vestigated. Whether thymectomy at birth achieves this altered immunological state through humoral or cellular mechanisms requires further study.

Whatever the final explanation, it seems clear that at least in certain donor-host strain combinations of mice isogenic with regard to the H-2 histocompatibility locus but homologous with respect to other histocompatibility genes, thymectomy of the host performed either at birth or at an early age produces a marked prolongation of the skin homograft survival.

Summary. The effect of thymectomy performed in mice either at birth or at 30 days of age on survival time of homologous skin grafts was studied. The results demonstrate that in certain donor-host strain combinations isogenic at the H-2 locus such as DBA/2 with (Balb/C × DBA/2)F_1 and Z(C_3H) with Ce, previous thymectomy of the host resulted in prolonged survival of the skin homografts. However, in other strain combinations differing at the H-2 locus such as Z(C_3H) with (A × Z)F_1 hybrid, thymectomy of the recipient resulted in a slight and perhaps unsignificant prolongation of skin homograft survival.

While this manuscript was in press similar observations were reported by Miller(21).

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Independence of Water Content and Respiration in Rabbit Erythrocytes.* (27150)

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Previous studies on the effects of changing extracellular osmolarity on erythrocyte respiration have shown varying results depending on the animal investigated, degree of extracellular hypo- and hyperosmolarity, and control of substrates. Hunter(1) demonstrated irreversible depression of respiration of chicken erythrocytes in markedly hypertonic solutions and little or no effect in hypotonic solutions. Tipton(2) found no significant change in respiratory activity of chicken

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erythrocytes in hypotonic and slightly hypertonic solutions of NaCl. Ray(3) found that both hypertonic and hypotonic solutions decreased O_2 consumption of dog erythrocytes. The respiration rate of erythrocytes studied in many species by Tipton(2) showed a maximum rate usually at that osmolarity isotonic with the erythrocytes studied although the differences were small, and it appeared that respiration of erythrocytes were generally uninfluenced by wide variations in the osmolarity of the suspending media. Hunter(4) found no change in O_2 consumption of chicken erythrocytes nor change in anaerobic glycolysis of beef erythrocytes upon swelling in aqueous solutions. Olmstead(5) found a decrease in respiration of rabbit erythrocytes suspended in hypertonic solutions containing glucose and methylene blue; however, some of the fall in O_2 uptake could have been due to dilution of substrate and methylene blue during the experimental procedure.

The purpose of the present study was to determine the effect of increase and decrease of erythrocyte water on the accelerated respiration of rabbit erythrocytes in presence of methylene blue. The changes in erythrocyte water were accomplished by suspension of erythrocytes in hypertonic and hypotonic solutions without changing the extracellular concentrations of substrate (glucose) or methylene blue.

Method. Blood, drawn by cardiac puncture from female rabbits weighing between 1.5 and 2.5 kg, was placed in heparinized tubes and centrifuged for 30 minutes at 1400 g. The supernatant and buffy coat were withdrawn and the cells suspended in phosphate buffered NaCl pH 7.4, 6.8, or 8.0 with a freezing point depression of 300 mos which approximates the freezing point depression of the serum of adult rabbits. Centrifugation was continued for 15 minutes at 1400 g, the supernatant again withdrawn, the cells washed in buffered NaCl as above, and centrifuged at 1400 g for 30 minutes. The supernatant was withdrawn and the cell surface gently blotted with a cotton applicator.

One ml of erythrocytes was added to 3 ml of a buffered NaCl solution in Warburg flasks. These solutions were adjusted to pH 6.8, 7.4 or 8.0, 300 mos, and contained 5.6 μM glucose/ml and 10^{-4}M methylene blue. One ml of various solutions containing the same concentrations of glucose and methylene blue but differing concentrations of buffered NaCl were placed in the sidearms to change or maintain the osmolarity of the extracellular solution when tipped into the main compartment. Standard Warburg technics were used throughout. Twenty percent KoH was placed in the center well of the flasks which were then gassed with O_2 for 5 minutes and equilibrated at the desired water bath temperature for 10 minutes before closing stopcocks. Manometers were read at 30-minute intervals. Qo_2 in this study refers to microliters of O_2 consumed by one ml rbc.

Results. Fig. 1 shows the effect on O_2 uptake of decreasing the intracellular water by increasing osmolarity of the extracellular phase. There are no significant differences in rates of cumulative oxygen uptake in the hypertonic solutions when compared with erythrocytes in solutions isotonic with mammalian plasma (300 mos). Reduction of available substrate to 1.4 μM/ml reduces the mean cumulative Qo_2 independently of the cell water content. Fig. 2 demonstrates the effect on O_2 uptake of increasing the intracellular water by suspension of red cells in hypotonic solutions (240 mos). No significant change occurred in the mean cumulative Qo_2 in the presence of increased intracellular water at either glucose concentration, although decrease in the latter reduced Qo_2.

Increasing extracellular pH to 8.0 reduces the Qo_2 in rabbit erythrocytes (Fig. 3) when compared with cells respiring at pH 7.4. However, this reduction in Qo_2 is independent of the cell water content occurring in cells with increased water (240 mos) and decreased water (360 mos) as well as with cells respiring in solutions of 300 mos. Red cell respiration was not significantly affected by reducing pH to 6.8.

Increase in temperature regularly decreased the mean cumulative Qo_2 with cessation of oxygen uptake after 90 minutes (Fig. 4). This decrease in cumulative Qo_2 as a function of temperature is again of similar

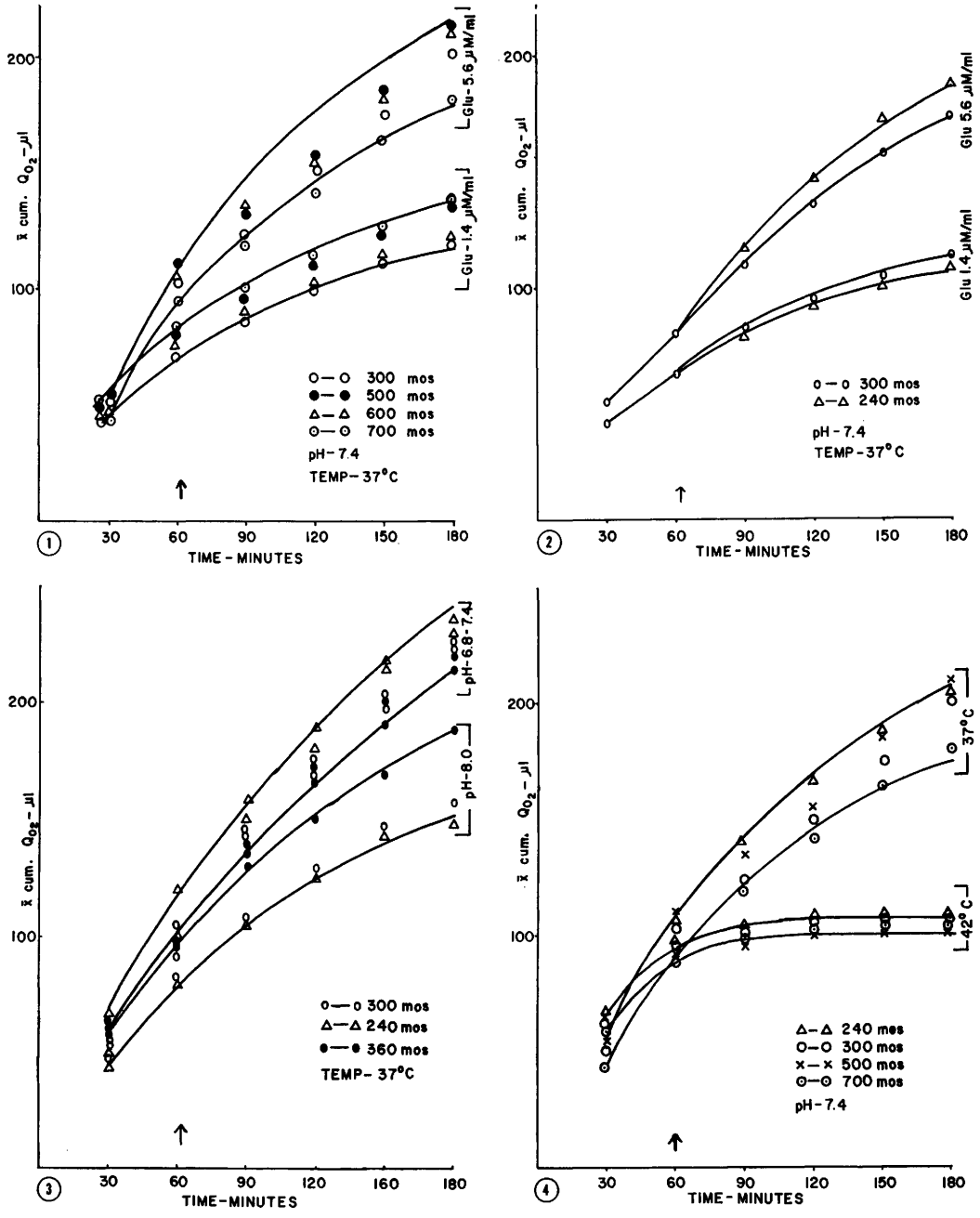

FIG. 1. Effect of decrease in intracellular H_2O on cumulative Q_{O_2} of RBC. Each point represents a mean of 6 determinations. Arrow indicates time osmolarity of extracellular solution was changed from 300 mos by tipping sidearm into main compartment of Warburg flask.

FIG. 2. Effect of increase in intracellular H_2O on cumulative Q_{O_2} of RBC. Each point represents a mean of 6 determinations. Arrow indicates time osmolarity of extracellular solution was changed from 300 mos by tipping sidearm into main compartment of Warburg flask.

FIG. 3. Effect of pH on Q_{O_2} of RBC. Note that increasing pH reduces Q_{O_2} of RBC independently of cell water content. Each point represents a mean of 6 determinations. Arrow indicates time osmolarity of extracellular solution was changed from 300 mos by tipping sidearm into main compartment of Warburg flask.

FIG. 4. Effect of temperature on Q_{O_2} of RBC. Note that raising temperature to 42°C results in cessation of Q_{O_2} at 90 min. independently of cell water content. Each point represents a mean of 6 determinations. Arrow indicates time osmolarity of extracellular solution was changed from 300 mos by tipping sidearm into main compartment of Warburg flask.

TABLE I. Magnitudes of Water Exchange by Rabbit Erythrocytes as Function of Extracellular Osmolarity at pH 7.4, 38°C.

Osmolarity of extracellular solution	H_2O efflux	H_2O influx
	ml/100 ml RBC	
240		26.8
300	.0	.0
400	9.9	
500	20.3	
600	24.9	
700	29.4	

magnitude in cells suspended in solutions of 240 mos as well as those suspended in solutions of 700 mos.

The actual changes in erythrocyte water in hypo- and hypertonic solutions measured by methods previously described(5,9,10) are listed in Table I. The rabbit erythrocyte contains about 70 ml H_2O/100 ml erythrocytes. Removal of 29.4 ml (42%) from or addition of 26.8 ml (38%) to this intracellular water may be accomplished without affecting erythrocyte respiration in presence of methylene blue.

Harrop and Barron(6) first demonstrated the accelerating effect of methylene blue on erythrocyte O_2 uptake. Recent studies by Brin and Yonemoto(7) and Huennekens *et al.*(8) showed that mammalian erythrocytes in presence of methylene blue metabolize glucose largely *via* the hexose monophosphate shunt.

Metabolism of glucose *via* the hexose monophosphate shunt being an overall ordered chemical reaction should vary in rate as concentrations of enzymes and substrates vary with changes in intracellular water content. However, it is clear from the above data that accelerated erythrocyte respiration in the presence of methylene blue, while dependent on the substrate concentration, pH and temperature, is independent of intracellular water content within rather wide limits.

It is possible that concentrations of intracellular enzymes are of such large magnitude that their concentration and dilution within the cells do not affect the velocity of the overall metabolic reaction. This explanation would be in agreement with the generally accepted fact that in the normal living animal enzymes are not the limiting factors in the velocity of metabolic activity. Another explanation suggests that the intracellular enzymes and substrates are active in that fraction of the erythrocyte water which is "bound" *i.e.*, not available for transfer across the erythrocyte membrane in response to increasing osmostic gradients. It has been demonstrated that as much as 43% of the rabbit erythrocyte water is "bound" in this manner (9).

Summary. Accelerated O_2 uptake of rabbit erythrocytes was measured in the presence of methylene blue and glucose during changes in erythrocyte water content. Although dependent upon substrate concentration, temperature, and pH, O_2 uptake was independent of rather large changes in cell water content.

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