are unexplained as yet; and that the error in the present Gregorian rule should amount to a day only after some 3000 years. They suggest that by that time the length of the year, and changes in it, should be known much more accurately than at present. Hence, they have recommended leaving revision of the Gregorian rule to future generations.

The Gregorian rule for leap year is "Years divisible by 4 are to be leap years unless they are also divisible by 100, in which case they are to be leap years only if divisible by 400." This rule obviously expresses the length of the year in days as

$$365 + 1/4 - 1/100 + 1/400$$

or in decimals as

$$365 + 0.25 - 0.01 + 0.0025$$

Combining, we obtain 365.2425 as the length of the Gregorian year.

The true length of the tropical year is 365.24220. Comparing with the Gregorian year, we see that the Gregorian rule is in error by 0.0003 day per year, or 3 days in 10,000 years.

Walker proposes the rule "Years divisible by 4 are to be leap years unless they are also divisible by 120, in which case they are to be common years." This expresses the length of the year in days as

$$365 + 1/4 - 1/120$$

or in decimals

$$365 + 0.25 - 0.00833 \ldots$$

Combining, we obtain 365.24166 . . . as the length by the proposed rule. Comparing this length, 365.24166 . . . , with the adopted length 365.24220, we see that it is too short by more than 5 days in 10,000 years.

Since both the Gregorian rule and the proposed rule start with "Years divisible by 4 are to be leap years . . ." one applying either rule starts with a mental division of the last two figures of the year number, to see whether it is divisible by 4. For the next step, almost any high-school student who has read the Gregorian rule recently should be able to figure mentally that the century years 1800, 1900, and 2100 are not divisible by 400 and, hence, are leap years; but 2000 is divisible by 400 and, hence, is not a leap year. The Gregorian rule can be applied mentally, but how many high-school students would be able to figure without pencil and paper which years between 1900 and 2100 are divisible by 120 and, hence, would not be leap years by the proposed rule?

There have been many proposals for correcting the Gregorian rule, but the only one usually mentioned in textbooks on astronomy was proposed several generations ago. It is that "but years divisible by 4000 shall not be leap years" be added to the Gregorian rule. This would make the length of the calendar year 365.24225 days and, on the assumption that the present figure for mean length of the year of seasons is correct, reduce the error to half a day in 10,000 years. This rule also continues the use of the division by 4, and has no effect on the present rule before the year 4000.

It has been suggested also that, if it develops that our present figure for the mean length of the year is appreciably too large, "but years divisible by 2000 shall not be leap years" be added to the Gregorian rule. This should be applied first in the year 2000; with our present marvelously accurate crystal clocks, astronomers may well know before the year 2000 whether this addition would improve the rule.

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Determination of Phosphocreatine and Other Phosphorylated Intermediates with Dowex-1 Resin

The analysis of phosphocreatine (PCr) in animal tissues has long been beset with certain difficulties. Interfering labile substances such as phosphopyruvate and triose phosphates are usually present in various amounts. In addition, the problem of isolating phosphocreatine chemically for radioactive studies is particularly acute since the laborious technique of removing orthophosphate (P) by magnesium precipitation (*1*) results in a loss of PCr and other compounds. Paper chromatography (*2*) has been used, but resolution is poor and spontaneous hydrolysis is a problem. The present report describes the use of the anion exchange resin Dowex-1 in the quantitative separation of PCr. Dowex-1 resins have been extensively applied to the analysis of nucleotides (*3, 4*), but little attention has been given PCr and other phosphorylated intermediates.

After the discovery that hydrolysis of labile phosphates occurred more readily with finer mesh resins (200 to 400 mesh), a porosity of 100 to 200 mesh Dowex-1 X8 resin was employed and the elution was performed at 0°C. The resin, which was stored in concentrated formic acid, was washed in 6 by 0.7-cm columns with 10 volumes of concentrated formic acid (plus 1*M* ammonium formate if other intermediates were to be separated) and then with distilled water until the effluent was neutral. After the neutralized extract was run through, the column was washed with 10 ml of water. Elution of the phosphorylated intermediates was begun with 0.05*N* formic acid, and the collection rate was 5 ml/10 minutes per tube, using the automatic fraction collector.

A typical separation of some intermediates is presented in Fig. 1. With the exception of hexose phosphates, most of the other substances are clearly separable. There is a tendency for most hexose phosphates as well as phosphoglyceric acid to be eluted simultaneously. At present, some success in separation has been achieved by combining silicic acid with Dowex-1. When only PCr was to be

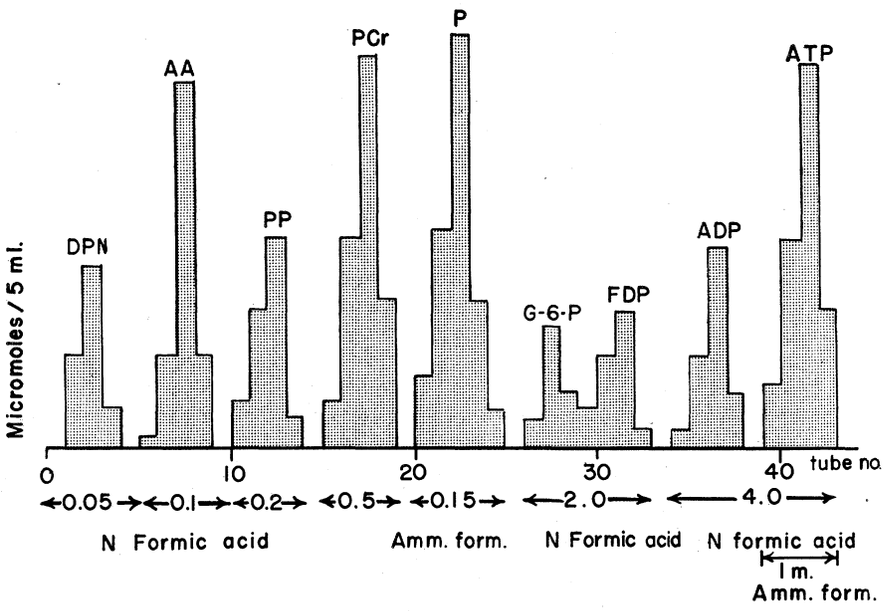

Fig. 1. Separation of some phosphorylated intermediates by ion-exchange chromatography. DPN, diphosphopyridine nucleotide; AA, adenylic acid; PP, inorganic pyrophosphate; PCr, phosphocreatine; P, orthophosphate; G-6-P, glucose-6-phosphate; FDP, fructose-1,6-phosphate; ADP, adenosinediphosphate; ATP, adenosinetriphosphate. Further details of the method are described elsewhere (*5*).

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*).

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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,

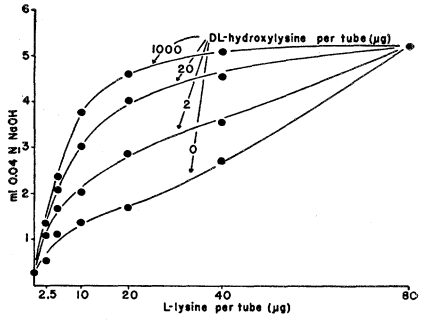

Fig. 1. Effect of addition of racemic hydroxylysine to the basal medium on the response of *Streptococcus faecalis* to increments of L-lysine.