the effect of GSSG was studied with purified enzyme and cofactor, the reversal of the inhibition of glucose-6-phosphate dehydrogenase by NADPH cannot be attributed to the glutathione reductase mechanism.

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The effect of oxidized glutathione on NADPH inhibition of glucose-6-phosphate dehydrogenase is indirect

Eggleston & Krebs (1974) provided evidence that oxidized glutathione (GSSG) and an unidentified cofactor could overcome the potent inhibition, by physiological concentrations of NADPH, of rat liver glucose-6-phosphate dehydrogenase. This appeared to provide an attractive mechanism for the physiological control of this enzyme and, hence, of the hexose-monophosphate shunt. Experiments reported by Rodriguez-Segade *et al.* (1978, 1979, 1980) appeared to support the findings of Eggleston & Krebs (1974) in rat liver extracts, to extend them to extracts from mussel hepatopancreas, and to identify and partially characterize the cofactor. In our own attempts to extend these findings (Levy & Christoff, 1983) we were unable to show any direct effect of GSSG on rat liver glucose-6-phosphate dehydrogenase or any evidence for the existence of a cofactor that mediates such an effect. Instead, we presented evidence that the earlier findings were partly the result of two artifacts: (a) the use of ZnCl_2 to inhibit hepatic glutathione reductase, which causes artifactual absorbance changes easily mistaken for glucose-6-phosphate dehydrogenase activity, and (b) the incorrect use of controls lacking glucose 6-phosphate. We presented evidence that in experiments *in vitro* GSSG appears to relieve NADPH inhibition of glucose-6-phosphate dehydrogenase by oxidizing NADPH to NADP^+ , catalysed by glutathione reductase (Levy & Christoff, 1983).

Rodriguez-Segade *et al.* (1985) now report anomalous absorbance changes, of about the same magnitude as those we described, when ZnCl_2 is added to rat liver

extracts. They also state that, in their reversal assays, net absorbance changes (in which one of a pair of cuvettes contains no substrate) differ by only approx. 5% from results in which absorbances in the presence or absence of substrate are read separately against air. These two methods for subtracting the absorbance change in the absence of added substrate must, of course, give the same result. The important point we made (Levy & Christoff, 1983), and which Rodriguez-Segade *et al.* (1985) appear to disregard, is that it is incorrect to subtract the absorbance change in the cuvette lacking substrate at all. This absorbance change is caused by glucose 6-phosphate in the extract, but because the concentration of glucose 6-phosphate used in the assay saturates the enzyme, any additional substrate added with the extract cannot alter the reaction velocity (Levy & Christoff, 1983). The interpretation of the effect of GSSG and NADPH inhibition of glucose-6-phosphate dehydrogenase in untreated rat liver extracts is completely erroneous if this 'correction' is made, as can be seen by examining our data for the high-speed supernatant in Table 1 of our paper (Levy & Christoff, 1983).

Rodriguez-Segade *et al.* (1985) state that they were unable to understand our reasoning that inhibition of hepatic glucose-6-phosphate dehydrogenase by GSSG is only apparent, since we admit to observing apparent inhibition in experiments in which no NADPH is added. Attempting to settle this point, they show that Hg^{2+} (which they add, as we did, to inhibit glutathione reductase without introducing the artifactual absorbance changes caused by Zn^{2+}) and GSSG, added separately, each inhibit glucose-6-phosphate dehydrogenase, whereas when they are added together they do not. This is precisely what one would expect and, in fact, what we also reported (see our Table 2 and our statement that $2 \mu\text{M-Hg}^{2+}$ inhibits the enzyme by approx. 15%; Levy & Christoff, 1983). Contrary to what Rodriguez-Segade *et al.* (1985) state, the failure to add NADPH to their assay mixtures does not eliminate interference from glutathione reductase activity. The NADPH generated from NADP^+ by the action of glucose-6-phosphate dehydrogenase is rapidly reoxidized by GSSG and glutathione reductase, diminishing the net absorbance increase at 340 nm, which can be erroneously interpreted as inhibition of glucose-6-phosphate dehydrogenase.

The role played by glutathione reductase in these experiments is central to the correct interpretation of the effect of GSSG. In this regard it is not clear, as Rodriguez-Segade *et al.* (1985) claim, why 9.6 units of glutathione reductase present in their rat liver extracts can only consume 1.4 nmol of NADPH in 10 min. In every rat liver extract we have examined, glutathione reductase activity is very high. To assess its role in experiments on glucose-6-phosphate dehydrogenase, one must assay glutathione reductase under the same conditions as those used in those experiments. We noted, for example, that low concentrations of EDTA in the glucose-6-phosphate dehydrogenase assay mixture profoundly stimulate glutathione reductase (Levy & Christoff, 1983). The large effect of glutathione reductase on absorbance changes in glucose-6-phosphate dehydrogenase assays in rat liver extracts is clearly illustrated in Table 1 of our paper (Levy & Christoff, 1983), by comparing the effects of increasing GSSG concentrations in cuvettes lacking glucose 6-phosphate but containing NADPH, with those in cuvettes containing glucose 6-phosphate but lacking

NADPH. It is also illustrated by the effects of $0.66 \mu\text{M-Hg}^{2+}$ (which inhibits glutathione reductase incompletely) and of $2 \mu\text{M-Hg}^{2+}$ (which inhibits completely) on analogous assays in our Table 2 (Levy & Christoff, 1983).

In our previous paper (Levy & Christoff, 1983) we reported absorbance changes during 10 min assays so that our results could be compared directly with those of Eggleston & Krebs (1974). All our assays, however, were conducted with a recording spectrophotometer. This provided additional opportunities to observe the opposing effects of glucose-6-phosphate dehydrogenase and glutathione reductase on absorbance changes at 340 nm and to confirm our conclusion that GSSG acts only indirectly on the former. When GSSG was added to our glucose-6-phosphate dehydrogenase assay mixtures, the absorbance increase was distinctly biphasic, with a slow rate preceding a faster one. The duration of the slow phase was proportional to the concentration of GSSG; it was abolished by adding $2 \mu\text{M-Hg}^{2+}$; and it did not occur in partially purified preparations lacking glutathione reductase. The most reasonable interpretation of this phenomenon is that the slow phase reflects the net absorbance change resulting from the simultaneous actions of glucose-6-phosphate dehydrogenase and glutathione reductase on the interconversion of NADP^+ and NADPH. When GSSG, which is always present in concentrations much lower than that of glucose 6-phosphate, is completely reduced, the production of NADPH from NADP^+ proceeds more rapidly, unhindered by the opposing, glutathione reductase-catalysed, NADPH reoxidation.

The communication of Rodriguez-Segade *et al.* (1985) demonstrates, once again, that one must use great caution in interpreting the effects of GSSG on glucose-6-phosphate dehydrogenase in a crude system that contains glutathione reductase. As we stated earlier (Levy & Christoff, 1983), the possibility that GSSG modulates animal tissue glucose-6-phosphate dehydrogenases is an attractive one that should not be summarily dismissed. Unfortunately, however, our earlier statement is still true: evidence for such a mechanism has yet to be established.

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Nomenclature: a possible solution to the 'peptidase anomaly'

The term 'peptidase' is used in two distinct senses in the general biochemical literature, and also in IUB-recommended nomenclature. This situation gives rise to confusion, and should be resolved if possible.

In one sense, 'peptidase' is taken to be synonymous with exopeptidase, i.e. to refer to a peptide (bond) hydrolase that acts specifically near the *N*- or *C*-terminus of a polypeptide. This usage can be traced back to the early German literature (Grassmann & Dyckerhoff, 1928), and has continued to be popular because exopeptidases often seem to be active preferentially against 'peptides' – small polypeptides. The reason for this is often simply that at a given weight concentration a small polypeptide affords a higher molar concentration of the susceptible end-groups than a larger one. Nevertheless, this is a usage of the term peptidase that has historical priority and is recognized by the Nomenclature Committee of the IUB (NC-IUB, 1984, line 10).

The second way in which 'peptidase' is used is as a short form of 'peptide (bond) hydrolase'. This sense was introduced by Bergmann (Bergmann & Ross, 1936; Bergmann & Fruton, 1937), who also used the root '-peptidase' in coining the terms 'exopeptidase' and 'endopeptidase' which also form part of the IUB recommended nomenclature (NC-IUB, 1984).

The conflict between the two meanings of peptidase is clearly seen if one considers the question 'Is an endopeptidase a peptidase?'. In Grassmann's terminology it is not, but in Bergmann's it is.

Hence it is suggested that we follow Bergmann in using peptidase as a general term for 'peptide (bond) hydrolase', and do not restrict it to enzymes acting preferentially on small polypeptides. Put another way, peptidase should be synonymous with 'protease' and not with 'exopeptidase', and would be analogous to 'glycosidase' for the hydrolases of glycosidic links. The original narrow use of peptidase by Grassmann was based on an imperfect understanding of the reason for the apparent selectivity of the enzymes for small polypeptides, and can reasonably be thought of as obsolete.

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