Table 1. Phosphocreatine of mouse tissues as determined by chemical and chromatographic methods. Mice were thrown into a dry ice-acetone mixture and tissues were removed in frozen state with chisel and hammer. Values are expressed in micromoles per gram of wet tissue and are an average of four determinations varying within 8 percent.

Tissue	Method		
	Chemical	Chromatographic	Chromatographic (unfrozen)
Skeletal muscle	6.48	5.75	5.15
Brain	3.05	4.25	3.00
Kidney	3.80	3.67	3.15
Liver	2.75	1.96	2.00

analyzed, the column was washed with 25 ml of 0.05*N*, followed by 25 ml of 0.15*N* formic acid before elution of the PCr with 0.5*N* formic acid. Presumably no other known phosphorylated intermediates are eluted with 0.5*N* formic acid. Inorganic pyrophosphate (PP), which is usually removed with 0.15*N* formic acid, if present, could be removed by collecting an additional 10 to 20 ml with 0.15*N* acid.

A number of tissues have been analyzed for PCr by both the chemical and chromatographic technique (Table 1). In general, the agreement between both methods is good, although the values are usually higher with the latter method. Tissues removed without rapid freezing of the whole animal had somewhat lower values for PCr, with the exception of liver. The reason for preference of the chromatographic over the chemical technique in the separation of PCr in isotopic experiments is clearly indicated in Table 2. The variation in specific activity among two determinations was considerably more than 100 percent in all instances with the chemical method and less than 25 percent with the chromatographic method, with the exception of liver. Preliminary studies reveal that the phosphocreatine of liver varies considerably, depending on the nutritional state of the animal.

Table 2. Comparison of specific activity of PCr of mouse tissues determined chromatographically and chemically. Mice were injected intraperitoneally with 100 μc of P^{32} (orthophosphate) and sacrificed 30 minutes later. Each value is an average of two determinations in two separate experiments.

Tissue	Specific activity (counts/min μmole P)	
	Chromatographic	Chemical
Kidney	9,700, 8,500	15,000, 6,000
Brain	705, 950	200, 1,500
Liver	9,650, 18,000	4,500, 12,000
Muscle	1,680, 2,050	1,500, 3,800

The present chromatographic method has been successfully used in the separation of PCr and other phosphorylated intermediates in phosphorylation studies with isolated frog nerve (*5*) and mouse brain *in vivo* (*6*). In addition to being quantitative, the method is rapid and simple, especially, when merely P and PCr are to be analyzed. A manifold with as many as six outlets has been successfully used to operate an equal number of columns from a common reservoir, either by hand or with an automatic fraction collector (*7*).

L. G. Abood
Division of Psychiatry, Neuropsychiatric Institute, University of Illinois College of Medicine, Chicago

References

1. M. Friedkin and A. L. Lehninger, *J. Biol. Chem.* 177, 775 (1949).
2. P. C. Caldwell, *Biochem. J. London* 55, 458 (1953).
3. W. E. Cohn, *J. Am. Chem. Soc.* 72, 1471 (1950).
4. R. B. Hurlbert *et al.*, *J. Biol. Chem.* 209, 23 (1954).
5. L. G. Abood and E. Goldman, *Am. J. Physiol.*, in press.
6. L. G. Abood, in preparation.
7. This work was supported by the Office of Naval Research.

22 September 1955

Biological Effect of Hydroxylysine

Although it is well established that hydroxylysine (2,6-diamino-5-hydroxyhexanoic acid) occurs regularly in collagens of terrestrial and marine animals and the gelatins derived from these, little is known of its biological significance. Despite its obvious structural similarity to lysine, there is no evidence in the reported results of *in vivo* studies that the hydroxylated compound is in any way related metabolically to this more widely distributed amino acid. Thus, Lindstedt (*1*) reported that dietary supplements of synthetic hydroxylysine were ineffective in promoting growth of lysine-deficient rats. Bergstrom and Lindstedt (*2*) found that hydroxylysine was unable to replace lysine for growth of *Leuconostoc mesenteroides* P-60.

The results of our own studies concerning the ability of hydroxylysine to support growth of the lysine-requiring bacteria *Streptococcus faecalis* (A.T.C.C. No. 9790) and *Leuconostoc mesenteroides* P-60 (A.T.C.C. No. 8042) are in general agreement with those of the afore-mentioned workers in that the addition of hydroxylysine to a lysine-free medium failed to allow growth of either of these organisms. However, while hydroxylysine was unable to support growth in the complete absence of lysine, it appeared to have considerable ability to lower the lysine requirements of these bacteria. Some typical results illustrating this effect are given here.

The composition of the lysine-free basal medium and the techniques for measurement of growth responses of the organisms to increments of lysine were the same as those described by Henderson and Snell (*3*). The L-lysine HCl and racemic hydroxylysine HCl used in this work were synthetic products (*4*). Hamilton and Anderson reported (*5*) that racemic hydroxylysine from the same source contained approximately 40 percent hydroxy-DL-lysine and 60 percent allohydroxy-DL-lysine. The results in Fig. 1 show the effects of additions of 2, 20, and 1000 μg of racemic hydroxylysine per milliliter of double-strength basal medium on the responses of *Streptococcus faecalis* to increments of lysine.

It is evident that the presence of only 2 μg of the racemic hydroxy compound (supplying only about 0.4 μg of the natural isomer) per tube gives an apparent reduction of more than 20 μg in the amount of L-lysine required for half-maximum growth of the organism. This suggests that hydroxylysine has some effect other than, or in addition to, that of serving as a nutrient that can, under conditions of suboptimal lysine supply, be converted into lysine or be exchanged for lysine in protein synthesis.

Somewhat similar effects of hydroxylysine addition to the basal medium on the apparent lysine requirement of *Leuconostoc mesenteroides* P-60 were observed, using the method of testing described in a previous paragraph. However, with this organism, the relationship between apparent lysine requirement and hydroxylysine concentration is more complex. Although small amounts of hydroxylysine allow maximum growth of the organism at reduced levels of lysine,

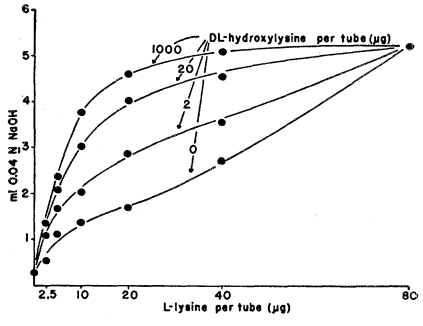

Fig. 1. Effect of addition of racemic hydroxylysine to the basal medium on the response of *Streptococcus faecalis* to increments of L-lysine.

high levels of the hydroxy compound (1 or 2 mg per tube) show a marked growth-depressing effect. This growth depression by high levels of hydroxylysine is eliminated by addition of larger amounts of L-lysine.

Both *Leuconostoc mesenteroides* P-60 and *Streptococcus faecalis* are commonly used for microbiological assay of lysine in hydrolyzates of foods and tissues and each of these organisms has been accepted, on the basis of earlier work (*6*, *7*), as having a highly specific requirement for lysine. However, in view of the results reported here, it seems likely that the presence of hydroxylysine in sample hydrolyzates could interfere with the quantitave microbiological determination of lysine when these organisms are used with basal media that contain no hydroxylysine.

C. S. PETERSEN
R. W. CARROLL

Research Laboratories,
Quaker Oats Company, Chicago, Illinois

References and Notes

1. S. Lindstedt, *Acta Physiol. Scand.* 27, 377 (1953).
2. S. Bergstrom and S. Lindstedt, *Acta Chem. Scand.* 5, 157 (1951).
3. L. M. Henderson and E. E. Snell, *J. Biol. Chem.* 172, 15 (1948).
4. Both were CfP grade products obtained from California Foundation for Biochemical Research, Los Angeles.
5. P. B. Hamilton and R. A. Anderson, *J. Biol. Chem.* 213, 249 (1955).
6. A. D. McLaren and C. A. Knight, *ibid.* 204, 417 (1953).
7. J. L. Stokes *et al.*, *ibid.* 160, 35 (1945).

13 September 1955

What Are Variables and Constants?

The notion *variable* has not in the literature attained the degree of clarity that would justify the almost universal use of that term without explanations. Recent investigations (*1*) have resolved it into an extensive spectrum of meanings, some pertaining to reality as investigated in science, others belonging to the realm of symbols studied in logic—two altogether different worlds. As a corollary, these distinctions yield a clarification of the notion *constant*.

Science and applied mathematics. By *quantity* we mean a pair of which the second member (or *value*) is a number while the first member (or *object*) may be anything. We call a class of quantities *consistent* if it does not contain two quantities with equal objects and unequal values. If $p(\gamma)$ denotes the pressure (in a chosen unit) of a gas sample γ (*2*), then the pair $[\gamma, p(\gamma)]$ is a quantity, and the class of all such pairs for any γ is consistent. This class reflects the physicist's idea of gas pressure, p. Gas volume, v, and temperature, t, can be defined similarly. Consistent classes of quantities, such as p, v, and t, are what scientists and mathematicians in applied fields mean by *variable quantities* and what Newton called *fluents*. The class of all gas samples will be referred to as the *domain* of p; the class of all numbers $p(\gamma)$ as the range of p.

A variable quantity whose range consists of a single number is said to be *constant*. An example is the gravitational acceleration g at a definite point on the earth, defined as the class of all pairs $[\alpha, g(\alpha)]$ for any object α falling in a vacuum, where $g(\alpha)$ denotes the acceleration of α. For any α, this value of g is found to be equal to one and the same number, g. (Throughout this paper, symbols for fluents are italicized and symbols for numbers are printed in roman type.) A less important, because less comprehensive, example is the distance traveled by a specific car C while C is parked. (The distance traveled by C is the class of all pairs $[\mu, m(\mu)]$ for any act μ of reading the mileage gage in C, where $m(\mu)$ is the number read as the result of μ.

In a plane, the coordinates relative to a chosen Cartesian frame are variable quantities whose domain is the class of all points (*3*). The abscissa x is the class of all pairs $[\pi, x(\pi)]$ for any point π. In the equation of the straight line $x - y = 3$ the *3* denotes the constant fluent consisting of the quantities $(\pi, 3)$ of value 3 for each point π.

Of paramount importance are the consistent classes of quantities whose domains consist of numbers. An example is the class of all pairs [x, *log* x] for any number $x > 0$, called the logarithmic function or the function *log*. It is capable of connecting consistent classes of quantities—for example, $y = log\ x$ along a logarithmic curve, and $w = log\ v$ for an isothermic expansion of an ideal gas, if w denotes the work in a proper unit. That is to say, $y(\pi) = log\ x(\pi)$ and $w(\gamma) = log\ v(\gamma)$ for any point π on the curve and any gas sample γ pertaining to the process. Moreover, *log* connects functions—for example, the exponential with the identity function, and *cos* with *log cos*. Because of their connective power, functions are omnipresent in science as well as in mathematics (*4*). Variable quantities such as p and v (whose domains do not contain numbers or systems of numbers) lack this power and therefore are confined to special branches of science such as gas theory. Denying the significance of this difference would be denying the role of mathematics as a universal tool in quantitative science.

Logic and pure mathematics. The formula

$$3^2 - 1 = (3 + 1) \cdot (3 - 1)$$

is a statement about specific numbers designated by 3 and 1. In the more general statement

$$x^2 - 1 = (x + 1) \cdot (x - 1) \text{ for any number } x,$$

the remark "for any number x" stipulates that, in the formula, x may be replaced by the designation of any number, for example, by 3 or $\sqrt{5}$, each replacement yielding a valid statement about specific numbers. A symbol that, in a certain context and according to a definite stipulation, may be replaced by the designation of any element of a certain class is what, following Weierstrass, logicians and pure mathematicians call a *variable*. The said class is called the *scope* of the variable. The scope of x in the formula $log\ x^2 = 2\ log\ x$ for any $x > 0$ is the class of all positive numbers. In $(\pm\sqrt{3})^4 = 9$, the symbol $\pm\sqrt{3}$ is a variable whose scope consists of the two numbers $\sqrt{3}$ and $-\sqrt{3}$.

A variable whose scope consists of a single number designates that number and, in pure mathematics, is referred to as *a constant*. Examples include numerals (1, 3, . . .); e, designating the base of natural logarithms; and brief designations of numbers with unwieldy symbols, such as $2^{\sqrt{2}} + e^{e}$—abbreviations introduced for the purpose of just one discussion involving repeated references to that number. As such *ad hoc* constants, one customarily uses the letters a, b, c, . . . , which, just as x and y, serve as variables in other contexts, for example, in the statement involving two variables

$$x^2 - a^2 = (x + a) \cdot (x - a) \text{ for any x and any a.}$$

Because of its vicarious character, a variable may always be replaced, without any change of the meaning, by any otherwise unused letter, for instance, a by y or x by b.

Variables whose scopes are classes of numbers are called *numerical variables*. The definitions of p and g make use of variables γ and α whose scopes consist of gas samples and falling objects, respectively. Statements that are valid for many fluents are conveniently expressed in terms of *fluent variables*; for example,

$$\text{If } z = log\ u, \text{ then } dz/du = 1/u \text{ for any two fluents } u \text{ and } z \quad (1)$$

In Eq. 1, u and z may be replaced by x and y: if $y = log\ x$ (which holds along the logarithmic curve), then $dy/dx = 1/x$; or by v and w: if $w = log\ v$ (which holds for an isothermic expansion), then $dw/dv = 1/v$. But u and z in Eq. 1 must not be replaced by numbers such as e and 1: although $1 = log\ e$ is valid, $d1/de = 1/e$ is nonsensical.

Confusion in the literature. No clear distinction has heretofore been made between numerical and fluent variables. Moreover, in the literature, numerical variables and variable quantities, not-