or altered histochemical reactivity due to cell necrosis, changes in membrane permeability, or deranged intra and extracellular ionic concentrations as a result of hypovolemia. The increased enzyme activities relate logically to altered carbohydrate metabolism and fluid dynamics in shock.

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Dose Response Relationships for the "Early" and "Late" Response to Erythropoietin* (32879)

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The response to erythropoietin (EP) in fasted rats measured 24 hours after injection ("early" response) varies markedly with the state of the animal at the moment of injection: the response measured 48 hours after ("late") does not (1).

In order to further characterize the "early" and "late" response to EP a dose response study was carried out.

Methods. A × C male rats 160–180 gm, were used. Ten rats were injected intravenously 1 day after start of fasting with 1, 2, 4, 8, and 16 units of (St A) Armour erythropoietin preparation Lot AL 038b kindly supplied by N.I.H. Hematology study section. In half of the rats a ^{59}Fe distribution study (1) was carried out 24 hours after EP injection: "early" response; and in other half, 48 hours after: "late" response.

Results. In the case of the 48-hour response (Fig. 1a) there is a highly significant ($p<0.001$) linear correlation between the

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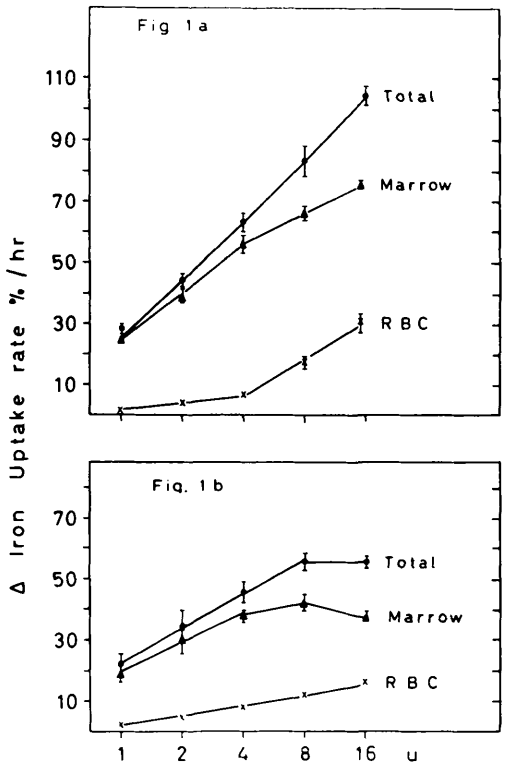

FIG. 1. Increase in iron uptake rate above that of controls (ordinate) as a function of dose of erythropoietin (abcissa) measured (a) 48 hours ("late"), and (b) 24 hours ("early"), after erythropoietin injection. I = ± one Standard E; when not indicated it falls within the symbol. RBC = red blood cells. Iron uptake rate (1): (a) Marrow: $K \times (7 \times {}^{59}\text{Fe}$ Femurs)/(100-pl). (b) RBC = $K \times ({}^{59}\text{Fe}$ RBC)/(100-pl). Total = sum of (a) and (b).

iron uptake rate of total erythroid tissue, and $\log_2$ dose of EP for the whole range of doses used. In the 24-hour response (Fig. 1b) there is no further increase in uptake on increasing the dose from 8 to 16 units. The distribution of iron uptake between marrow and RBC is not uniform throughout the dose range. In the 48-hour response the marrow curve follows the total iron uptake curve initially but starts to level off at 4 units of EP. At this point there is a significant increase of the slope of the RBC uptake curve. In the 24-hour response marrow uptake begins to level off at 4 units then drops at 16. The RBC uptake does not, however, show any change in slope. The slope of the total iron uptake curve of the 48-hour response, 19.4 ± 2.73 increase in iron uptake rate for a doubling in dose, is significantly greater than that of the 24-hour response 10.7 ± 1.84 for each doubling in dose.

Discussion. The dose response curves for the "early" and "late" responses to EP are clearly different in slope and in magnitude. In the early response, iron uptake by erythroid tissue, shows saturation above 8 units, and no change in slope of the RBC uptake with increasing dose. In the "late," 48-hour iron uptake rate by total erythroid tissue does not saturate at 16 units and there is an increase in slope of the curve relating RBC Fe uptake dose to $\log_2$ uptake above 4 units. These differences serve to further characterize these two responses, the "early" one, which would reflect mainly the effect of EP on RBC precursors already present at the moment of EP injection and the late one; the production of new RBC precursors as a consequence of differentiation of stem cells. The linear log dose response curve, for iron uptake by RBC, for the "early" response suggests that it might reflect the reticulocyte releasing action of EP (2). The increase in marrow uptake with doses up to 8 units would reflect in small part increase in new precursors as a consequence of differentiation (3) but more importantly, increase due to increased proliferation of those RBC precursors already present at the moment of injection.[1] Maximum effect on these precursors would be obtained with about 8 units of EP, further increase in dose would only be reflected in a change of distribution in uptake between marrow and RBC, due to increase in reticulocyte release with concomitant drop in marrow uptake.

The sharp increase in RBC iron uptake 48 hours after injection of doses of EP greater than 4 units, coinciding with a drop in marrow uptake, probably reflects a shortening of marrow transit time and the appearance in the blood of larger less mature reticulocytes (4).

Summary. The dose response curves measured 24 hours ("early") after erythropoietin injection differ significantly from those measured 48 hours after ("late"). The slope of the curve relating increase in iron uptake by

[1] Hodgson, G. and Eskuche, I., Submitted for publication.

total erythroid tissue to EP dose is greater ($p<0.01$) in the 48-hour response. Total uptake saturates above 8 units in the "early" response but not in the "late." Marrow uptake drops at higher doses (16 units) in the "early" response but does not do so in the "late." The curve for uptake by circulating erythrocytes shows only one slope in the "early" but two distinct slopes in the "late" response: one from 1–4 units the other much steeper from 4–16 units. The results are discussed in relation to the possibility of different mechanisms being involved in the two responses. The "early" one reflecting the effect of EP on proliferation of recognizable erythroid precursors, the late differentiation of "stem cells."

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Bone Salt Mobilization Effected by Ascorbic Acid* (32880)

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It is accepted that ascorbic acid plays a role in normal bone growth (1). The extent of the physiological function of vitamin C in bone has not been clarified; however, its requirement is usually considered to be limited to the part it plays in matrix formation. In this regard, an influence on both collagen formation (2) and alkaline phosphatase activity (3) has been demonstrated. These (2,3) are only selected references since both effects have been shown by a number of investigators and have been reviewed elsewhere (4). The possibility that ascorbic acid influences bone calcification directly has been a point of research interest, but no conclusive evidence has been presented illustrating such a role. Similarly, there is no unequivocal demonstration linking ascorbic acid to bone catabolism. Recently some work has been reported which suggests that ascorbic acid may play a role in bone destruction (5,6). Those reports suggested that ascorbate enhanced the *in vitro* release of both calcium and phosphate (5) and resulted in bone ash reduction in rachitic chicks given this compound (6). The latter results imply that bone demineralization may have been increased by the ascorbic acid, although it could be that the reduced ash value was effected by a stimulation of matrix formation.

In the present case, results are presented which suggest that exogenous ascorbic acid can cause the mobilization of skeletal ^{45}Ca. Since the isotope was given several days prior to the vitamin, it may be assumed that much of the ^{45}Ca was mainly located in mature crystals when the ascorbate was administered. If so, it follows that the increase in blood ^{45}Ca following ascorbic acid administration resulted because bone salts in the so-called nonexchangeable fraction (7) were mobilized.

Materials and Methods. Three separate experiments were conducted to test the idea that ascorbic acid could influence bone salt mobilization. Procedures similar in these studies are as follows. Male leghorn chicks were placed on a diet used previously (8) at 1 day of age and given this feed until the experiment was completed. When 8 days of age, each chick was given ^{45}Ca intramuscularly. The isotope, administered in solution with normal saline, was given at a level of 20 μC/100 gm of body weight in the first study and 10 μC/100 gm in the last two cases. When 14 days old, the animals were injected intraperitoneally with

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