Table II. Estimated distribution of donor and host CFU for the different experimental treatment

Treatment	Donor		Host
0 R	Marrow cells inoculum	10^7	Cells in total marrow 4×10^8
	CFU	12×10^2	CFU in total marrow 50×10^3
			CFU in circulating pool 110–150
650 R[a]	Marrow cells inoculum	10^7	Surviving CFU in total marrow 100–200
	Surviving CFU	2–5	Surviving endogenous spleen CFU 4–8
			Surviving CFU in circulating pool <0.3

[a] Surviving fraction: 0.002–0.004.

10:1; consequently, bone-marrow as well as spleen are rapidly and permanently colonized by the donor cells. This is in agreement with previous observations by other authors[7,8].

When cells from an irradiated donor are injected into an irradiated recipient, the ratio donor to host cells is in order of 1:40. Accordingly, very few of the dividing cells in the bone-marrow are of donor origin. On the other hand, the proportion of cells from donor origin in the spleen is larger (76%) than expected from Table II (30 to 50%). The bone-marrow appears to be repopulated with the same efficiency by exogenous and endogenous cells whereas the spleen is repopulated with a higher efficiency by exogenous cells. One must, however, consider that in an irradiated recipient, the circulating pool of CFU is very small ($<$ 0.3 CFU), and that colony seeding from host bone-marrow would proceed over a longer period of time than occurs when donor cells are injected directly into the blood.

Observations in thymus. From experiments based on morphological observations[8,17,18], as well as from those involving injected bone-marrow cells (with chromosome markers)[2,7,8], it has been assumed that the thymus of sublethaly or supralethaly irradiated animals repopulates in two phases. The first phase (from day 4 to day 12) is characterized by the proliferation of surviving precursor cells in the thymus. The second and final phase starts 2–3 weeks after irradiation and features proliferation of small lymphocytes derived from the bone-marrow.

Our data indicate, however, that injected bone-marrow cells can directly enter the thymus of AKR/T1Ald mice and there proliferate at early times after exposure (6 days). This occurs regardless of whether AKR donors have been previously irradiated or not. The early period of thymus repopulation by donor cells is followed by a second one during which the proportion of donor cells in the thymus decreases. Evidently, the host cells crowding out the donor cells cannot come from the bone-marrow which at this time is already populated by cells of donor origin. Several explanations are possible for this observations but no definite explanation can be proposed since the cells involved in regeneration of the thymus and their cellular parameters are still insufficiently known.

a) The donor cells which have been proliferating and maturing in the thymus during the initial 6-day-period migrate out from the thymus afterwards. A more primitive type of precursor cells not present in the bone-marrow transplant but which survives in the thymus after irradiation starts to divide and mature. Most likely the latter cells are incapable of bringing on full regeneration of the gland, since finally repopulation of the thymus by cells originating in bone-marrow takes over. Such a pattern would correspond to the biphasic type of regeneration found for thymus by certain authors[8–17].

b). The bone-marrow donor cells rest in the thymus and divide more slowly than precursor cells endogenous to the thymus. Again in this case, thymus would have to contain cell types not present in the bone-marrow transplant and our results therefore suggest that thymocytes can stem from 2 different cell types, as has already been proposed[7]. It should finally be pointed out that the strains of mice utilized in our experiment (AKR, AKR/T1Ald) display certain particularities such as have been reported with respect to isogenic restauration in irradiated AKR mice[19] and leukemogenesis in AKR/T1Ald isogeneic radiochimaera[20].

Résumé. L'utilisation d'une race de souris (AKR/T1Ald) ne possédant que 38 chromosomes a permis de montrer que les cellules de moelle que l'on injecte à un individu irradié repeuplent directement la moelle, le thymus et la rate du receveur. Si le donneur est irradié, la pénétration des cellules n'est importante que dans la rate du receveur.

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Effect of Metabolic Stress on Activation of Glutathione Reductase by FAD in Human Red Cells

Glutathione reductase (GR) plays an important role in the protection of protein in red cells against oxidation. Increased activity of GR has been reported in red cells in patients with severe metabolic disorders, e.g., severe cases of uremia[1] and of cirrhosis of liver[2]. Increased GR activity has also been observed in red cells with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency[3]. The reason for these increases in GR activity is unknown.

Recently, it has been reported that GR is present in at least 2 forms, the active form which is associated with fla-

vine adenine dinucleotide (FAD), and the inactive form not associated with FAD[4–6]. Therefore, the control of GR activity in red cells in various pathological situations may be related to its activation by FAD.

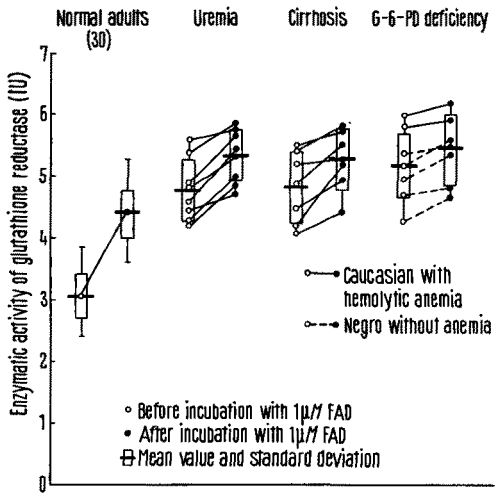
In this report, the effect of metabolic stress on GR activity in relation to these 2 forms was studied in red cells in patients with severe metabolic disorders due to various causes, e.g., severe uremia (blood urea nitrogen level over 100 mg/100 ml), severe cirrhosis of liver, and G-6-PD deficiency.

Hemolysates were prepared as previously described[7]. GR activity was assayed at 37 °C according to the method of LONG and CARSON[8] in hemolysates with 130 m*M* *Tris* EDTA buffer (pH 7.6), 5.3 m*M* oxidized glutathione (GSSG), and 0.29 m*M* reduced nicotine adenine dinucleotide phosphate (NADPH) using a Gilford Model 2000 Automatic Recording Photometer. Total GR activity in hemolysates was measured after 30 min of incubation at 37 °C with 1 μM FAD according to the method of BEUTLER[6]. The amount of the inactive form of GR represents the increase in GR activity after incubation with FAD. The saturation percentage was calculated by dividing the GR activity of the active form by the total activity after incubation with FAD.

Results are shown in the Figure. Mean GR activity in red cells from 30 normal adults was 3.04 $\pm$ 0.36 IU. (the active form). After addition of FAD to the hemolysates, this increased to 4.38 $\pm$ 0.37 IU, an increase of 1.34 $\pm$ 0.37 IU (the inactive form). Thus, in normal adults, 69.4% of GR is in the active form, and 30.6% of GR is in the inactive form. Similar results were also obtained in red cells from patients with cerebrovascular disease, pneumonia, hereditary spherocytosis, acute psoriasis, and sickle cell anemia.

In contrast, in the red cells of 8 severe uremics, GR was 4.76 $\pm$ 0.49 IU in the active form. After addition of FAD, this increased to 5.33 $\pm$ 0.40 IU, an increase of 0.57 $\pm$ 0.24 IU (the inactive form). Thus, in uremics, 89.3% of GR is in the active form, and 10.7% of GR is in the inactive form.

In the red cells from 7 severe cirrhotics, GR was 4.82 $\pm$ 0.58 IU (the active form). After additon of FAD, this increased to 5.28 $\pm$ 0.49 IU, an increase of 0.46 $\pm$ 0.31 IU (the inactive form). Thus, in severe cirrhotics, 91.3% of GR is in the active form, and 8.7% of GR is in the inactive form.



Effect of FAD on glutathione reductase activity of red cells of normals compared to those of uremia, cirrhosis of liver, and G-6-PD deficiency.

In 7 cases of G-6-PD deficiency, GR was 5.18 $\pm$ 0.51 IU (the active form). After addition of FAD, this increased to 5.44 $\pm$ 0.57 IU, an increase of 0.25 $\pm$ 0.15 IU (the inactive form). Thus, 95.2% of GR is in the active form, and 4.8% of GR is in the inactive form. Of these 7 cases, 2 were Caucasians with chronic hemolytic anemia and 5 were Negro males without anemia. The 2 Caucasians had a higher level of GR (original and total) and a higher degree of saturation with FAD than the Negro group.

From these results, it is apparent that total GR activity is increased and that GR is almost completely saturated with FAD in red cells in patients with severe metabolic disorders or G-6-PD deficiency.

Recently, FLATZ[9] has suggested that the metabolic anomalies of G-6-PD deficient red cells may increase red cell FAD content or facilitate the binding of FAD by the GR enzyme. However, the presence of increased GR activity with a high degree of saturation with FAD in red cells of our patients with uremia and cirrhosis indicates that this enhanced association with FAD is not specific for a particular disorder. The increase of saturation percentage of GR with FAD may represent a regulatory mechanism to maintain the integrity of red cells in severe metabolic disorders, as well as in G-6 PD deficiency.

In conclusion, there is increased GR activity with a high degree of saturation with FAD in red cells of severe uremia, cirrhosis of liver, and G-6-PD deficiency. This enhanced association with FAD appears to be an important controlling mechanism for GR activity in red cells of patients with severe metabolic disorders and is independent of their various causes[10].

Zusammenfassung. Bei 8 Fällen schwerer Urämie, 7 Fällen schwerer Lebercirrhose und 7 Fällen mit G-6-PD-Mangel war die aktive, an Flavin-Adenin-Dinukleotid gebundene Form der Erythrozyten-Glutathionreduktase beträchtlich vermehrt.

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