TABLE III. Statistical Analysis of α- and β-D-Galactosidase Activities in Rat Thyroid and Spleen.

Organ and enzyme	Male	Female	Difference	S.E. of difference	Probability of larger difference (t-test)
Thyroid					
2-naphthyl-α-D-galactoside	551.00 (9)*	549.89 (9)	1.11	107.42	.99
6-bromo-2-naphthyl-α-D-galactosidase	539.78 (9)	502.12 (8)	37.66	86.54	.66
2-naphthyl-β-D-galactosidase	529.44 (9)	572.11 (9)	−42.67	111.08	.69
Spleen					
2-naphthyl-α-D-galactosidase	544 22 (9)	499.56 (9)	44.66	91.91	.62
6-bromo-2-naphthyl-α-D-galactosidase	592.22 (9)	693.75 (8)	−101.53	74.96	.20
2-naphthyl-β-D-galactosidase	693.22 (9)	801.67 (9)	−108.45	102.86	.31

* Numbers in parentheses are numbers of animals.

The need for special sampling of tissue under the dissecting microscope should be emphasized.

Summary. Rat thyroid gland and spleen contain levels of α and β-D galactosidases which show no significant sex-linked differences. We have no satisfactory explanation for the discrepancy in results from those of others, except that the parathyroid glands, very active for the two galactosidases, are closely related to the thyroid. It is suggested that careful dissection may be required to avoid inadvertent dilution or enrichment of enzyme source by adjacent anatomic structures. The quantitative data reported herein confirm previous histochemical observations of intense reactivity of rat thyroid gland for α- and β-D galactosidases. The differences in values obtained for spleen may reflect variations in numbers of heterophiles and megakaryocytes which showed high α-D and β-D galactosidase activity by histochemical technics.

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1. Monis, B., Tsou, K. C., Seligman, A. M., *J. Histochem. & Cytochem.,* 1963, v11, 653.

2. Gillman, S. M., Tsou, K. C., Seligman, A. M., *Arch. Biochem. & Biophys.,* 1952, v98, 229.

3. Wallenfels, K., Malhotra, O. P., *Advances in Carbohydrate Chemistry,* 1961, v16, 239.

4. Conchie, J., Fundlay, J., Levvy, G. A., *Biochem. J.,* 1959, v71, 318.

5. Conchie, J., MacDonald, D. C., *Nature,* 1959, v184, 1233.

6. Levvy, G. A., McAllan, A., Hay, A. J., *J. Endocrinol.,* 1961, v23, 19.

7. Alvarez, A., Sas, J., *An. Fac. Med. Montevideo,* 1961, v46, 92.

8. Chauncey, H. D., Quintarelli, G., *Am. J. Anat.,* 1961, v108, 263.

9. Oka, R., Okamoto, Y., Go, J., Nagasuma, H., *Gann,* 1961, v52, 305.

10. Robins, E., Fisher, K., Lowe, I., *J. Neurochem.,* 1961, v8, 96.

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Distribution of 5,5-Dimethyl-2,4-Oxazolidinedione (DMO) Between Plasma and Erythrocytes. (29312)

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Waddell and Butler(1) described a procedure for evaluating intracellular pH based on distribution of the weak acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO), between the extracellular and intracellular compartments of skeletal muscle. Among other things, the assumption was made that the muscle cell membranes are freely permeable to the

undissociated form of the acid and impermeable to the anion. Thomason(2) applied the principle to determination of the intracellular pH of erythrocytes from human subjects without success. In adapting the so-called DMO procedure to determination of intracellular pH in our laboratory, preliminary data from experiments with rat blood were found to be consistent with the hypothesis that rat red blood cell membrane is permeable to the DMO anion. The experiments to be reported were designed to show that in rat blood the DMO anion is distributed across the erythrocyte membrane in a manner similar to a Donnan distribution.

Materials and methods. Pooled heparinized blood collected by direct cardiac puncture from normal adult male rats of the Wistar strain was used. To a given quantity of the blood was added a saline solution of DMO to give a concentration of DMO approximately 100 μg per ml. In some experiments either Tris, $NaHCO_3$ or HCl was also added in amounts corresponding to 10 to 20 meq per liter of whole blood. The sample was then transferred to a tonometer and equilibrated for 30 minutes in a water bath at 38°C with a CO_2-air mixture having partial pressure of CO_2 varying between 20 mm and 100 mm Hg pressure. Upon completion of the equilibration the sample was transferred anaerobically to a centrifuge tube under oil. An aliquot was removed to serve for the analyses on whole blood. The remainder was contrifuged to obtain plasma for analysis.

Plasma pH was determined at 38°C by means of a Cambridge model R pH meter with a water-jacketed glass electrode. The water contents were obtained by drying to constant weight 1 ml quantities of material, at 105°C. The hematocrit was determined employing Wintrobe tubes and centrifugation at 3,000 rpm for one hour. The Wilson and Ball(3) procedure was employed for the chloride determinations and the DMO concentrations of plasma and whole blood were determined as described by Waddell and Butler(1) for plasma. There was good agreement between the determined values for whole blood DMO and the expected values based on the quantity of DMO added to the whole blood.

Cell concentrations were calculated from the whole blood and plasma values by substitution in the following expression: Whole blood = [Plasma (100-hematocrit)/100] + [cell (hematocrit)/100]. The concentration of DMO^- in plasma was calculated from the total DMO concentration by solving the following statement: $(DMO^-) = (DMO)_T [1/(1+10^{6.13-pH})]$ where pH represents the pH of plasma and 6.13 the pK′ for DMO in plasma. The concentration of undissociated DMO (DMOH) was assumed to be the same in plasma and cell water. Hence, concentration of erythrocyte DMOH was evaluated by subtracting the plasma DMO^- from the plasma total DMO. Concentration of DMO^- in the erythrocyte was then obtained by subtracting the erythrocyte DMOH from the erythrocyte total DMO.

Results and discussion. In Table I are shown the DMO anion concentrations found in plasma and erythrocytes of oxygenated rat blood to which DMO had been added. The blood was equilibrated with varying partial pressures of CO_2, and in some samples with added HCl, $NaHCO_3$ or Tris, to provide deviations of plasma pH from 7.07 to 7.68. In bloods with a reduced plasma pH the concentration ratios $(DMO^-)_c/(DMO^-)_p$ tended to approach a value of 1. Further, as plasma pH increased the concentration ratios were reduced. It is noteworthy that these changes in concentration ratios of DMO^- are similar to those found with the Cl^- concentration ratios, the chloride anion being known to be distributed between the plasma and erythrocytes according to Donnan's law (4). To establish this similarity further, in Fig. 1 are presented graphs relating anion concentration ratios as ordinate against the plasma pH as abscissa. Two lines were constructed from the data of Fitzsimons and Sendroy(5) for oxygenated human whole blood; namely, $rCl^- = (Cl^-)_c/(Cl)_s = 3.208 - 0.344$ pHs, and $rHCO_3^- = (HCO_3^-)_c/(HCO_3^-)_s = 4.163 - 0.471$ pHs. Regression lines were constructed from the data of this report representing $rDMO^-$

TABLE I. DMO Concentrations in Plasma and Erythrocytes.

Sample No.	pH	Plasma DMO⁻, meq/kg	Plasma Cl⁻, meq/kg	Plasma H_2O, g/l	Erythrocytes DMO⁻, meq/kg	Erythrocytes Cl⁻, meq/kg	Whole blood Hematocrit, %	Whole blood H_2O, g/l	$(DMO^-)_c/(DMO^-)_p$	$(Cl^-)_c/(Cl^-)_p$
1	7.07	.748	118.5	947	.684	94.8	41.9	862	.91	.80
2	7.10	.781	118.5	957	.892	110.5	43.0	829	1.14	.93
3	7.16	.936	124.0	955	.806	111.8	46.7	822	.86	.90
4	7.18	.878		941	.728		47.8	843	.83	
5	7.22	.922		960	.750		48.0	842	.81	
6	7.23	.952		953	.781		45.7	833	.82	
7	7.24	.949		941	.696		45.8	842	.73	
8	7.25	1.052		934	.829		51.3	837	.79	
9	7.33	.932		934	.747		46.2	826	.80	
10	7.39	1.031	110.9	950	.789	79.5	43.4	852	.76	.72
11	7.40	1.928*		941	1.336		46.0	832	.69	
12	7.42	.994		957	.745		44.4	852	.75	
13	7.42	1.035		960	.630		45.0	857	.61	
14	7.49	1.193		945	.903		47.0	837	.76	
15	7.63	.857	114.5	950	.578	66.7	37.0	857	.67	.58
16	7.64	1.059		952	.637		44.4	852	.60	
17	7.66	1.004	118.8	939	.600	66.4	38.2	848	.60	.56
18	7.68	1.121		936	.728		42.7	848	.65	

* In this sample 200 μg of DMO per ml of whole blood was added.

$= (DMO^-)_c/(DMO^-)_p$ and $rCl^- = (Cl)_c/(Cl^-)_p$.

It is realized that with the system under study the concentration ratios for DMO^- at equilibrium would simulate a Donnan distribution even though the erythrocyte membrane is permeable only to the undissociated DMOH. The following should be obtained at equilibrium,

$(DMOH)_c \times K_c = (H^+)_c \times (DMO^-)_c$ and,

$(DMOH)_p \times K_p = (H^+)_p \times (DMO^-)_p$

If $(DMOH)_c \times K_c = (DMOH)_p \times K_p$, as is assumed by application of the Waddell and Butler formula(1), then

$$[(H^+)_p]/[(H^+)_c] = = [(DMO^-)_c]/[(DMO^-)_p].$$

In preliminary experiments involving the direct pH determination of hemolyzates from rat erythrocytes prepared according to the procedure of Thomason(2) it was found that the concentration ratio $(H^+)_p/(H^+)_c$ was definitely lower than the concentration ratio $(DMO^-)_c/(DMO^-)_p$ at the same plasma pH. Thomason's findings(2) are similar in that the pH of erythrocytes from human subjects as determined by direct glass electrode measurement of hemolyzed erythrocytes was found to be consistently lower than the pH determined by DMO distribution on the same samples. A "t" test on these data shows the differences to be highly significant. It

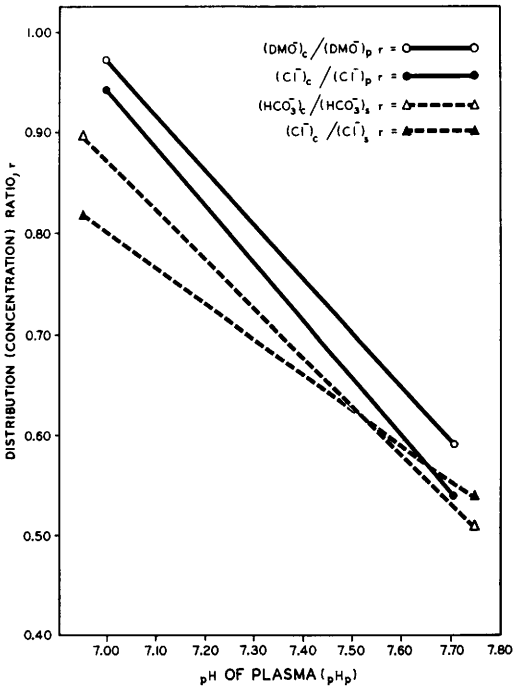

FIG. 1. Variation of erythrocyte to plasma concentration ratios with plasma pH for oxygenated rat blood. Equations for the regression lines are as follows: O——O, $r = (DMO^-)_c/(DMO^-)_p = 4.74 - 0.540$ pHp, standard deviation $= \pm 0.078$ and ●——●, $r = (Cl^-)_c/(Cl^-)_p = 4.82 - 0.555$ pHp, standard deviation ± 0.061. △-----△, and ▲-----▲ represent, respectively, regression lines for $rHCO_3^-$ and rCl^- of oxygenated human blood based on data of Fitzsimons and Sendroy(5) (see text).

is evident that the Waddell and Butler(1) principle for determining intracellular pH cannot be applied to determination of the erythrocyte pH. The latter principle assumes that the pK′ for DMO in plasma and erythrocytes is equal. Experiments are in progress to test the correctness of this assumption.

Summary. *In vitro* experiments on rat blood to which DMO was added indicated that the DMO^- becomes distributed between the plasma and the erythrocytes in a manner similar to a Donnan distribution. It was concluded that DMO cannot serve as a test substance for determination of the erythrocyte pH employing the principle proposed by Waddell and Butler.

1. Waddell, W. J., Butler, T. C., *J. Clin. Invest.*, 1959, v38, 720.

2. Thomason, R., *Skandinav. J. Clin. and Lab. Invest.*, 1963, v15, 45.

3. Wilson, D. W., Ball, E. G., *J. Biol. Chem.*, 1928, v79, 221.

4. Van Slyke, D. D., Wu, H., McLean, F. C., *ibid.*, 1923, v56, 765.

5. Fitzsimons, E. J., Sendroy, J., Jr., *ibid.*, 1961, v236, 1595.

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Hemagglutination by Rheumatoid Factor of Cells Coated with Animal Gamma Globulins.* (29313)

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The rheumatoid factor is a gamma globulin, usually of high molecular weight (19S), found in the sera of most patients with rheumatoid arthritis and of some patients with other diseases(1). It is characterized by its unique reactivity with low molecular weight (7S) human gamma globulin (HGG) and rabbit gamma globulin (RGG). It has been suggested that the rheumatoid factor may represent an "autoantibody" with primary specificity for 7S HGG and with cross-reactivity with RGG(2,3). If rheumatoid factor is an antibody to 7S HGG with cross-reactivity with RGG, it might be expected to cross-react with gamma globulins of other species, analogous to the cross-reactivity of classical antibodies to HGG with gamma globulins of other species. The present study was therefore undertaken to investigate the reactivity of rheumatoid factor with gamma globulins of species other than human.

The bis-diazotized benzidine (BDB) hemagglutination technique(4) was found to be more satisfactory than the tanned sheep cell method(5) for use in this study. Using the BDB method, it has been demonstrated that rheumatoid sera react with the gamma globulins of several mammalian species and that this reactivity can be completely absorbed from rheumatoid sera with a preparation of insoluble HGG. The reactivity of rheumatoid factor with these mammalian gamma globulins is consistent with the hypothesis that rheumatoid factor is an antibody to HGG with cross-reactivity with rabbit, bovine, equine and other mammalian gamma globulins.

Materials and methods. *Sera.* Sera were obtained at intervals over a 2-year period from patients with classical rheumatoid arthritis. The sera were stored at —20°C. Prior to use in the tanned sheep cell hemagglutination system, sera were inactivated at 56°C for 30 minutes and absorbed with washed sheep erythrocytes. Inactivation and absorption were not necessary in the BDB hemagglutination system but were sometimes carried out for convenience in the comparison of results obtained with the tanned cell sys-

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