Seasonal Variations in Red Cell Glutathione Levels with Aging in Mental Patients and Normal Controls* (34517)

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Glutathione, γ-glutamylcysteinylglycine, is widely distributed in animal and plant cells. It occurs most commonly in its reduced form (GSH) or, more rarely, as the disulfide (GSSG). In human erythrocytes GSH is found in sizeable amounts. It is known to play an important part in the maintenance of the cell's integrity and in many of its biochemical reactions.

Abnormal erythrocytic GSH levels were claimed as concomitants with many physical or mental disease conditions. Significantly depressed blood levels were found in schizophrenic patients by Altschule and by Martens (1, 2), whereas studies by other authors have failed to reveal significant variations between mental patients and normal controls (3, 4). Red cell GSH was correlated with aging by Bertolini, who detected a steady increase in healthy subjects between the ages of 20 to 70 years (5), whereas diminution was reported within a similar age range by Chow (6). This paper represents an attempt at correlating observations on red cell GSH levels in mental patients and controls with data on such changes as may be caused by aging.

Materials and Methods. Venous blood was obtained from fasting and nonmedicated patients resident at Creedmoore State Hospital. Coagulation was prevented with small amounts of Liquaemin (1% isotonic heparin sodium solution, Organon, Inc.). GSH levels were determined by a recent method (7) and expressed as GSH index, or milligrams of GSH per 100 ml of red blood cells (1). Hemoglobin was estimated spectrophotometrically by a standard cyanmethemoglobin method. Microhematocrits were read on an illuminated microcapillary reader (Model CR, International Equipment Co.) after 4 min of centrifugation in a microhematocrit centrifuge.

Subjects of this study were patients with institutional diagnoses of paranoid or catatonic schizophrenia and of psychoses with senility or alcoholism. Excluded from this study were individuals with anemia or known physical ailments and subjects deficient in red cell glucose-6-phosphate dehydrogenase (G-6-PD). Bearers of this hereditary trait, which is commonly known as primaquine-sensitivity, may constitute 20% of the Negro population. They were recognized by subnormal values of GSH index and/or stability and of erythrocytic G-6-PD, as described in a previous paper (8).

Control specimens were drawn from employees at Creedmoor State Hospital, since this category was believed to represent the closest practicable approach to nutritional parallels of the patient population. They were subjected to the same analyses and exclusions as were described above.

Evaluation. Findings on patient and control populations were subdivided by sex and race (Negro and non-Negro). The data were thus distributed within eight groups (male Negro and non-Negro patients and controls, female Negro and non-Negro patients and controls). Correlation coefficients (r) pertaining to changes in GSH index with age were determined on an IBM System 360 Model 50 computer.

Averages of GSH index per decade of age

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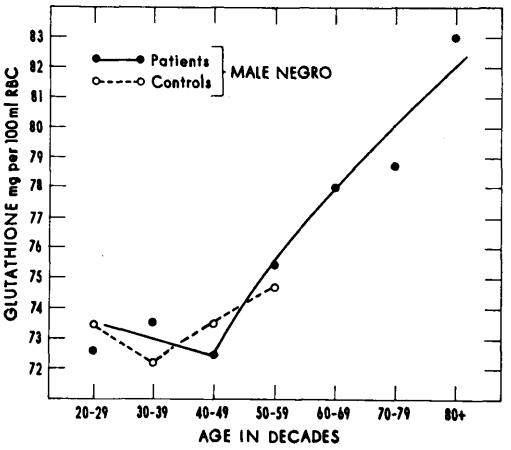

FIG. 1. Changes in blood glutathione levels with aging in male Negro patients and controls. 248 patients, avg. N per point 35.4 $\pm$ 6.3; 73 controls, avg. N per point 18.2 $\pm$ 4.1.

were determined for each group and were plotted against age. In order to detect any seasonal influence on GSH levels, the data were subdivided into "summer readings" that were taken from March 22 through September 21, and "winter readings" that were obtained during the rest of the year. Such seasonal data were treated and plotted by decade as described above.

Results obtained on Negro subjects. GSH indices were determined on 248 male and 177 female Negro patients, and on 73 male and 90 female Negro controls. Increase in GSH index with age is seen in male Negro patients (Fig. 1). Significant positive correlation was obtained ($r = .28, p < .01$). Positive and insignificant correlation was found in male Negro controls ($r = .105, p < .10$). No Negro male controls were available after the sixth decade. No significant seasonal variations were observed in either patients or controls. No systematic changes in GSH index with age are observed when averages per decade are plotted for female Negro patients and controls. In the fourth decade, the value is significantly higher in patients than in controls (t 2.140, df 56, $p < .05$) (Fig. 2).

Some significant variations appear in female Negro patients' averages during winter (Fig. 3). Descent in GSH value is encountered from the fourth to fifth decades (t 2.496, df 36, $p < .02$), and from the seventh to eighth decades (t 2.319, df 18 $p < .05$), whereas a rise is obtained between the two oldest groups (t 2.826, df 16, $p < .025$). The patients' summer level is significantly higher than the controls' in the sixth decade (t 2.212, df 27, $p < .05$).

Results obtained on non-Negro subjects. GSH indices were determined on 163 male and 147 female non-Negro patients, and on 125 male and 111 female non-Negro controls. Significant correlation of GSH index with age was found in male patients ($r = .257, p < .01$), and in their controls ($r = .293, p < .01$). The difference in r values is insignificant ($z = .32$).

When average values were plotted against age, ascending curves were obtained for male patients and controls (Fig. 4). Patient values significantly exceed the corresponding average in controls in the fifth decade (t 2.096, df 44, $p < .05$). Significant ascent is observed in controls between the fifth and the sixth decades (t 2.646, df 51, $p < .02$).

Division of the data obtained on non-Negro males by season and plotting them as averages per decade results in some significant slopes (Fig. 5). Increase is seen during winter in controls between the fifth and sixth decades (t 3.521, df 26, $p < .005$), followed by descent to the next age group (t 2.126, df

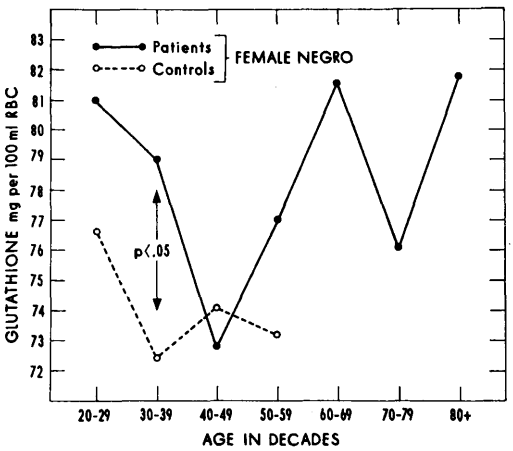

FIG. 2. Changes in blood glutathione levels with aging in female Negro patients and controls. 177 patients, avg. N per point 25.3 $\pm$ 2.3; 90 controls, avg. N per point 22.5 $\pm$ 2.5.

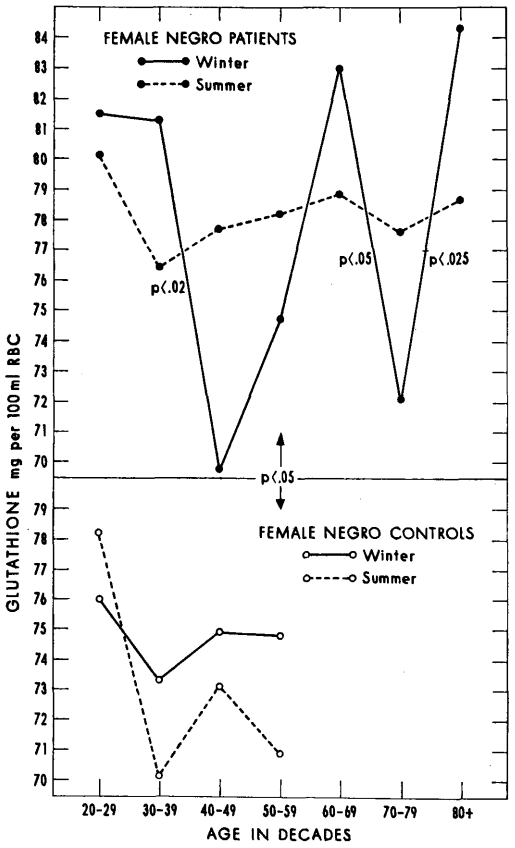

FIG. 3. Seasonal influence on changes in blood glutathione levels with aging in female Negro patients and controls. Patients: 92 winter readings, avg. *N* per point 13.1 ± 2.0; 85 summer readings, avg. *N* per point 12.1 ± 1.7. Controls: 54 winter readings, avg. *N* per point 13.5 ± 2.8; 36 summer readings, avg. *N* per point 9.0 ± 1.2.

19, $p < .05$). At the height of this peak, in the sixth decade, winter readings in controls significantly exceed their summer average (t 2.3665, df 27, $p < .025$). Significant ascent is observed in patients in summer between the two oldest groups (t 2.245, df 20, $p < .05$). Patients' values exceed controls in the fifth decade during winter (t 2.203, df 20, $p > .05$).

No correlation of GSH levels with age is seen in non-Negro female patients and controls, since both graphs appear nearly plateau shaped (Fig. 6). No seasonal variations were observed in either group.

Discussion. Since the data acquired for this paper were categorized by age, sex and race of patients and controls, simple correlation between blood GSH levels and mental disease, as claimed by other authors (1, 2) could not be expected. In some age groups, however, average GSH indices were significantly higher in female Negro and in male non-Negro patients than in the corresponding control groups (Figs. 2–5). GSH indices were within normal range in patients and in controls of all groups.

Positive correlation of GSH index with aging could be established in all of the male groups, with the exception of Negro controls, whose age range was insufficient to permit such a comparison. In the three residual male populations, GSH indices in the oldest cohorts were significantly higher than in the youngest. This is comparable to findings on 88 healthy males by Bertolini, who explains such increase with the rising need of the senile system for additional sulfhydryl groups in order to maintain the stability of hemoglobin by preventing its oxidation into methemoglobin (5).

The findings on females differ from those on males through absence of any systematic changes in GSH index with age; the averages obtained from the oldest cohorts resemble those of the youngest. Since dependence of

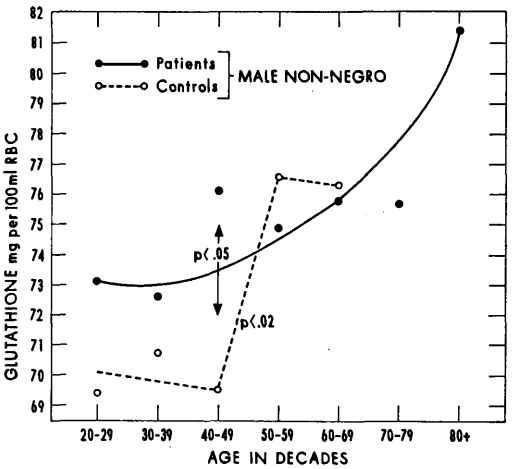

FIG. 4. Changes in blood glutathione levels with aging in male non-Negro patients and controls. 163 patients, avg. *N* per point 23.3 ± 1.6; 120 controls, avg. *N* per point 24.0 ± 1.4.

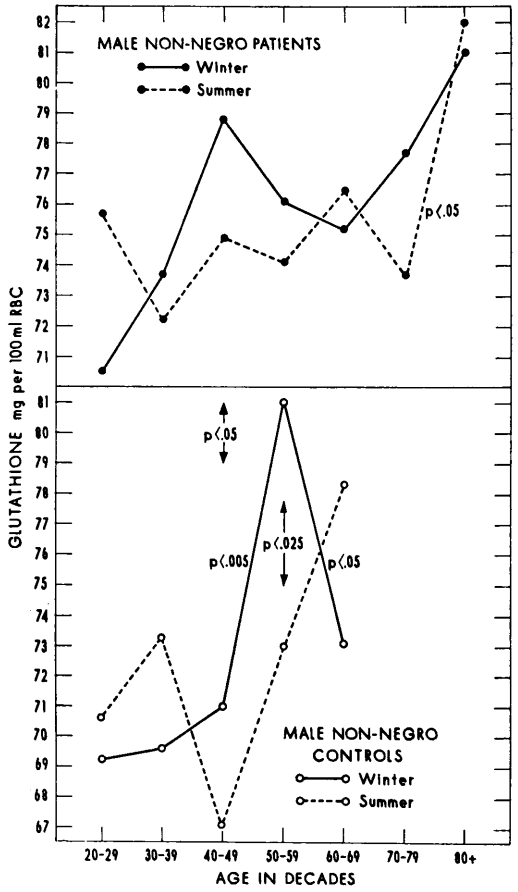

FIG. 5. Seasonal influence on changes in blood glutathione levels with aging in male non-Negro patients and controls. Patients: 76 winter readings, avg. *N* per point 10.9 ± 1.6; 87 summer readings, avg. *N* per point 12.4 ± 0.8. Controls: 72 winter readings, avg. *N* per point 14.4 ± 2.0; 48 summer readings, avg. *N* per point 9.6 ± 2.1.

red cell GSH levels on estrogenic hormone cycle was established by Tokunaga (9), it would appear convenient to attempt explanation of the differences in male and female patterns in hormonal terms. On this basis, it could be suggested that the significant fluctuations observed in female Negro patients during winter could be caused by an imbalance in estrogenic hormone function. Since there does not appear to be sufficient evidence connecting sex hormones with mental disease, or linking them with seasonal and/or racial variations in GSH levels to support such a hypothesis, it may be admissible briefly to discuss some other hormones which are known to be influenced by some of these factors.

Dependence of red cell GSH level on thyroid function was claimed by a number of authors, most of whom believed the former to be inversely affected by the latter (10, 11). There is strong proof of seasonal variation in thyroid function in some races. Osiba found protein-bound iodine levels and basal metabolic rates in Japanese males to be significantly higher in winter than in summer, and quotes a number of Japanese authors claiming similar results. This author remarks that, contrarily, most American workers established nearly constant basal metabolic rates in their subjects throughout the year (12). Racial differences in seasonal variations were established by Kaname, who detected significant negative correlation between basal metabolic rate and environmental temperature in Japanese, whereas this effect was lacking in Canadians resident in Japan (13).

A considerable body of work deals with attempts at linking adrenocortical steroid functions to mental disease (14). Studies on seasonal variations in hormonal output resulted in biphasic curves, suggesting the highest degree of adrenocortical activity to take place during winter, with an additional peak during very hot weather (15). Since treatment with adrenocortical hormones was claimed by various authors to influence red cell GSH levels in either direction (10), it would appear possible to include temperature stress effects on adrenocortical hormones

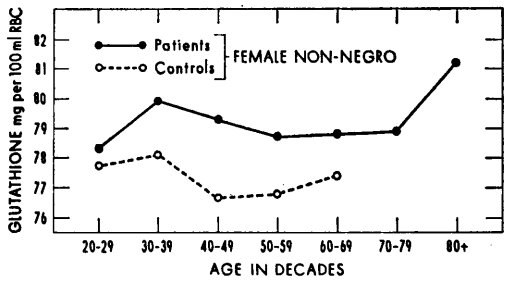

FIG. 6. Changes in blood glutathione levels with aging in female non-Negro patients and controls. 147 patients, avg. *N* per point 21.0 ± 0.7; 111 controls, avg. *N* per point 22.2 ± 3.4

among the factors that may cause such significant seasonal variations in GSH index as were observed on female Negro patients, and on male non-Negro patients and controls.

Summary. Determinations of red cell glutathione index and stability, and glucose-6-phosphate dehydrogenase were performed on 735 mental patients resident at Creedmoor State Hospital, with institutional diagnoses of paranoid or catatonic schizophrenia, and psychoses with senility or alcoholism. This population was matched with 399 nonpatient controls that were drawn from hospital employees. Patients and controls were grouped and matched by sex and race (Negro and non-Negro.

Significant positive correlation between GSH index and age was obtained in male Negro and non-Negro patients, and in male non-Negro controls. When averages of GSH index per decade were obtained for each group and plotted against age, some significant differences were observed between female Negro and male non-Negro patients and their controls.

When the data on GSH indices were subdivided according to the season during which they had been obtained, and plotted by decade against age, some significant slopes were observed in female Negro and male non-Negro patients, and in male non-Negro controls. Some interpretations of these findings are discussed.

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