(results not shown)." Thus no effect of pH was reported for glutamate, and one would expect the K_m of glutamate to be different from that of 2-OG. Perhaps Dr. McCormack carried out the pH experiment with glutamate and remembers the unreported results. A clue to a possible explanation can be found in a later set of data (Table 4), where kinetic parameters for the effects of Ca^{2+} on the reduction of $NAD(P)^+$ induced by different substrates in intact liver mitochondria are reported. Among the substrates used was glutamate, and the effect of Ca^{2+} on the glutamate K_m for NAD(P)H oxidation is reported. In this experiment, the effect of glutamate on redox was reported only in the presence of 0.5 mM malonate and no added malate. Malonate would have prevented regeneration of oxaloacetate and thereby stopped transamination. We proposed (Smith et al., 1992) that the change in pH-sensitivity of 2-OGDH when transamination was active was due to an interaction between glutamate–oxaloacetate transaminase (GOT) and 2-OGDH. This interaction has been reported previously by others (Fahien et al., 1988). If Dr. McCormack had done the experiment testing the effect of pH on [1-^{14}C]glutamate oxidation in the presence of 0.5 mM malonate, we would predict that oxidation would have been stimulated by H^+ ions, since the dehydrogenase did not have access to 2-OG generated by transamination.

Dr. McCormack further suggests that we should have been able to observe Ca^{2+} and pH effects with excess ADP in State 3. He suggests that our lack of success was because we saturated the enzyme by providing two sources for the appearance of 2-OG in the matrix, neither of which alone might have been saturating. That is perhaps a possibility for the liver, but not for the kidney, where the 2-OGDH activity is more than 3-fold higher than in liver. Thus the lack of effect of pH and Ca^{2+} is difficult to explain in the case of kidney mitochondria provided with excess ADP. In addition, we wish to point out that, under similar assay conditions, we had no problems observing Ca^{2+} effects on 2-OGDH in intact rat heart mitochondria (Wan et al., 1989) in the presence of excess ADP. The best explanation seemed to be that perhaps the intramitochondrial ATP/ADP ratios in intact liver and kidney mitochondria are lower in State 3 than in heart.

At any rate, saturation cannot explain the State 4 (Figure 2a of Smith et al., 1992) data, since significant stimulations by Ca^{2+} are observed with both liver and kidney mitochondria, whereas an increase in H^+ produces opposite effects in liver compared with kidney. These data were repeated many times over a period of several years and are remarkably reproducible.

Despite the problems with the relatively unphysiological State 3 (excess ADP) condition, the remaining conditions of substrate concentration, pH and Ca^{2+} concentration are all within reasonable physiological range. The conclusions drawn are that the high ratio of GOT/2-OGDH activity in the liver compared with kidney favour GOT/2-OGDH complex-formation, and within the complex, flux through 2-OGDH is significantly influenced by GOT activity. McCormack's (1993) suggestion about using [1-^{14}C]glutamate to assess flux through 2-OGDH is a useful one, as is the suggestion about repeating the experiments at 37 °C. We will, of course, make use of these suggestions as we continue to test the hypothesis, and hope that others will continue to scrutinize our work.

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Bacterial oxidative-stress substance is 2-*C*-methyl-D-erythritol 2,4-cyclopyrophosphate

The growing interest in the oxidative-stress response manifested by the 40th Harden Conference to be held in Britain this year stems from the evident involvement of oxidative stress in many pathological and normal biochemical processes, reviewed, for example, in [1]. The widely accepted parts of the response are the appearance of micromolar amounts of signalling nucleotides and biosynthesis of a family of stress proteins with diverse and mainly unknown functions [2,3]. However, recently an unusual reaction to oxidative-stress agents was noticed [4] in a group of bacteria, which accumulate, sometimes in large amounts, a new phospho compound [5], determined to be 2-methylbutanetetra-1,2,3,4-ol 2,4-cyclopyrophosphate (MTCPP) on the basis of two-dimensional n.m.r. spectroscopy, mass spectrometry and other analyses [6–8]. Simultaneously a similar compound was detected in a strain of the anaerobic bacterium *Desulfovibrio desulfuricans* [9], and its chemical structure was published in a Research Communication in the *Biochemical Journal* [10] as 3-methyl-1,2,3,4-tetrahydroxybutane 1,3-cyclic bisphosphate, with *RS* relative configuration of two chiral centres. The identity of the constitutive compound of the anaerobe with the cyclopyrophosphate accumulating in other micro-organisms [5] under forced oxidative conditions is still to be proved, and this required additional information from our side, which is provided in this Letter.

Synthesis of the organic skeleton of the compound from precursors of known chirality reveals its identity for both aerobic and anaerobic bacteria and demonstrates its D-*erythro* absolute configuration (2*S*,3*R*).

MTCPP was isolated from *Corynebacterium* (*Brevibacterium*) *ammoniagenes* A.T.C.C. 6872 cells cultivated in a medium supplemented with benzyl viologen and purified by repeated ion-exchange chromatography as described in [7].

To obtain the tetraol from MTCPP, the substance was incubated for 24 h at room temperature in 1 ml of 48% HF, followed by neutralization with AB-16-CT (OH^-) resin. A 67 mg portion of tetraol thus obtained was dissolved in 1 ml of pyridine plus 1 ml of acetic anhydride and incubated for another 24 h with 20 mg of dimethylaminopyridine. Then methanol was added and the sample was dried repeatedly with toluene and purified by chromatography in benzene (yield 135 mg). Synthesis of 2-*C*-methylerythritol was performed by conventional procedures starting from a known pentose [11–14].

^{1}H, ^{13}C and ^{31}P n.m.r. spectra were recorded on Bruker WM-250, AM-300 and AMX-400 instruments, with acetone for ^{1}H and for ^{13}C and sodium ethylenediamine tetraphosphonate for ^{31}P (12.8 p.p.m.) as standards. A JSASCO DIP-360 instrument was used for polarometry.

The carbon skeleton of MTCPP stripped of its phosphate groups and esterified by four acetyl groups is characterized by

strong rotation of polarized light: $[\alpha]_D^{25} = +22.5°$ (c 1.0 in $CHCl_3$) and +19.6° for tetraol (c 1.0 in water). Previous data for 2-*C*-methylerythritol were +21.4° [15] and +23.7° [16].

As the published data [10] were in favour of the *RS* configuration of the compound under study, the D-*erythro* isomer was chosen as the first candidate for the chemical synthesis. The optical characteristics of the final product (tetra-acetate) are in good agreement with those of the derivatives of the natural compound isolated from *C. ammoniagenes*, $[\alpha]_D^{25} = +21.5°$. ^1H and ^{13}C n.m.r. parameters for the tetraol and its derivatives are similar to the published data [17], with an accuracy of 0.05 p.p.m. for ^1H and 0.4 p.p.m. for ^{13}C n.m.r. Thus we believe that the *RS* configuration calculated from n.m.r. data for the substance from *D. desulfuricans* [10] can also be ascribed to that extracted from *C. ammoniagenes*, and the full name of this bacterial oxidative-stress substance is 2-*C*-methyl-D-erythritol 2,4-cyclopyrophosphate (MECYPP), that is (2*S*,3*R*)-methyl-1,2,3,4-tetrahydroxybutane 2,4-cyclic bisphosphate. It is not yet clear whether MECYPP in the bacterial cytoplasm is present only in free ionized form or as binary or tertiary complexes, due to its affinity to polyvalent cations [18]. There are some indications that protein-denaturing agents are able to increase the n.m.r.-visible portion of the phosphocompound.

Another problem is the nature of the immediate products of transformation of MECYPP. The usual contaminant of the compound noticed in the course of the purification procedure is most possibly a new cyclic phosphate readily formed at pH values above 7. Our suggestion is that MECYPP is liable to hydrolysis, concomitant with formation of the smaller cycle where a phosphate group is connected with the first and the second carbon atoms [6].

We were pleased to learn that another derivative of the methylerythritol, 2-methyl-2,3,4-trihydroxybutanoic acid 1,4-lactone, was involved in the water-stress reaction of some Australian legume plants [19]. Meanwhile, 2-*C*-methyl-D-erythritol itself accumulates in a tulip-tree, *Liridendron tulipifera* [16], in the autumn, which is also a stress. So it seems reasonable to suggest that a certain common precursor (isopentenyl pyrophosphate, or a UDP-sugar?) is under the regulation of the stress genes.

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