ter, although estimations of pre-glomerular resistance showed no rise throughout the autoregulatory period. The observation that ureteral pressure is greater than tissue pressure suggests that Bowman capsule extravascular pressure is an effective compressing force on the renal vascular bed. These data also suggest that tubules possess considerable structural rigidity.

Mechanisms of autoregulation, according to the present study, appear to operate primarily in association with glomerular filtration and also extend their effects through the post-glomerular segment. The "clamping" effect of extravascular pressure on intra-parenchymal arterial vessels has not been evaluated.

Summary. The present investigation has been concerned with mechanisms explaining autoregulation of renal blood flow. Data from experiments on the isolated perfused dog kidney suggest that as renal artery pressure is elevated, blood flow regulation is brought about by (a) combined effects of increased tissue pressure and Bowman capsule extravascular pressure, and (b) increases in post-glomerular viscosity resulting from glomerular filtration.

1. Hinshaw, L. B., Day, S. B., Carlson, C. H., *Am. J. Physiol.*, 1959, v197, 309.
2. Winton, F. R., *Trans. Third Conf. on Renal Function, N. Y.*, Macy, 1952, 51.
3. Selkurt, E. E., *Am. J. Physiol.*, 1946, v147, 537.
4. Waugh, W. H., *Circ. Res.*, 1958, v6, 363.
5. Weiss, C., Passow, H., Rothstein, A., *Am. J. Physiol.*, 1959, v196, 1115.
6. Hinshaw, L. B., Ballin, H. M., Day, S. B., Carlson, C. H., *ibid.*, 1959, v197, 853.
7. Thompson, D. D., Kavaler, F., Lozano, R., Pitts, R. F., *ibid.*, 1957, v191, 493.
8. Malvin, R. L., Wilde, W. S., Sullivan, L. P., *ibid.*, 1958, v194, 135.
9. Winton, F. R., *Harvey Lectures*, N. Y., Academic Press, 1951-52, 21.
10. Whittaker, S. R. F., Winton, F. R., *J. Physiol.*, 1933, v78, 339.

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Influence of Sodium Thiosulfate on Reducing Capacity of Human Erythrocytes *in vivo*.* (25525)

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Ability of the red blood cell to reduce iodine is, to a great extent, a function of its glutathione content(1). We observed that, during intravenous infusion of sodium thiosulfate, there occurs a diminution in reducing capacity of red blood cells. We established an index determined by the ratio between this diminution and amount of sodium thiosulfate infused. Erythrocytes of normal women had a reductivity index (R.I.) which was more than double that of normal men.

Method. One hundred ml of 10% $Na_2S_2O_3 \cdot 5\ H_2O$ was infused intravenously as described by Cardozo and Edelman(2). Average duration of infusion was 10 minutes. Venous blood was withdrawn in heparinized syringes at 0, 25, 35, 45, and 60 minutes after beginning of infusion. Each blood specimen was divided into 2 portions. The first part was used for iodimetric titration of whole blood, the second portion for iodimetric titration of serum(3). Volume of iodine used for each titration is employed to determine reducing capacity of the sample at each time interval and is measured in terms of arbitrarily devised reductivity units (r.u.). Ten r.u. are equal to that volume of N/1000 iodine standard required to titrate 0.5 mg of sodium thiosulfate standard. The N/1000 iodine solution was prepared from N/10 iodine(4) by dilution. One g KI/100 ml was then added to stabilize final solution as recommended by

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Brun(3). The N/1000 iodine solution was standardized against N/400 sodium thiosulfate(3), which was stabilized with 2-3 g of borax/liter. Fresh standards were prepared monthly. Whole blood reducing capacity at each time interval was measured as follows: A 1:10 dilution of original blood sample was prepared by laking 1 ml of heparinized blood in 5 ml of distilled water with gentle agitation. The proteins were then precipitated with 4 ml of freshly prepared Folin-Wu reagent (1 vol. 10% sodium tungstate: 1 vol. ⅔ N sulfuric acid: 2 vol. distilled water). After precipitation, specimens were centrifuged for 7-10 minutes at 2500 rpm, and the protein-free supernatant was filtered through Whatman #12 paper. One ml of protein-free filtrate was then titrated by direct iodimetry at room temperature, using 1% starch reagent to establish the end-point. Just prior to each titration, 1 ml of 2 N HCl was added to the titration tube. Reducing capacity of serum at each interval was measured by making a 1:5 dilution of the original sample as follows: heparinized blood was centrifuged 10 minutes at 2500 rpm. One ml of serum was combined with 4 ml of Folin-Wu reagent to precipitate proteins. After precipitation, specimens were centrifuged for 7-10 minutes at 2500 rpm. One ml of protein free supernatant was then titrated by direct iodimetry as described above. All titrations of blood and serum were done in duplicate, subtracting blank values of tubes containing similar volumes of water. No more than 2 hours were permitted to elapse between each venipuncture and final titration of its sample. To calculate red blood cell reducing capacity at a specific time (t) the following formula was used: $R_t = \frac{B_t - (1 - HCT)\S S_t}{HCT}$. R_t is reducing capacity of red blood cells/ml rbc. B_t is reducing capacity of whole blood/ml whole blood. S_t is reducing capacity of serum/ml serum. At conclusion of each experiment, R_t was plotted as ordinate on graph paper, and time (min.) plotted as abscissa (Fig. 1). Clear portion represents loss in red blood cell

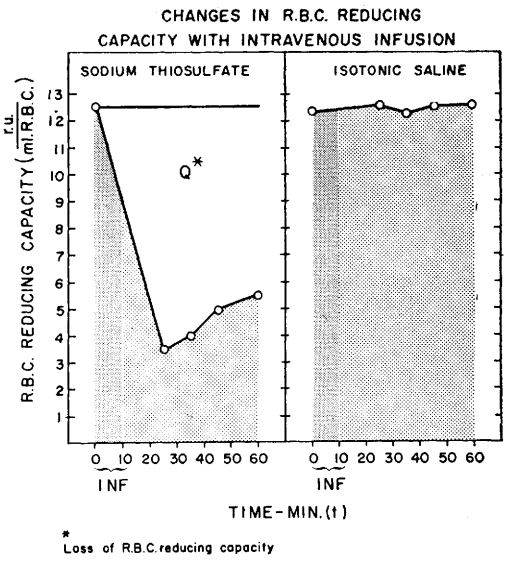

FIG. 1.

reducing capacity in one hour. When this area (Q) is divided by amount of sodium thiosulfate infused, the reductivity index (R.I.) is established.

$$\text{R.I.} = \frac{\text{Area represented by Q in unit-min.}}{\text{Total amount of sodium thiosulfate (g)}}:$$

Amount of thiosulfate infused is calculated by diluting 1 ml aliquot of the 100 ml infusion in 1 liter volumetric flask using borax as preservative and titrating 1 ml of the 1/1000 dilution by iodimetry as described for protein-free filtrates above. Simultaneous calculations were made to determine extracellular fluid volume(2) and to calculate disappearance rate (k) of sodium thiosulfate from plasma according to the formula: $k = \frac{0.693\|}{T\,½}$ where T½ is half life of sodium thiosulfate in plasma. Red blood cell reducing capacity pattern following intravenous infusion of 100 ml of isotonic saline is demonstrated for control purposes. Reductivity indices were established in 14 adult white females and 10 adult white males. In addition, 3 female patients were given 75 mg testosterone propionate daily intramuscularly for 7 days. Indices were measured prior to and following course of testosterone. Two additional male patients were given 5 mg stilbesterol tablets

§ HCT is hematocrit.

‖ Nat. log of 2.

TABLE I. Erythrocyte R.I. in 14 Women.

Age range (yr)	R.I. range	Avg R.I.	S.D.
29-71	68-127	87	17

daily for 1 week. Reductivity indices were measured prior to and following this estrogen. Indices were also determined in 3 boys and 1 girl during puberty, a 25-year-old man with hyperparathyroidism, a 49-year-old man with carcinoma of bladder, and a 50-year-old castrated man with carotid artery occlusion and hemiplegia. A final study was performed by administering testosterone propionate to this castrate in dosage listed above, and control R.I. was compared with post-testosterone R.I.

Results. Adult women (Table I) had an average R.I., 87, which was significantly greater ($p<.001$) than the average R.I., 34, of normal adult men (Table II). Administration of testosterone propionate (Table III)

TABLE II. Erythrocyte R.I. in 10 Men.

Age range (yr)	R.I. range	Avg R.I.	S.D.
30-63	28-42	34	5

resulted in considerable depression of R.I. in 2 of 3 women and in the castrated man. The opposite effect was obtained following administration of stilbestrol to 2 normal men (Table IV). Differences in R.I. could not be attributed to variations in extracellular fluid. Sodium thiosulfate estimation of the extracellular compartment in men averaged 18.8% of total body weight; in women, 19.6% of total body weight. Two patients given 100 mg cortisone acetate orally daily for one week showed 3 liter increases in extracellular fluid but no change in R.I. A third patient, given 100 μg l-triiodothyronine orally daily demonstrated 16% fall in R_o¶ after one week, but no change in R.I. No sex difference was found in (k) rate of disappearance of sodium thiosulfate from plasma which averaged 0.016/min. In 4 patients undergoing pubertal changes, one 14-year-old girl and one 14-year-old boy showed R.I. values of 69 and 34 respectively, consistent with normal adult values. The other R.I.'s are intermediate. High R.I. values were encountered in 2 men with neoplastic disease. Average R_o for women and men were 15 and 17 r.u./ml rbc respectively equivalent to 0.93 and 1.05 mg GSH/ml rbc.

TABLE III. Effect of Testosterone on Erythrocyte R.I.

Age	Sex	Pre testosterone	Post testosterone
80	♀	92	32
54	♀	94	37
64	♀	93	78
50	♂ (castrate)	71	46

¶ $R_o = R_t$ when $t = 0$

TABLE IV. Effect of Stilbestrol on Erythrocyte R.I.

Age	Sex	Pre stilbestrol	Post stilbestrol
38	♂	37	78
41	♂	29	41

Discussion. Reducing capacity of red blood cells under normal conditions is, in large measure, a function of reduced glutathione (GSH) content and does not vary appreciably during one hour, nor does it change following intravenous infusion of isotonic saline (Fig. 1). This capacity falls significantly following intravenous infusion of sodium thiosulfate. The absolute and relative diminution in this capacity differs between sexes in normal adults, resulting in differences in R.I. Since there is no sex difference in erythrocyte mean corpuscular volume(5), size of red blood cell is probably not a factor. Preliminary studies suggest that circulating androgen and estrogen may play a role in response of erythrocyte reducing capacity to intravenously administered sodium thiosulfate. Diminution in this capacity reflects changes in intracellular oxidation-reduction of glutathione favoring the oxidized form. The roles of glucose-6-phosphate dehydrogenase and glutathione reductase have not yet been evaluated in the R.I. phenomenon. It has previously been shown(6,7) that intracellular glyoxalase activity influences glutathione stability, and that interferes with this stability by inhibiting glyoxalase activity. Since arsenate and thiosulfate behave similarly in reduction-oxidase systems, it is conceivable that R.I. may be a function of similar process.

Further investigation is proceding to elucidate the mechanism of the R.I. phenomenon and to see whether it relates to neoplasia.

Summary. Reducing capacity of erythrocytes has been measured. Followng intravenous infusion of sodium thiosulfate, there occurs a significant diminution in this capacity. A reductivity index (R.I.) was established relating diminution in this capacity with amount of sodium thiosulfate infused. R. I. of erythrocytes of women is more than double that of normal men. Preliminary studies suggest that estrogen and androgen may influence R.I.

1. Woodward, G. E., Fry, E. G., *J. Biol. Chem.*, 1932, v97, 465.
2. Cardozo, R. H., Edelman, I. S., *J. Clin. Invest.*, 1952, v31, 280.
3. Brun, C., *J. Lab. & Clin. Med.*, 1950, v35, 152.
4. Pierce, W. C., Haenisch, E. L., *Quantitative Analysis,* John Wiley & Sons, N. Y., 1948, 241.
5. Wintrobe, M. M., *Clinical Hematology,* Lea & Febiger, Philadelphia, 1951, 95.
6. Klebanoff, S. J., *Biochem. J.,* 1956, v64, 425.
7. ———, *ibid.*, 1957, v65, 423.

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Dietary Vitamin K Requirement of the Rat. (25526)

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Recent reports by Barnes and Fiala(1) and by Mameesh and Johnson(2) have shown that a dietary Vit. K deficiency can be produced in the non-coprophagic rat. Prior to the finding of the role of coprophagy in supplying intestinally synthesized Vit. K to the rat(1,2), it had not been possible to determine the quantitative Vit. K requirement of the rat for maintenance of a normal prothrombin level. The experiments reported herein were conducted to measure the Vit. K requirement of the growing, non-coprophagic, male rat.

Methods. Weanling male albino rats of the Sprague-Dawley strain were randomly divided into groups of 10 rats each. The animals were individually housed in wire-bottomed cages in a temperature-controlled laboratory. Food and water were given *ad libitum.* Daily feed records were kept, and the rats were weighed at weekly intervals. Coprophagy was prevented in all rats by the method of Barnes *et al.*(3). The composition of the basal diet is given in Table I. The diet was mixed every 2 weeks and stored in dark brown glass jars under refrigeration. To reduce bacterial growth, the daily feed refused was discarded and feed dishes and water bottles were washed every other day with hot water and a household detergent. Vit. K_1 (3 - phytyl - 2 - methyl - 1,4 - naphthoquinone, Mann Research Laboratories, Inc., New York) was dissolved in triolein and added to the diet at the time of mixing at predetermined levels. Evidences for inadequate Vit. K intake were: a) hemorrhagic deaths with one or more of the following symptoms ob-

TABLE I. Basal Diet.

Ingredient	g
Sucrose	66.9
Drackett-soy protein (Archer Daniels Midland Co., Cincinnati, O.)	20.0
DL-methionine (Dow Chemical Co., Midland, Mich.)	.5
Triolein	3.0
Vitaminized cerelose*	5.0
Salt mixture No. 446†	4.0
Methyl linoleate urea inclusion compound (25% methyl linoleate, Hormel Foundation, Austin, Minn.)	.6
α-Tocopherol acetate	.012
Vit. A (Nopcay, 250,000 USP units per g, Nopco Chemical Co., Harrison, N. J.)	.008
Vit. D_3 (Super Nopdex 15,000 I units/g, Nopco Chemical Co., Harrison, N. J.)	.013

* Mameesh and Johnson(2). † Spector(5).