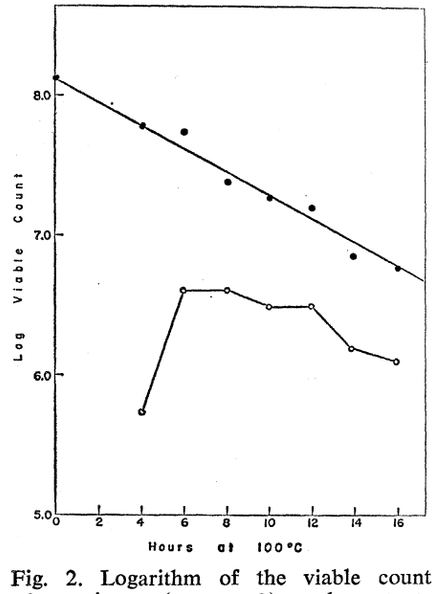

Fig. 2. Logarithm of the viable count of survivors (●———●) and mutants (○———○) as heating period increases. All heating temperatures are 100°C.