the source of carbohydrate in a thiamine deficient diet‖ which they gave to 9 young adolescent males over an 18-21 month period. The present results may explain in part the long period required to bring out the thiamine deficiency symptoms in most of the subjects, and that one failed even after 20 months to develop deficiency symptoms.

*Summary.* 1. Twelve female rats kept on a diet in which dextri-maltose constituted the sole source of nourishment survived on the average 85 days. 2. These rats lived 48 days longer than rats of the same weight kept on a single food diet of dextrose or sucrose; and 11 days longer than rats kept on dextrose with access to the 0.02 per cent solution of thiamine hydrochloride. 3. Their food intake was higher and they lost weight at a slower rate than the dextrose and thiamine rats. Their activity, water intake and vaginal smears were essentially the same. 4. It was concluded that the dextri-maltose contains sufficient amounts of thiamine to utilize to its fullest the available carbohydrate.

[8] Najjar, V. A., *et al.*, *J. A. M. A.*, 1944, **126**, 357.

‖ Vitamin-free casein, Crisco, dextri-maltose, a mineral mixture and a vitamin mixture.

## 15052

## Plasma Chloride and Bicarbonate after Potassium Administration.

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After nephrectomy, the plasma chloride of dogs falls gradually to low levels.[1] The anion is not lost to the exterior and must undergo redistribution. Since, after nephrectomy, potassium accumulates, it may carry chloride and bicarbonate as the potassium salt into tissue cells, such as muscle, according to the Boyle-Conway theory.[2,3] This situation has been mimicked over short periods of time by the injection of potassium nitrate dissolved in a minimum of water to avoid dilution of extracellular chloride and bicarbonate.

*Methods.* Mongrel male dogs given only water overnight were nephrectomized through the mid-ventral abdominal wall under intraperitoneal sodium amytal (55 mg of the acid per kilo). The potassium nitrate, 2.5 mM per kilo of dog, was injected intraperitoneally as a solution containing 500 mM $KNO_3$ per liter. Blood was collected from the femoral artery: before, and 2 hours, and 4 hours after the $KNO_3$ injection. Heparin was mixed into the blood which was collected under oil and covered with a rubber stopper before chilling and centrifugation.

Plasma was analyzed as in previous work[4] for water, dry residue (protein), chloride, and potassium. Sodium was weighed as the triple acetate in the Jena crucible according to Butler and Tuthill.[5] The bicarbonate as carbon dioxide of plasma was determined in duplicate, or in triplicate if checks were unsatisfactory, by the manometric method of van Slyke and Neill,[6] employing the minor modifications of Peters and van Slyke.[7] The pH of plasma was measured from whole

[1] Atchley, D. W., and Benedict, E. M., *J. Biol. Chem.*, 1927, **73**, 1.

[2] Boyle, P. J., and Conway, E. J., *J. Physiol.*, 1941, **100**, 1.

[3] Wilde, W. S., *Bull. Math. Biophysics*, 1944, **6**, 105.

[4] Wilde, W. S., *Am. J. Physiol.*, 1945, **143**, 666.

[5] Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

[6] van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

[7] Peters, J. P., and van Slyke, D. D., *Quantitative Clinical Chemistry*, Methods, 283 *et seq.*, 292, Baltimore, 1932.

TABLE I.

Plasma Constituents in mM per Liter of Water; Actual Value Before (0) and Change During First 2 Hours ($\Delta_1$) and During Second 2 Hours ($\Delta_2$) After Peritoneal Injection of 500 mM $KNO_3$. Blood Cl values in mM per liter of blood.

| Dog No. | Plasma Cl | | | Plasma K | | | Plasma Na | | | Blood Cl | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | $\Delta_1$ | $\Delta_2$ | 0 | $\Delta_1$ | $\Delta_2$ | 0 | $\Delta_1$ | $\Delta_2$ | 0 | $\Delta_1$ | $\Delta_2$ |
| 1 | 116.8 | —1.7 | | 5.38 | +4.60 | | | | | | | |
| 2 | 120.7 | —3.5 | —1.1 | 4.50 | +6.52 | | 161.5 | —7.2 | | | | |
| 3 | 121.5 | —3.3 | —0.7 | 4.04 | +6.86 | —0.58 | | (159.3) | —1.0 | 98.6 | —13.9 | +0.6 |
| 4 | 125.1 | —2.4 | | 4.57 | +5.25 | | | | | 90.8 | — 7.8 | |
| 5 | 119.7 | —4.9 | —0.6 | 5.09 | +5.82 | +0.79 | 159.0 | —5.3 | —2.2 | 91.5 | — 6.0 | —0.8 |
| 6 | 122.3 | —3.0 | —0.1 | 4.15 | +7.03 | +0.76 | 159.3 | —6.8 | +5.9 | 92.1 | — 5.2 | +0.8 |
| 7 | 127.0 | —3.4 | +0.6 | 5.00 | +6.07 | —0.59 | 162.4 | —4.4 | —0.4 | 95.7 | — 7.6 | —1.1 |
| Mean | | —3.17 | —0.38 | | +6.03 | | | | | | | |
| * $\sigma$ | | 0.93 | 0.58 | | 0.78 | | | | | | | |

* $\sigma$ = Standard deviation.

blood drawn directly without anticoagulant into the micro chamber of the Coleman glass electrode. All concentrations are expressed in millimoles mM per liter of plasma water.

*Results.* The change $\Delta_1$ in plasma chloride (Table I) during the first 2 hours after the potassium injection was only —3.17 mM. While by "t" test[8] the probability is less than 0.0014 that this represent a random variation, it is far below the change predicted mathematically[3] from Boyle-Conway. In fact to explain the small chloride decrement on such basis would require that the chloride of potassium enter cell water equal to only 17% of the body weight. Muscle water amounts to about 30% of body weight.

While the corresponding increment $\Delta_1$ in potassium is of course significant statistically it bears no significant correlation[8] to the chloride decrement $\Delta_1$ (r, 0.465).

Had plasma chloride and sodium been diluted together by some common mechanism (The sodium concentration also fell. Table I), chloride would have had the value $\Delta_1$ of —4.6 instead of —3.17. In such a comparison we must remember of course that sodium bound as sodium proteinate in plasma can vary because of possible disturbance of capillary hydrostatic pressure relations in potassium poisoning or that some of the protein-bound Na can be released by ion exchange with the elevated K.

In preliminary experiments the insensible water loss as measured with an Ohaus balance for the 4 hours averaged 5 cc per kilo. This, as planned, is the quantity of water injected as 500 mM $KNO_3$. While the initial effect of any hypertonic solution placed in the peritoneal cavity is a dilution of extracellular electrolytes,[9] the final result with $KNO_3$, if, as is likely, it is completely diffusable,[4] is that the water injected as a solvent diffuses everywhere. Since this equals the insensible loss in volume we would expect no net change in electrolyte concentrations.

The peritoneal fluid at the end of 4 hours averaged 2.3 cc per kilo. This approximates the volume found normally after intraperitoneal amytal.

While the dry residue or protein of plasma varied in either direction the hematocrit ratio of erythrocytes in blood rose spectacularly (Table II) and continued to rise during the third and fourth hours. Since the water per unit dry weight of corpuscles did not change, the increased corpuscular volume represented hemoconcentration.

Though the hemoconcentration progressed into the second period, the electrolytes had reached equilibration in 2 hours as evidenced by the insignificant values of $\Delta_2$ (Table I).

The possible exchange between injected nitrate and blood corpuscular chloride might be expected to raise the extracellular chloride. However in calculations of the concentration of chloride in corpuscular water in dogs 6

[8] Treloar, A. E., *An Outline of Biometric Analysis*, t on p. 151, r on p. 171, Minneapolis, 1938.

[9] Darrow, D. C., and Yannet, H., *J. Clin. Inv.*, 1935, **14**, 266.

TABLE II.
Plasma Bicarbonate in mM per Liter of Water, pH of Plasma, Dry Residue of Plasma, and Hematocrit Ratio. Definition of 0, $\Delta_1$, $\Delta_2$ in Legend of Table I.

| Dog No. | Plasma bicarbonate | | | Plasma pH | | | Dry residue of plasma, g/kg $H_2O$ | | | Hematocrit, % erythrocytes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | $\Delta_1$ | $\Delta_2$ | 0 | $\Delta_1$ | $\Delta_2$ | 0 | $\Delta_1$ | $\Delta_2$ | 0 | $\Delta_1$ | $\Delta_2$ |
| 1 | 24.6 | —0.6 | | | | | 75.5 | +2.0 | | 39.7 | + 5.7 | |
| 2 | 22.0 | —2.2 | | 7.38 | 0.00 | | 79.6 | —2.2 | | 34.7 | + 0.3 | |
| 3 | 23.2 | —2.5 | —1.0 | 7.39 | +0.11 | +0.01 | 86.8 | —1.6 | —1.0 | 38.1 | +11.9 | +3.2 |
| 4 | 21.8 | —7.2 | | 7.40 | | | 82.5 | +6.7 | | 51.2 | +12.2 | |
| 5 | 23.9 | —5.4 | —0.4 | 7.41 | +0.09 | +0.15 | 93.0 | —1.3 | —0.4 | 34.6 | + 6.2 | +1.5 |
| 6 | 21.1 | —0.6 | —1.1 | 7.51 | —0.03 | —0.02 | 87.8 | —4.0 | —0.5 | 38.7 | + 4.0 | +5.8 |
| 7 | 19.0 | —4.5 | —1.7 | 7.47 | —0.01 | —0.03 | 85.7 | +6.4 | —1.0 | 40.6 | + 8.1 | +3.6 |

and 7, $\Delta_1$ fell only 3.0 and 2.3 mM respectively. Since corpuscular water did not change we estimate that the chloride lost from the erythrocytes of these dogs could have raised the extracellular chloride 0.19 mM.

The sharp fall $\Delta_1$ in the chloride of whole blood (Table I) is referable mainly to the increased proportion of corpuscles, which contain less chloride than plasma.

The plasma pH, measured in order to calculate bicarbonate and complicated by artificial respiration in dogs 5, 6, 7, is listed in Table II. The bicarbonate fell faster than chloride throughout, probably in relation to accumulating metabolic acids. However during the first 2 hours its value declined so much more than chloride in proportion to the normal concentrations of each as to suggest a special relation to the potassium administered. Whether the effect was related to a release of lactic acid by the potassium tetany or to some other factor remains unknown.

*Summary.* In nephrectomy a sudden experimental elevation of the extracellular potassium did not lower the plasma chloride as much as predicted from the Boyle-Conway concept. The chloride fell 3.17 mM while the potassium rose 6.03 mM and the correlation 0.465 between these is insignificant. The proportional fall in plasma sodium slightly exceeded that for chloride. The bicarbonate of plasma fell so rapidly initially as to suggest a special relation to the administered potassium.

## 15053

## Shadowed Electron Micrographs of Bacteria.*

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In a recent note[1] we have described a procedure, based on metallic shadow-casting,[2] for enhancing the visibility of very small particles under the electron microscope and making apparent many details of their shapes which are not seen in other ways. This technic consists in obliquely depositing in a vacuum a thin layer of a relatively structureless metal such as chromium onto the electron microscopic preparation. The distribution of thickness of this layer, which should be of such an average thickness as to be partially transparent to the electron microscope beam, is determined by the contours of the preparation.

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1 Williams, R. C., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 265.

2 Williams, R. C., and Wyckoff, R. W. G., *J. Applied Physics*, 1944, **15**, 712.