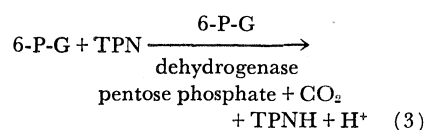
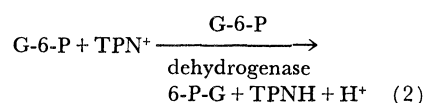
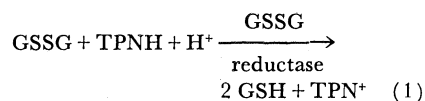


Fig. 1. Milligrams of reduced glutathione formed from oxidized glutathione as a function of milliliters of dialyzed hemolysate. Solid curve, primaquine-sensitive subject; dashed curve, normal subject. (a) Assay of GSSG reductase, reaction 1; (b) assay of G-6-P dehydrogenase, reaction 2 + 1; (c) assay of 6-P-G dehydrogenase, reaction 3 + 1.

a function of cell age (3), and, therefore, is self limited (4). The red blood cells of susceptible individuals have a low glutathione level (5) and an increased tendency to form Heinz bodies (6). Hemolysis can also be induced by certain other aniline derivatives (7). In this preliminary report, we present evidence of an abnormality in glucose-6-phosphate dehydrogenase activity in hemolysates of primaquine-sensitive erythrocytes.

The abnormality has been demonstrated by investigation of the following reactions in hemolysates of red blood cells from primaquine-sensitive and normal subjects (8).



Rall and Lehninger have indicated that glutathione reductase is present in human red blood cells (9). Reduced triphosphopyridine nucleotide serves as coenzyme. Glucose-6-phosphate and 6-phosphogluconate are successive intermediates in the direct oxidation of glucose. Both substrates have been used to form a coupled system—by providing TPNH—for demonstration of glutathione reductase (10).

Heparinized blood from two normal men, one Caucasian and one Negro, was compared with blood from four sensitive Negroes. After the red blood cells had been centrifuged and washed with 0.145M NaCl, they were hemolyzed with 5 volumes of H₂O for 1 hour

at 4°C. Each hemolysate was cleared by centrifuging at 1400g for 90 minutes. (Stromata were not completely removed.) Maximum temperature during centrifugation was 37°C. Fifty milliliters of hemolysate were dialyzed for 12 hours at 4°C in 2000 ml of 0.67M phosphate buffer at pH 7.4. Dialysis serves to remove glutathione and other substrates and coenzymes from the hemolysate.

To determine the presence, in dialyzed hemolysate, of enzymatic activity capable of catalyzing each of the three reactions, we mixed aliquots of dialyzed hemolysate with those constituents appropriate to each reaction. Final reaction mixtures contained 14 ml buffered to pH 7.4 in 0.036M tris(hydroxymethyl)-aminoethane and were incubated for 15 minutes at 22°C. The concentration of oxidized glutathione was always $1 \times 10^{-4}M$. When used, the concentration of TPNH was $1.5 \times 10^{-4}M$; TPN, $1 \times 10^{-5}M$; G-6-P, $5 \times 10^{-4}M$; and 6-P-G, $2 \times 10^{-4}M$.

The reaction was stopped by adding 10 ml of 4.5-percent metaphosphoric acid. Reduced glutathione was determined by the method of Grunert and Phillips (11). Glutathione reductase activity was measured by the amount of oxidized glutathione converted to the reduced form. Appropriate controls were run.

Figure 1a shows results obtained for direct demonstration of glutathione reductase (reaction 1), expressed as milligrams of reduced glutathione formed versus milliliters of hemolysate used. The curves show equal activities of glutathione reductase in hemolysates of normal and primaquine-sensitive red cells.

Because glutathione reductase is TPNH-dependent, similar glutathione reduction should be demonstrable in coupled reactions 2 + 1 or 3 + 1, if the respective dehydrogenases are normally active. When glucose-6-phosphate and TPN were used as the sole source of

TPNH for activation of glutathione reductase (reaction 2 + 1), hemolysate from the sensitive subject showed a striking failure to reduce oxidized glutathione (Fig. 1b). This failure indicates that hemolysate from the sensitive subject was deficient in glucose-6-phosphate dehydrogenase activity. Hemolysates from three other primaquine-sensitive subjects showed virtually identical inability to reduce oxidized glutathione when glucose-6-phosphate was the substrate.

To determine whether the succeeding step in the direct oxidation of glucose was intact, 6-phosphogluconate was added instead of glucose-6-phosphate (reaction 3 + 1). The results (Fig. 1c) indicate equal 6-phosphogluconic dehydrogenase activity.

We interpret these data as demonstrating an abnormality in the direct oxidation of glucose in the red blood cells of primaquine-sensitive subjects. This abnormality affects glucose-6-phosphate dehydrogenase activity (12).

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5. E. Beutler *et al.*, *ibid.* 45, 286 (1955).
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8. The following abbreviations are used: GSSG, oxidized glutathione; TPNH, reduced triphosphopyridine nucleotide; GSH, reduced glutathione; G-6-P, glucose-6-phosphate; TPN, oxidized triphosphopyridine nucleotide; 6-P-G, 6-phosphogluconate.
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12. This work was supported by the research and development division, Office of the Surgeon General, Department of the Army; by Winthrop Laboratories, Inc.; and by E. R. Squibb and Sons.
13. We wish to thank B. Vennesland and E. S. G. Barron for their advice and helpful criticism in this work.

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Ord Kangaroo Rat in Captivity

Methods recently developed in our faunal colony now make possible the rearing of kangaroo rats in the laboratory. These methods were evolved during the development of a program designed to provide laboratory-reared rodents native to the western Utah desert for use in laboratory studies (1). Several of the common species of the family Cricetidae

have been reared successfully. The heteromids are more difficult. The Ord kangaroo rat, *Dipodomys ordii*, was judged to offer the best possibilities as a laboratory animal because of its gentle nature and the ease with which it could be maintained in captivity.

The standard techniques usually employed for breeding laboratory animals have required drastic modification to fit the ecological requirements and the specific behavior patterns of the kangaroo rat. In nature, each adult rat is thought to be solitary in habit and to defend its burrow and food caches against intrusion by other kangaroo rats and other species of rodents. This inherent behavior pattern has been the greatest obstacle to successful matings in captivity. The failure of trial-and-error techniques, including a number of unsuccessful cage designs, led to the study of the rat's behavior. It was obvious that in order to reproduce in nature, there must be a period during which the sexes are attractive instead of repugnant to each other. It was judged that this behavior would be associated with sex hormonal effects.

Work initiated in this laboratory by William P. Jollie (2) led to successful mating and the rearing of a brood of four. Daily vaginal smears, autopsies, and histological studies led Jollie to conclude that the female estrous cycles were of 5 or 6 days duration and that they occurred at least from early February to late June or early July. More recent studies have shown that some wild-trapped females contained embryos as early as December and January, and wild young rats have been found in September and October, indicating a more extended polyestrous period. Whether or not the male reproductive pattern is cyclical in response has not been established.

Jollie assumed that the estrum phase of the cycle (characterized by a heavy predominance of enucleated, cornified, squamous epithelium in the vaginal smear) signified the period when attraction might replace repugnance in the female. From examination of vaginal smears, he made several pairings when females were presumed to be receptive and was successful in obtaining one litter. This and one other litter resulting from breeding of a captive pair (3) comprise the reported successful attempts of breeding kangaroo rats in the laboratory.

Following Jollie's lead, further studies were made from 1 January to 1 October 1955, in which five wild-trapped females and five captive-reared females were used in studying the breeding procedures. They were held singly in separate metal mouse cages until they were deemed ready for mating. Then each female was introduced simultaneously with a male into a larger breeding pen (30 by 30 by 10 in.) in which neither had established territorial ownership. If compatible, they

were left together for 1 to 5 days, depending on observed evidence of cohabitation or copulation, after which they were separated and returned to individual cages.

The disadvantages of using the vaginal smear technique for determining estrum were overcome by associating a characteristic swelling and inflammation of the vulva with the heat period. By this method, six of the females (three wild-trapped and three captive-reared) produced ten litters with a total of 30 young. The number of young in the litters ranged from two to four. The postnatal mortality rate was low and a good growth rate was observed.

A limited amount of data suggests that the gestation period is 29 to 30 days. The most accurate measurement occurred when the presence of what appeared to be a gelatinous plug was evident 20 hours after the male and female were paired. The animals were immediately separated, and 29 days later the female gave birth to four young.

Thus far, captivity has failed to alter appreciably the behavior patterns of kangaroo rats born in the laboratory. However, some litter mates have lived harmoniously in a cage for months without the usual strife and mortality. This suggests that the antagonistic behavior pattern that has kept production of these animals on an experimental level may be altered by selection of the more gregarious individuals from successive generations.

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Relation between Weight-Lifting Totals and Body Weight

According to a suggestion of T. F. Young (1), the weight-lifting ability of a trained athlete may be expected to be proportional to the two-thirds power of his body weight. This follows immediately from the assumption that the length of a limb should be proportional to the cube root of the body weight and the cross-sectional area proportional to the two-thirds power of the body weight.

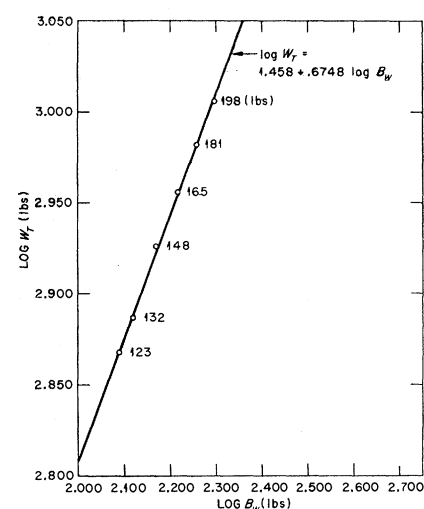


Fig. 1. Plot of $\log W_T$ (world-record weight-lifting total) versus $\log B_W$ (body-weight class).

If strength is proportional to the cross-sectional area of a limb or the torso, weight-lifting ability should be proportional to the two-thirds power of the body weight. Hence

$$W = a B_W^{2/3} \quad (1)$$

where W is the weight that can be lifted in any one or in any combination of lifts, a is a constant, and B_W is the body weight. In order to test this relationship, it is convenient to express Eq. 1 in logarithmic form

$$\log W = \frac{2}{3} \log B_W + \log a \quad (2)$$

Then a plot of $\log W$ versus $\log B_W$ should be linear, with a slope of approximately $2/3$ or 0.67 . Implied in the treatment is the assumption of constancy of form factor for weight lifters over the entire range of body-weight classes.

Weight-lifting championships are usually decided on the basis of the total weight lifted by an athlete in three different lifts: the press, the snatch, and the clean and jerk. Taking the total of the three lifts is effectively an averaging process, whereby the advantage one lifter might have over another because of more favorable leverage in one lift is counterbalanced in the other lifts. Hence, as a test of Eq. 2, the world-record totals in the three lifts mentioned should be better than the records in any one lift. Figure 1 shows a plot of $\log W_T$ (where W_T is the world-record total established at each body weight i) against $\log B_W$. As can be seen, the points fall in a straight line as predicted. By the method of least squares, the best equation describing the points was obtained and is given as Eq. 3.

$$\log W_T = 1.458 + 0.6748 \log B_W \quad (3)$$

The slope of the line, 0.6748 , is very close