II. ON THE QUANTITY OF FREE WATER IN THE RED BLOOD CORPUSCLES.

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THE quantity of free water in the red blood corpuscles is of great significance for a wide field of research, and particularly for the study of the distribution of diffusible substances between the corpuscles and plasma.

In all previous work in this field [Warburg, 1922; Van Slyke, 1926; Henderson, 1928], Ege's [1922, 1] figure of 35–40 % for the disperse phase of the corpuscle, *i.e.* 65–60 % for the free water, has been the basis of calculation. With this value directly or indirectly inserted in the formulae, it has been shown that the distribution of Cl^- and HCO_3^- is inversely proportional to the distribution of H^+ . The value of free water only enters the calculation of concentrations of the first named ions, as $[\text{H}^+]$ is measured directly as the activity of the blood cell interior. Netter [1929] in the same way has shown that the distribution of NH_4^+ and H^+ are directly proportional. $[\text{H}^+]$ here is likewise measured directly, $[\text{NH}_4^+]$ computed from the analyses with the value of free water taken as 65 %.

This is all quite in accordance with the Gibbs-Donnan theorem and forms, I think, a strong argument in favour of the above-mentioned value or the free water.

Determination of the quantity of free water in the erythrocytes can be made in several ways, all of them indirect. That most commonly used is the measurement by the hematocrit method of the volumes of the same mass of red corpuscles in several hypo- and hyper-tonic solutions of the same salt. The values found are inserted in the equation for van't Hoff-Boyle-Mariotte's law: $(v - x)p = (v_1 - x)p_1$, where v and v_1 are the volumes of the cells in solutions of osmotic pressures p and p_1 respectively; the volume of the blood in its own plasma being taken as 100, x then represents the disperse phase and $100 - x$ the free water. Now, Krevisky [1930] has determined this quantity once more and found it to be only 35 %, thereby confirming an older finding by Gough [1924]. Ege [1927] showed that the technique employed by Gough was not irreproachable, giving too high volume values and therefore apparently too small a quantity of free water.

Krevisky uses a high-speed centrifuge until constant volume is reached, but he has only applied highly hypertonic (3–20 %) NaCl solutions as exterior

fluid. In such solutions Koeppe's criterion (*i.e.* a transparency of the column in the hematocrit tube) does not appear and haemolysis very often occurs. The cause of the non-appearance of Koeppe's phenomenon is perhaps that the blood-corpuscles in such solutions assume "thorn-apple" form and therefore cannot pack so closely that all fluid between them is pressed out, this being the condition for the appearance of transparency. The volume values in such concentrated salt solutions are higher than expected according to the above equation, from the values found for v and x in hypo-, iso- and slightly hyper-tonic solutions of the same salt—even if centrifuging is carried on for long periods. Krevisky, in order to make this law valid, enlarges the value of x up to 65 %. This is only what Ege [1922, 2] already observed. The latter author found the following values with dog's blood centrifuged at 8000 r.p.m. for 3–4 hours:

NaCl %	Δ	vol.	x calculated by present author
0.91	0.56	100	—
1.67	1.00	75	43
5.0	2.95	62.4	53.6
6.7	4.95	68.2	64
10.0	5.90	67.4	64

With Ege's volume values in concentrated NaCl solutions inserted in the formula, one finds apparent x values of exactly the same magnitude as Krevisky found in the same solutions. It is not however permissible to assume the extension of the validity of these values to any other range of concentration, be it higher or lower, especially when this has not been investigated. All volume-concentration curves not exceeding about $1\frac{1}{2}$ times the isotonic concentration (Krevisky's lowest is about 3 times isotonic) yield an x value of about the same order of magnitude as Ege's (35–40 %) [*cf.* Christensen and Warburg, 1929].

Whether this discrepancy between the values of x in high and low ranges of concentration is due to a real alteration in the magnitude of x or only to the impossibility of getting correct volume values in concentrated salt solutions by the hematocrit method, I am not at present able to say.

SUMMARY.

The volume values of blood corpuscles in isotonic, hypotonic and slightly hypertonic salt solutions, when inserted in the equation for van't Hoff-Boyle-Mariotte's law, yield values of about 65 % for the quantity of free water. The same blood in highly hypertonic solutions of the same salt gives lower values for the quantity of free water—down to 35 %. The first-named value has been employed in all theoretical work up till now with good result; the value of 35 % must be either fallacious, or only valid in very concentrated solutions.

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