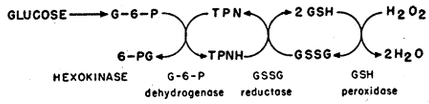

Fig. 2. The glucose-linked pathway of hydrogen peroxide detoxification.

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.

This procedure is similar to the classical one for determining the excitability cycle of nerve in that it uses paired stimuli; it also differs, in that response magnitude, rather than threshold, is the indicator.

There have been almost no studies of human cortical reactivity cycles because of the difficulty in detecting evoked potentials with scalp electrodes. These potentials are very small at the scalp and are obscured by the much larger "spontaneous" brain rhythms. Gastaut, Corriol, and Roger (*1*) were able to make some determinations from the visual cortex in subjects with unusually large responses to light flash, but their data suggest that they were measuring a secondary component of the evoked potential. Purpura *et al.* (*2*) studied three patients, two schizophrenic, with brain exposed at operation; their results suggested delayed recovery to direct cortical stimulation in the schizophrenic. We applied Dawson's principle of averaging to extract evoked potentials from scalp recordings (*3*); in a few subjects we showed that the records so obtained could be used to plot a cortical reactivity cycle.

In the study reported here, cortical potentials evoked by electrical stimulation of the ulnar nerve at the wrist were extracted by means of a photographic averager. Shipton has described the apparatus (*4*). The electroencephalographic recording from the somatosensory area contralateral to the stimulus is led to a cathode ray oscilloscope, whose beam is modulated so that fluctuations of brightness in a horizontal line correspond to amplitude variations in the usual *Y*-axis. The vertical position of the beam is systematically shifted for each sweep by a raster. We photograph 100 sweeps routinely on Polaroid slide film. The film is optically analyzed by moving it across a slit through which a beam of light passes onto a photomultiplier, the output of which is written out on an *X-Y* plotter. Figure 1 shows a film and the corresponding tracing; it also indicates the difficulty of detecting evoked potentials in the electroencephalogram. Electrical stimuli were brief (usually 0.1 msec) and of sufficient intensity to elicit a twitch in the little finger. The time between successive pairs of stimuli was 1.3 seconds.

The film record in Fig. 1 demonstrates that the primary evoked potential is consistently present and of constant latency. Upward deflection in the tracings indicates relative positivity at the active electrode. The primary complex begins with a negative deflection, maximal at latency of 17 to 23 msec; positivity follows, peaking at 21 to 30 msec. There may be two positive components in the complex. A more variable series of secondary components, extending to 350 or 400 msec, generally follows the primary complex.

Reactivity cycles were obtained in 13 apparently healthy nonpatients and in 92 psychiatric patients of all types. The cycles are based only on primary responses, measured from peak negativity to peak positivity. Mean amplitude was about 3 μv. Pairs of stimuli were separated, from 2.5 to 200 msec, by 26 intervals, with separations in steps of 2.5 msec from 2.5 to 20 msec and in steps of 10 msec thereafter.

The tracings in Fig. 2 illustrate recovery of responsiveness for one subject with unusually large responses and very little secondary activity. Ordinarily the secondary components must be separated from the second primary re-

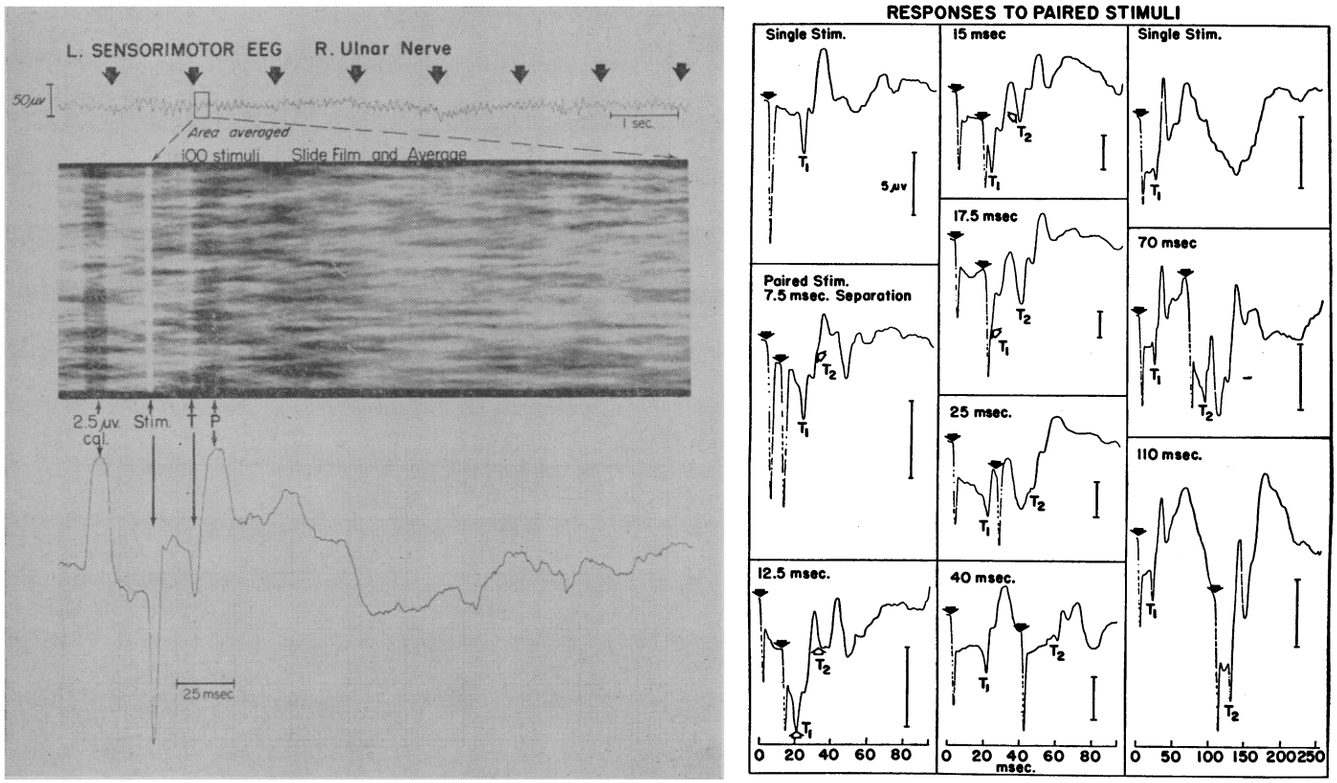

Fig. 1 (left). (Upper trace) Effect of single shocks to the right ulnar nerve on a conventional electroencephalogram from the left sensorimotor area. (Bottom trace) Optical analysis, giving an average of 100 responses from slide film (film and trace on identical time scale). *T*, Maximum initial negativity; *P*, peak of positive component. Fig. 2 (right). Tracings from one subject to illustrate development and amplitude fluctuation of responses to the second of a pair of stimuli separated by varying intervals. The time scale is longer in the right-hand column; T_1 and T_2, points of maximum negativity for the first and second responses.

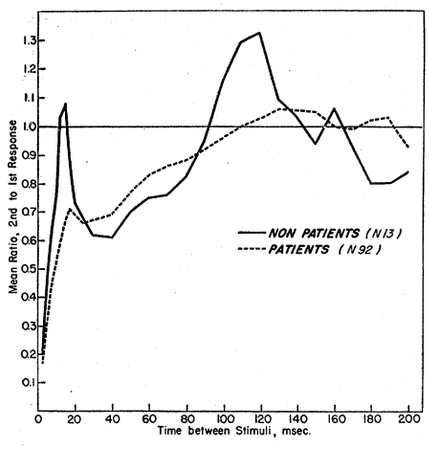

Fig. 3. Mean reactivity cycles of patients and nonpatients. A ratio of 1.0 indicates full recovery of responsiveness.

sponse by subtracting the changes at corresponding times subsequent to an unpaired stimulus. It may be noted in Fig. 2 that the second response increases until it is as large as the first at a separation of 17.5 msec. A subsequent brief period of response greater than normal is then followed by diminished responsiveness until the second response is again greater than the first, at 110 msec. This biphasic pattern characterized the recovery curves of all nonpatients, as indicated in the mean curve (Fig. 3).

Figure 3 also shows the mean reactivity cycle for the patients. Although the biphasic pattern was also the predominant one in the individual curves of most of the patients, this is not clear in the mean curve because the amount of recovery was less than in nonpatients and there was greater dispersion in timing. The greatest difference between patients and controls was in the amount of recovery by 20 msec. All nonpatients, except one with a peak recovery ratio of 0.95, showed full recovery by 20 msec. Only 27 patients (29 percent) showed full recovery—a highly significant difference ($P < .001$). It may be noted that two-thirds of the patients whose recovery ratios overlapped those of the controls were diagnosed as psychoneurotic, whereas for psychotics there was almost no overlap. The greater reactivity of nonpatients from 100 to 120 msec was also statistically significant ($P = .03$). Reliability, on retest, of the measure of peak recovery by 20 msec was 0.78 in 17 subjects. No significant age or sex differences were found.

The mean time for initial recovery of reactivity in the nonpatients was 12.5 msec. This is more rapid than the recovery time reported for any animal and suggests that initial recovery time may be phylogenetically determined. It is also of interest that the major differences between patients and nonpatients in cortical reactivity occurred during this early phase of recovery. The differences in findings for patients and for controls indicate that research designed to determine factors governing the cortical reactivity cycle may be of great importance to psychiatry. Information about the anatomical locus and neurohumoral mechanisms underlying the cycle may help to clarify the pathophysiology of disturbed behavior. As the reactivity cycle is easy to determine in animals, relevant experimentation with implanted electrodes, with drugs, and with surgery may readily be carried out (*5*).

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Choline Sulfate in Higher Plants

Abstract. Choline sulfate, the sulfate ester of choline, is widely distributed in plant species and tissues. It constitutes up to one-third of the labeled metabolic products of radiosulfate uptake by roots of sulfur-deficient corn, barley, and sunflower plants. This neutral, nonabsorbed zwitterion appears to be a useful reservoir for sulfur in plants.

O-Choline sulfate has previously been identified in mycelia and conidiospores of certain fungi (*1*), in a genus of lichens (*2*), and in a red alga (*3*). The analogous choline phosphate occurs in higher plants and is involved in phosphorus transport by the sap (*4*).

In this study (*5*) corn (*Zea mays*), barley (*Hordeum vulgare*), and sunflower (*Helianthus annus*) were grown in water or in sand with either a complete Hoagland solution or one lacking sulfate. The roots were cut at different times, allowed to take up radiosulfate during periods of several hours, and extracted with hot 80-percent ethanol. Two-dimensional paper chromatography (*6*) and autoradiography revealed a major radioactive product ($R_F = 0.89$ in phenol and water [100 : 40 wt./wt.]; $R_F = 0.37$ in *n*-butanol, propionic acid, and water [142 : 71 : 100 vol/vol]). It was identified as choline sulfate by cochromatography with synthetic choline sulfate-S^{35}, by the identity of the hydrolysis rates of the natural product and synthetic choline sulfate-S^{35} (half-time for hydrolysis is 33 minutes in 1.0*N* HCl at 100°C), and by repeated cocrystallization with synthetic choline sulfate (*7*).

In order to ascertain that the formation of choline sulfate was not due to microorganisms associated with the plant roots, corn and barley were grown on agar under sterile conditions. Choline sulfate-S^{35} was formed as before.

Choline sulfate was the major labeled compound formed by roots of sulfur-deficient plants, constituting up to one-third of the incorporated S^{35}. In leaves of the deficient plants as well as in normal roots and leaves of all the higher plants examined, choline sulfate constituted 5 to 15 percent of all the soluble sulfur compounds.

The large amount of choline sulfate formed in roots of sulfur-deficient plants suggests its function as a major sulfur reservoir. Its neutral nature and high solubility in organic solvents suggest that it functions as an effective transport agent and that choline-containing membranes mediate in the transport mechanism.

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