ciferin in a concentration of $2 \times 10^{-11}M$ and found to be one quantum of light per molecule of luciferin oxidized, with an accuracy of $\pm$ 28 percent (*7*). The quantum efficiency of the *Cypridina* system remains to be determined. It is noteworthy, however, that a luciferin concentration of the order $10^{-11}M$, in both systems referred to above, is unusually low for a chemical test. The most sensitive qualitative tests, involving color reactions, precipitation, or fluorescence, are generally not useful at dilutions greater than 1 part in 10^{10}. Luminescence as an indicator for substances other than the specific enzyme or substrate is apparently not as sensitive; for example, the firefly system has been used to detect the cofactor adenosine triphosphate in concentrations down to about $10^{-9}M$ (*8*), and the "luminol reaction" to detect hydrogen peroxide in concentrations down to $10^{-7}M$ (*9*).

Quantitative data on the yield of pure luciferin (*3*, *10*) and luciferase (*5*) from *Cypridina* indicate that the living organisms, averaging about 4 mg (wet weight) each, contain at best 1 μg of luciferin and 1 μg of luciferase, in a molecular ratio of 100 to 1. The impressively bright luminescence of these small crustaceans, amply sufficient for a self-photograph (Fig. 1), is understandable in terms of the light-emitting potency of the system, despite the seemingly minute quantities of the essential components involved.

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10. We have recently obtained 60 mg of crystalline luciferin, in an approximately 50-percent yield, per kilogram (wet weight) of winter *Cypridina* preserved in Dry Ice.

14 August 1961

Glucose-6-Phosphate Dehydrogenase and Detoxification of Hydrogen Peroxide in Human Erythrocytes

Abstract. Human erythrocytes with deficient glucose-6-phosphate dehydrogenase levels are unable to maintain their levels of reduced glutathione in the presence of low-level, steady-state concentrations of hydrogen peroxide. This finding has bearing on the biochemical mechanisms of drug-induced hemolytic anemia.

Individuals with hereditary deficiencies in glucose-6-phosphate dehydrogenase (G-6-PD) are known to be sensitive to the hemolytic effects of fava bean and a wide variety of drugs, including primaquine, phenylhydrazine, and menadione (*1*). However, the biochemical mechanisms underlying the cellular damage induced by such compounds have not yet been clarified.

Suggestions that hydrogen peroxide might play a critical role in drug-induced hemolysis have in general been dismissed on the grounds that the high catalase activity of erythrocytes would result in rapid destruction of peroxide. Indeed, it may be readily observed that the addition of concentrated hydrogen peroxide directly to erythrocytes causes very little injury to the cells; in contrast, extensive methemoglobin formation and oxidation of reduced glutathione (GSH) may be noted when the addition of peroxide is preceded by the addition of a catalase inhibitor such as azide. However, the toxic effects of very low concentrations of hydrogen peroxide generated within cells cannot be judged from experiments such as that quoted above, namely, experiments in which much higher concentrations of peroxide are added directly to erythrocytes. For example, it has been demonstrated (*2*) that at low concentrations of hydrogen peroxide, the usual decomposition to water and oxygen, by catalase, yields way to a peroxidatic coupling of peroxide to various hydrogen donor molecules. In this regard, the erythrocyte enzyme glutathione peroxidase, recently reported by Mills (*3*), and which is capable of detoxifying hydrogen peroxide by coupling it to reduced glutathione, is of particular interest.

The primary importance of erythrocyte GSH levels in drug-induced hemolytic anemia is apparent from previous observations in erythrocytes deficient in glucose-6-phosphate dehydrogenase, of decreased average GSH levels (*4*), and instability of GSH in the presence of hemolytic agents such as acetylphenylhydrazine (*5*). We have therefore re-evaluated the effect of low-level, steady-state concentrations of hydrogen peroxide on the GSH levels of normal and G-6-PD deficient erythrocytes.

In order to mimic the conditions under which hydrogen peroxide might be generated within cells, either from metabolic processes or from the autoxidation of various drugs or their metabolites, a hydrogen peroxide diffusion technique was used (*6*). The peroxide was added to the center well of a Warburg flask and permitted to diffuse slowly to the erythrocytes in the main compartment. A grease ring at the top of the center well prevented any cross-contamination by "creeping." The rate of diffusion of peroxide could be controlled by adjusting the amount and concentration of peroxide placed in the center well. The diffusion rate was also dependent upon the temperature, the rate of shaking, and the geometry of the flask.

Heparinized blood was obtained from a group of individuals with erythrocytes deficient in glucose-6-phosphate dehy-

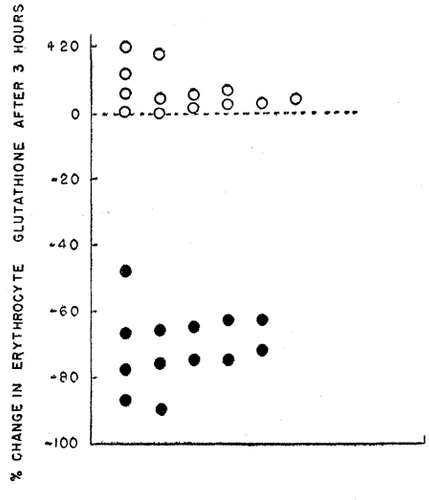

Fig. 1. Percentage change in reduced glutathione (GSH) in erythrocytes of 13 individuals with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (solid circles) and 13 individuals with normal G-6-PD activity (open circles) after 3 hours of hydrogen peroxide diffusion. Average initial GSH values were: 50 mg/100 ml (range 31 to 68 mg/100 ml) for the G-6-PD deficient erythrocytes, and 58 mg/100 ml (range 36 to 77 mg/100 ml) for the normal erythrocytes. Included in the G-6-PD deficient group were three subjects receiving isoniazid for treatment of tuberculosis; included in the normal G-6-PD group was one individual receiving isoniazid for treatment of tuberculosis, and one recovering from hemolytic anemia that was not drug-induced.

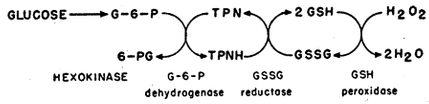

Fig. 2. The glucose-linked pathway of hydrogen peroxide detoxification.

drogenase (*7*) and from a group of normal volunteers. The blood, either freshly drawn or refrigerated for 12 to 24 hours, was diluted with two to three volumes of isotonic saline and centrifuged, and the supernatant fluid and buffy coat were removed by aspiration. The packed erythrocytes were then washed twice in four to six volumes of isotonic saline, and finally resuspended in three parts of isotonic saline at *p*H 7.4 (*8*), containing 250 mg of glucose per 100 ml. Three and a half milliliters of the erythrocyte suspension were placed in the main compartment of a manometric flask, and 0.25 ml of commercial 30-percent hydrogen peroxide was placed in the center well. The vessel was capped with Parafilm and incubated at 37°C in a Dubnoff metabolic incubator at a shaking speed of 90 to 100 oscillations per minute. Under these conditions, roughly 12 μmole of hydrogen peroxide were added to the main compartment per hour, as determined by collecting the peroxide in 1*N* H_2SO_4 and titrating with standardized 0.01*N* $KMnO_4$.

At the end of 3 hours, the contents of the main compartment were removed and analyzed for reduced glutathione by a modification of the technique of Grunert and Phillips (*9*). The value at 3 hours was compared with that at zero time, and the data are expressed as percent change in reduced glutathione in 3 hours (see Fig. 1). Losses of 50 to 90 percent were observed for erythrocytes obtained from 13 individuals deficient in the dehydrogenase; no losses were observed for erythrocytes with normal levels of the enzyme. No losses in reduced glutathione were noted in control samples incubated without hydrogen peroxide.

However, losses of reduced glutathione induced by hydrogen peroxide could be obtained with normal erythrocytes also, when they were incubated in the absence of glucose. These results are similar to those reported by Beutler *et al.* (*10*) for the hemolytic agent acetylphenylhydrazine; they demonstrate that the protective mechanism of normal erythrocytes is linked to the metabolism of glucose.

The loss in reduced glutathione was most probably due to its oxidation, as catalyzed by the erythrocyte enzyme, glutathione peroxidase (*3*). Coupling of hydrogen peroxide to reduced glutathione was not catalyzed by any peroxidatic activity of catalase, since the catalase inhibitor, azide, did not block this activity, but rather augmented it. The glucose-linked protective mechanism of normal erythrocytes may best be attributed to the reduction of oxidized glutathione by the triphosphopyridine nucleotide (TPNH) specific, glutathione reductase (*11*); the reduced triphosphopyridine nucleotide is supplied from the activity of the hexose monophosphate shunt (see Fig. 2) (*3*). In G-6-PD deficient erythrocytes, the severe limitation in TPNH production from the dehydrogenation of glucose-6-phosphate (and from the subsequent dehydrogenation of 6-phosphogluconate) results in a marked inability to maintain the level of reduced glutathione in the continuous presence of low-level, steady-state concentrations of hydrogen peroxide. The major importance of the glutathione peroxidase–G-6-PD pathway for the detoxification of hydrogen peroxide in erythrocytes is illustrated by the fact that once the GSH level has fallen, other changes, such as methemoglobin formation and increased osmotic fragility, become more and more evident under the influence of diffusing peroxide (*12*).

These data are consistent with a mechanism of drug-induced hemolysis in G-6-PD deficient erythrocytes, in which hydrogen peroxide plays a major role. It is suggested that the oxidative damage induced by hemolytic agents is caused in part by the intermediate generation of hydrogen peroxide in low concentration from the autoxidation of the active drugs or their metabolites (*13*).

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7. We are indebted to Dr. Ernst Jaffe of Albert Einstein College of Medicine, New York, for supplying four of these blood specimens, and to Dr. Paul Marks of Columbia University College of Physicians and Surgeons for supplying two of these specimens. The remaining G-6-PD deficient specimens were obtained by enzyme screening of a series of blood samples obtained from a randomly selected group of Negro male subjects from the Columbia-Presbyterian Medical Center; the cooperation of Pat Leitner of the out-patient department of Vanderbilt clinic is gratefully acknowledged. All of the G-6-PD deficient specimens contained less than 10 percent of the normal G-6-PD activity as measured by the technique of A. Kornberg and B. L. Horecker [in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955)].
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Reactivity Cycle of Somatosensory Cortex in Humans with and without Psychiatric Disorder

Abstract. With a photographic averager to extract evoked cortical responses, reactivity cycles of primary potentials were determined over 200 milliseconds in 105 subjects. The typical cycle was biphasic, with peaks before 20 and after 100 msec. Subjects who were not psychiatric patients showed full recovery of responsiveness during the initial peak phase, whereas most of the psychiatric patients did not.

The purpose of this report is to describe the reactivity cycle of the somatosensory cortex in man and to present evidence that quantitative alterations in this cycle occur in psychiatric illness.

The cortical reactivity cycle is determined by applying paired "conditioning" and "test" stimuli, separated by varying intervals, to evoke cortical potentials. The relative amplitude of the two potentials (ratio of the second to the first) gives an indication of changes in cortical responsiveness with time subsequent to the first stimulus.