scribed area in the rostral midbrain reduced the inhibitory aftereffects of ejaculation (postejaculatory interval) with a resulting increase in the number of ejaculations achieved in 1-hour tests. These lesions also interrupted the DNE bundle, as reflected in a 63 percent reduction of telencephalic NE (*10*).

The apparent relationship between NE depletion and altered copulatory performance may have resulted from (i) destruction of the DNE bundle, or (ii) destruction of a system in close proximity to the DNE bundle but functionally unrelated to it. Since telencephalic NE depletion has also been obtained after hypothalamic medial forebrain bundle lesions which impair copulation (*11*), it follows that the behavioral effects obtained in either the earlier study or the present one were produced by something other than DNE bundle damage. While a choice between these alternatives cannot be made on the basis of current information, we have observed that lesions of the type reported in the earlier study also produce substantial dopamine depletion as well as damage to the descending medial forebrain bundle (*12*).

If we assume that interruption of the DNE bundle was responsible for the behavioral changes found in the present study, this suggests that either (i) this system normally exerts a direct inhibitory effect on copulation, or (ii) the DNE system has an indirect effect on behavior through its modulation of other systems with which it is in a state of dynamic equilibrium. Partial damage of the DNE bundle may have disrupted this equilibrium, setting in motion compensatory changes in potent excitatory systems more directly involved in behavior regulation. When viewed in this way, the assignment of an excitatory or inhibitory role to the DNE bundle, as applied to a behavioral end point, loses much of its meaning. Such an interaction may occur between NE and dopamine systems, and is suggested by recent evidence at both the behavioral (*13*) and biochemical levels (*14*).

T. K. CLARK
Department of Biophysics and Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260
ANTHONY R. CAGGIULA
Departments of Psychology and *Pharmacology, University of Pittsburgh*
R. A. MCCONNELL
Department of Biophysics and Microbiology, University of Pittsburgh
SEYMOUR M. ANTELMAN
Western Psychiatric Institute and Clinic, Department of Psychiatry, University of Pittsburgh School of Medicine, and *Department of Psychology, University of Pittsburgh*

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7. The behavioral response which normally accompanies ejaculation in the rat can be easily identified as an intromission (mounting of the female and a brief series of rapid shallow and deep thrusts terminated by a quick dismount) in which the last deep thrust is intensified and prolonged and the dismount is slower.
8. Dopamine and norepinephrine were determined by a modification of the semiautomated fluorometric procedure of P. Waldmeier and L. Maître [*Anal. Biochem.* **51**, 474 (1973)]. These procedures are based on the trihydroxyindole process described by R. Laverty and K. M. Taylor [*ibid.* **22**, 269 (1968)]. The catecholamine-containing eluates from the alumina column were converted to trihydroxyindoles with the use of a Technicon AutoAnalyzer, and the fluorescence was measured with an Aminco-Bowman spectrophotofluorometer equipped with a flow cell. Assay values (μg/g) were as follows. Telencephalic NE: control (U + S), 0.32 ± 0.04, N = 4; lesion group A, 0.13 ± 0.01, N = 9; lesion group B, 0.24 ± 0.02, N = 3. Cortical NE: control, 0.27 ± 0.01, N = 5; lesion group A, 0.09 ± 0.01, N = 8. Caudate dopamine: control 8.2 ± 0.5, N = 5; lesion group A, 9.5 ± 0.7, N = 8.
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Glucose-6-Phosphate Deficiency and Inhibition by NADPH: A Self-Contradictory Argument

In his discussion of hemolytic anemia resulting from genetic deficiencies of human glucose-6-phosphate dehydrogenase (G6PD) (*1*) Yoshida cited the traditional explanation: These variants "cannot generate enough NADPH in red cells to maintain an adequate concentration of reduced glutathione." The impairment in ability of G6PD-deficient cells to maintain normal levels of reduced glutathione has been recognized for many years; and NADPH (reduced nicotinamide adenine dinucleotide phosphate), the product of G6PD, is known to be necessary for keeping glutathione in the reduced form. In his review, however, Yoshida argues that these same "hemolytic variant enzymes are strongly inhibited by NADPH of physiologic concentration . . ." and "therefore, in the presence of low concentrations of NADP and relatively high concentrations of NADPH as in the red cells, the hemolytic variants such as Manchester, Tripler, and Alhambra, can scarcely function (Table 4) although their 'red cell G6PD activity' is more than 20 percent of normal." The activities of normal and variant G6PD's in the presence of high ratios of NADPH to total NADP (0.82 to 0.99), such as are known to exist in normal red cells, were shown in Yoshida's table 4.

These statements are self-contradictory. The red cell cannot have levels of NADPH which are, at the same time, both normal and deficient. We would not ordinarily call attention to so obvious a discrepancy, except for the repetition of this contradiction (*2*).

Consideration of the maximum velocities and either table 3 or figure 2 in (*1*) reveals that the competitive inhibitor constants (K_i's) of the NADPH-sensitive variants are not low enough to explain the greater clinical severity of these variants, if the ratio of NADPH to total NADP is less than half the normal ratio. Although they are competitively inhibited by NADPH much more readily than other G6PD-deficient variants, their maximum activities are much greater. Thus, a high ratio of NADPH to the total NADP leads to the contradiction mentioned, whereas a low to moderate ratio of NADPH to total NADP (*3*) leads to refutation of a major point in the article, that concerning the significance of low K_i's in certain variants of human G6PD with clinically severe symptoms.

An argument might be made that Yo-

shida's own data shown in his table 3 or figure 2 should not be used to refute his point, since these may not have been obtained under the same conditions as exist within the cell. These are the only data, however, defending Yoshida's point at less than high ratios of NADPH to the total NADP. If they were offered as serious evidence in support of Yoshida's point, it would seem inappropriate to disregard the data when they are found to disprove the point.

HENRY N. KIRKMAN
Department of Pediatrics, School of Medicine, and *Child Development Institute, University of North Carolina, Chapel Hill 27514*

GIANFRANCO D. GAETANI
Department of Hematology, School of Medicine, University of Genoa, Genoa, Italy

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3. In the red cells of the two most prevalent G6PD-deficient variants, Mediterranean and A$^-$, the ratio of NADPH to the total NADP is only 0.2 to 0.4, even in the absence of imposed oxidative stress [G. D. Gaetani, J. C. Parker, H. N. Kirkman, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3584 (1974); H. N. Kirkman, G. D. Gaetani, E. H. Clemons, C. Mareni, *J. Clin. Invest.* **55**, 875 (1975)].
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I did not ignore the obvious possibility that the nicotine adenine dinucleotide phosphate (NADP) concentration in the glucose-6-phosphate dehydrogenase (G6PD)-deficient variant red cells could be higher than normal, thus compensating for part (or all) of the intrinsic red cell enzyme deficiency. The crucial problem is how high the NADP level (or how low the NADPH level) may be maintained in red cells without seriously impairing other metabolic pathways, levels of intermediate metabolites, and cell integrity. I previously estimated (*1, 2*) that NADP concentration may go up to about 10 μM (about 20 times normal) without damaging the red cells. This value was deduced from the following facts: (i) Activity of the hexose monophosphate shunt pathway of red cells from the Mediterranean variant subject, associated with severe enzyme deficiency but not associated with chronic hemolytic anemia, was about 30 percent that of normal red cells; (ii) from the activity of the variant enzyme measured under the simulated physiologic conditions, the concentration of NADP required for the physiologic shunt pathway activity was estimated to be 2 to 5 μM; and (iii) the Mediterranean G6PD is labile in red cells, and the enzyme activity of the older variant red cells is expected to be lower than that of unfractionated red cells. Thus, the concentration of NADP in the older variant cells could be higher than that of unfractionated cells.

On the basis of the measurement of the enzyme activity under simulated physiologic conditions in the presence of various concentrations (ranging from 0.5 μM to 10 μM) of NADP [table 4 in (*1*) and table 1 in (*2*)], we found that several G6PD variants associated with relatively mild enzyme deficiency and yet with chronic hemolytic anemia are expected to have lower G6PD activity in red cells even in the presence of 20 times the normal concentration of NADP, while several G6PD variants associated with severe enzyme deficiency but not with a chronic hemolytic problem are expected to have close-to-normal enzyme activity in red cells.

Kirkman and his associates have made a direct determination of the total NADP + NADPH and the NADPH of the normal, G6PD A$^-$, and Mediterranean G6PD red cells (*3*). Their values for NADP and NADPH of the normal red cells are in agreement with the values reported (*4*). But they found low concentrations of NADPH (10 to 25 μM) and high concentrations of NADP (25 to 40 μM) in red cells from the G6PD-deficient variant subjects. In accepting their values for the variant G6PD red cells, one has to face the following problems. In the presence of 25 to 40 μM of NADP, under the simulated physiologic conditions, the shunt pathway activity of the variant (A$^-$ and Mediterranean) red cells is expected to be much higher (more than ten times) than that of normal red cells. Since the shunt pathway activity of the variant red cells is below normal, one has to assume that (i) kinetic properties of the enzyme in red cells is entirely different from those determined in vitro, (ii) a putative suppression mechanism (or inhibitor) for G6PD activity is operating in the variant red cells, or (iii) NADP and NADPH are not homogeneously distributed in red cells. The G6PD activity of the normal red cells, estimated under simulated physiologic conditions in the presence of known concentrations of NADP (0.5 to 1 μM), NADPH (40 to 50 μM), and various metabolites, agrees with the shunt pathway activity measured by CO_2 generation. Therefore, none of the possibilities suggested above seems to exist, at least in the normal red cells.

NADPH is known to be easily oxidized to NADP, and avoiding the oxidation during the assay may not always be complete. Although the method now used is adequate for estimating NADPH concentration in the normal cells, it may not be adequate for protecting NADPH from the oxidation in the G6PD-deficient red cells. A new assay method for NADP and NADPH in the variant red cells may be necessary, particularly to resolve the problems described above.

AKIRA YOSHIDA
Department of Biochemical Genetics, City of Hope National Medical Center, Duarte, California 91010

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