CXX. THE ANION AND CATION CONTENTS OF NORMAL AND ANAEMIC BLOODS.

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(Received March 30th, 1936.)

IN the course of investigations on the permeability of erythrocytes, it was noticed that the potassium content and osmotic pressure, as judged from the total ionic concentration, of erythrocytes from cases of acholuric family jaundice, were much diminished, whilst the osmotic pressure of the plasma was about normal. In view of the contradictory nature of these observations, a more complete survey of the several constituents of the cells and plasma in various normal and abnormal conditions was undertaken.

The conditions investigated fall into five groups: normal; microcytic anaemia; macrocytic anaemia; myelosclerosis; acholuric family jaundice (congenital haemolytic icterus).

Potassium, sodium and chloride were determined in cells and plasma; haemoglobin and water were estimated in the cells and bicarbonate in the plasma, whilst cell bicarbonate was calculated from the formula

$$[\text{HCO}_3^-]_{\text{cell}} = \frac{[\text{HCO}_3^-]_{\text{plasma}}}{k} \times \frac{[\text{Cl}^-]_{\text{cells}}}{[\text{Cl}^-]_{\text{plasma}}}.$$

The value of k (which from the Donnan equation should be 1) is between 0.8 and 0.9. It has been given the value 0.85 and the error in cell HCO_3^- calculated in this way is not likely to exceed ± 1 m.eq.

Plasma water was assumed to be 93 %. Deviations from this average figure are almost certainly less than ± 1.5 %.

Further, in order to correlate the findings with erythrocyte destruction and regeneration, the percentage of reticulocytes, the plasma bilirubin and the erythrocyte fragility in solutions of sodium chloride were investigated.

Methods.

Heparinised blood was centrifuged under liquid paraffin at 4000 r.p.m. for 30 min. in tubes drawn out into sealed graduated and calibrated capillaries. Part of the supernatant plasma was removed for analysis and the residue washed away. The volume of cells was measured and the cells analysed. The object of the liquid paraffin was to prevent evaporation of plasma during centrifuging. To some extent it may also check loss of CO_2 , because its viscosity prevents mechanical agitation of the plasma surface.

Sodium was estimated by the method of Oberst [1935]. For plasma, 0.05 ml. was used, the error being ± 2 %. In the case of cells 0.15 ml. was used, the error being not greater than ± 5 % (equivalent to ± 0.5 % of the total base present). Oberst points out that plasma contains about twenty times as much sodium as do cells so that the presence of even a trace of intercellular plasma will give a figure for cell sodium which is relatively much too high. He recommends that the cells be washed with a sodium-free fluid consisting of dialysed plasma whose osmotic pressure is restored by addition of potassium chloride. The use of such

a wash-fluid, if not actually objectionable owing to the possibility of removing sodium from abnormal cells, is time-consuming and unnecessary, for if Oberst's own figures are examined it will be seen that the subtraction of 9.2 mg./100 ml. from the sodium content of unwashed cells gives a very close approximation to the sodium content of the corresponding washed cells. It can in fact be shown that the intercellular plasma is about 2.8 % by volume, which would correspond to an apparent increase in cell sodium of 9.2 mg./100 ml. Thus, if 0.1 ml. centrifuged cells is suspended in 2 ml. saline and the suspension re-centrifuged, the protein content of the supernatant saline (consisting of plasma washed out from between the cells) can be estimated and if the protein content of the corresponding plasma be known, the plasma content of the supernatant saline, and hence of the centrifuged cells before suspension, can be determined. The protein content of the supernatant saline was estimated by nesslerisation after oxidation, and also with Folin's phenol reagent, a blank determination being carried out on the supernatant saline after precipitation of the protein with sodium tungstate and sulphuric acid.

In thirteen of Oberst's experiments the sodium content of washed cells, calculated in the above way, differed from the estimated values as follows: +50, +19, +16, +8, +6, -5, -4, +2, +2, -2, -2, -1 and 0 %. If the first three results are excluded the differences between the calculated and estimated figures are well within the region of experimental error.

The technique of washing cells, moreover, is objectionable on other grounds; thus whilst it is true that intercellular plasma gives incorrect and weighted results for cell sodium, the same observations apply to other ions and as it is impossible by using different wash-fluids to obtain absolute figures for each of these constituents, it is not reasonable to attempt this in the case of sodium alone. The supernatant plasma above the centrifuged cells is presumably in equilibrium with intercellular plasma and this in turn is in equilibrium with the cells. For practical purposes of comparing and contrasting plasma and cell ion distributions it is essential to consider the supernatant plasma as one phase and to group the cells and intercellular plasma together as the other phase.

Potassium was determined on a protein-free filtrate by the method of Kramer and Tisdall [Maizels, 1935], duplicates on 0.1 ml. cells differed by less than 4 % and on 0.6 ml. plasma by less than 10 %. These results were 1 or 2 % lower than those obtained with washed cells. For chloride, the method of Claudius [1924] was used on 0.05 ml. plasma and 0.1 ml. cells, and for plasma bicarbonate that of Payne as described by Maizels *et al.* [1930] on 0.1 ml. material. Water was estimated by heating 0.1 ml. cells at 105° to constant weight. The errors of these three determinations were within ± 2 %.

Haemoglobin was estimated by the method of Haldane, the figure 100 % corresponding with 138 g. per litre of blood and from this and the haematocrit value the amount of haemoglobin per litre of cells was calculated. The amount is normally about 6 mM with respect to cell water, but as Adair [1928; 1935] has shown, this is osmotically equivalent to an approximately 12 mM solution.

In addition to the chemical estimations, the number of erythrocytes per μ l. of blood was counted and from this and the haematocrit value, the mean cell volume was derived.

There are certain advantages and disadvantages inherent in the methods employed of centrifuging blood in narrow graduated tubes. Intercellular plasma is reduced to a minimum and a very small volume of packed cells can be measured accurately so that it is possible to carry out all the estimations on about 1.5 ml. centrifuged cells instead of on 10 ml. and therefore, in the case of anaemic

patients, it suffices to withdraw only 6 ml. blood instead of 40 ml. On the other hand, loss of CO_2 is not prevented and only partial oxygenation of the haemoglobin of the venous blood may occur; since both these processes affect the distribution of water and electrolytes between cells and plasma the latter cannot be investigated with the accuracy achieved by Van Slyke and Henderson. However, the method permits certain conclusions to be drawn and it may be said that the p_{H} of blood after centrifuging under oil was between 7.7 and 7.8 at 17° , that the haemoglobin appeared to be well oxygenated and that results obtained on normals were consistent and agreed fairly well with those of other observers.

Results.

Normals (Table I, Nos. 1-6). The cases about to be described were either normal or showed a minor degree of anaemia in association with some condition like rheumatoid arthritis. The averages and ranges for the various findings were as follows: haemoglobin 91 % (82-102), erythrocytes 4.95 millions per $\mu\text{l.}$ (4.3-5.4), haematocrit 42.4 % (37-46), mean cell volume $86\mu^3$ (80-91). Haemoglobin per litre of cells 295 g. (272-313). Cell constituents were as follows: K 106 m.eq. (101-110), Na 12 m.eq. (10-15), Cl 51 m.eq. (48-56), water content 71 % (69-72). In the case of the plasma, the figures were: K 4.1 m.eq. (3.8-4.7), Na 139 m.eq. (136-142), HCO_3 25.5 m.eq. (23-27), Cl 102 m.eq. (97-106). The ratio of cell to plasma chloride concentrations, R , was 0.65.

Details of individual cases are given in Table I, but it must be noted that cell and plasma constituents are given in terms of their concentrations per litre of water, and if it be desired to convert concentrations into contents per litre of cell or plasma, it will be necessary to multiply the concentrations by the percentage of water present.

The figures for plasma K are a little lower than those of some observers, but agree with those of Laurent and Walther [1935] obtained by a different method. It is possible, that as Walther suggests, the high results of other workers are due to delay in separating cells and plasma.

The average concentrations of cell and plasma base per litre of cell water were respectively 166 and 154 m.eq.—a difference of 12 m.eq. From the latter figures, 3 m.eq. must be subtracted corresponding to an excess of 2 m.eq. cell Mg on the one hand, and an excess of 5 m.eq. plasma Ca on the other. Thus the excess of cell over plasma base is in the present series about 6 %. This excess of cell base is to be expected owing to the presence of the non-permeating Hb-present in the cell. Similarly the sum of plasma $[\text{Cl}^-] + [\text{HCO}_3^-]$ is greater than the sum of cell $[\text{Cl}^-] + [\text{HCO}_3^-]$ being 137 m.eq. compared with 93.

It is generally agreed that the osmotic pressures of erythrocyte and plasma are equal and therefore the sum of cell anion and cation should equal the sum of plasma anion and cation. In the present series the concentration in cell water of $\text{K} + \text{Na} + \text{Cl} + \text{HCO}_3$ is 258.5 m.eq. To this figure must be added about 12 m.eq. for cell Hb so that the total osmotic equivalence of the cell contents is about 270 m.eq. The corresponding figure in the case of the plasma is 291. (The osmotic pressure of the plasma proteins which is small has not been considered—for the purposes of the present discussion it may be neglected.) There is thus an apparent excess of at least 21 m.eq. of osmotically active substances in the plasma. Henderson [1928] remarks on this apparent excess of plasma constituents and suggests that it may be due to “differences between the two phases in activity coefficients of water and of dissolved substances” and “to unequal distribution of substances not accounted for in the balance sheet”. It is however at least as simple to assume that a proportion of cell water is not free, in the

Table I.

Table I.																								
Case No.	Date	Disease	Age	Sex	Haemo- globin %	Erythro- cytes per μl.	Colour index	Haema- tocrit	Mean cell vol. μ ³	Haemo- globin g. per litre	Van den Bergh units	Reticu- lated cells %	Cell concentrations					Plasma concentrations					Excess cell base over plasma base m.eq.	Excess plasma osmotic pressure over cell osmotic pressure* m.eq.
													K m.eq.	Na m.eq.	HCO ₃ m.eq.	Cl m.eq.	H ₂ O %	K m.eq.	Na m.eq.	HCO ₃ m.eq.	Cl m.eq.			
1	—	N	24	M.	83	4.32	0.96	39.4	91	291	0.3	0.1	153	14.3	22	67	72	4.3	148	29	104	15	17	
2	—	N	20	F.	97	5.00	0.97	44.1	88	304	0.5	0.2	150	16.6	22	74	71	4.3	152	28	110	10	18	
3	—	N	35	M.	102	5.12	1.00	45.0	88	313	0.5	0.1	147	17.4	20	73	71	5.0	148	26	114	11	22	
4	—	N	16	F.	82	4.50	0.91	37.5	83	301	0.4	0.2	151	15.1	21	71	69	4.0	149	28	113	18	18	
5	—	N	52	M.	91	5.40	0.84	43.0	80	292	0.5	0.1	146	21.5	20	78	71.5	4.5	152	25	113	6	21	
6	—	N	24	M.	90	5.44	0.82	45.7	84	272	0.3	0.2	146	16.8	21	72	71.5	4.6	147	27	108	11	19	
7	—	Mi	60	M.	46	4.03	0.57	29.5	73	215	0.2	1.4	152	17.5	21	71	75	4.5	154	28	109	6	31	
8	—	Mi	42	M.	53	4.12	0.64	29.8	72	245	0.1	0.2	147	20.5	21	74	70	4.0	150	27	110	19	14	
9	26. xi. 35	Mi	63	M.	28	3.3	0.42	18.8	57	205	0.8	2.0	148	18.3	19	70	77	4.9	150	25	111	11	28	
10	14. i. 36 2. xii. 35 30. xii. 35	Mi	44	F.	70	5.2	0.67	39.1	75	247	0.7	3.0	151	15.2	21	71	73.5	4.0	149	28	112	13	26	
11	—	Mi	42	F.	78	4.9	0.80	37.5	79	279	0.2	0.8	154	16.4	22	71	71.5	4.3	154	28	108	12	20	
12	—	Mi	38	F.	66	5.3	0.51	27.4	64	222	0.5	0	147	17.0	18	63	76.5	4.3	152	26	106	8	35	
13	—	Mi	41	F.	29	3.49	0.62	36.6	69	249	0.8	3.5	156	17.2	21	74	74	4.2	154	26	107	9	20	
14	—	Mi	54	M.	44	5.00	0.44	31.7	63	192	4.5	1.9	151	16.0	21	72	79	4.4	150	28	115	—	29	
15	15. xi. 35	Ma	73	M.	44	1.41	1.56	21.0	149	290	1.25	0.5	157	21	20	67	71.5	4.3	158	30	115	16	30	
16	24. xii. 35	Ma	69	F.	72	3.00	1.20	35.0	117	283	0.2	0.2	151	19	21	74	73	4.5	154	28	114	12	24	
17	4. xii. 35 27. xii. 35	Ma	44	F.	43	1.74	1.24	21.2	122	280	2.0	12.5	142	20	18	67	70	4.3	151	26	113	7	35	
18	—	Ma, S	36	F.	84	4.20	1.38	25.7	140	275	3.0	7.0	152	19	18	66	69	4.1	152	25	108	15	22	
19	—	Ma, S	64	F.	42	1.71	1.23	22.9	134	254	0.5	13.0	145	—	—	—	69	—	—	—	—	—	—	
20	—	Ma, S	52	M.	72	2.70	1.33	19.7	141	280	—	0	145	—	—	—	69	—	—	—	—	—	—	
21	—	My	38	F.	60	2.80	1.07	29.1	104	285	3.5	11.0	149	17	20	71	69	5.7	153	27	110	7	27	
22	—	My	56	F.	58	3.13	0.93	28.5	91	281	2.7	4.8	153	16	19	68	72	4.3	148	26	111	17	21	
23	9. x. 34 16. i. 35 11. i. 35	AJ	42	M.	94	4.80	0.98	37.3	78	348	—	—	108	—	—	66	67	—	—	—	104	—	—	
24	25. iii. 36 16. x. 34 2. ii. 35 11. iv. 35 26. ii. 36	AJ	20	F.	82	3.92	1.04	35.2	90	320	6.0	5.0	118	20	23	71	65	4.5	154	28	101	—	38	
25	—	AJ	11	M.	55	3.42	0.80	26.4	77	288	3.5	4.0	130	—	—	75	65	4.1	143	24	108	—	53	
26	16. v. 35 26. i. 36†	AJ	35	F.	82	3.97	1.03	34.6	87	326	4.0	12.0	134	—	—	80	66	—	—	—	—	—	—	
27	—	AJ	60	F.	54	2.40	0.97	49.7	92	292	0.4	0.1	136	22	24	80	69	—	—	—	—	—	—	
28	26. v. 35 26. i. 36†	AJ	21	M.	87	4.40	0.99	34.5	78	347	9.0	13.0	127	—	—	71	68	4.5	—	—	—	—	—	
29	27. v. 35 20. i. 36†	AJ	20	F.	106	5.32	1.00	44.7	84	329	0.25	1.4	125	13	21	70	69.5	4.2	145	28	111	—	45	
					76	3.82	1.00	31	81	339	12.0	18.0	124	18	20	65	68	5.0	149	28	108	—	47	
					110	5.42	1.01	49.6	92	306	1.5	0.4	126	17	22	74	70	4.6	148	27	106	—	34	

N = normal.

Mi = microcytic anaemia.

My = myeloid leucosis.

Ma = macrocytic anaemia.

† Post-operative record.

S = Sprue.

AJ = acholuric jaundice.

sense that it is not available for the solution of substances present in the cell. Thus if 8 % of cell water were bound then the discrepancy in the sums of osmotically active substances in cell and plasma would disappear. On general grounds it is to be expected that bound water is present in erythrocytes since as Adair and Adair [1934] have shown 1 g. Hb binds 0.2 g. water and hence the 30 g. Hb present in 100 ml. cells should bind about 8 % of the total water present. And whilst on the one hand there is direct experimental evidence that between 5 and 10 % of cell water is bound [Maizels, 1935], there is on the other hand no evidence at all that ionic activity is increased in the presence of haemoglobin [Adair and Adair, 1934].

The microcytic anaemias (Table I, Nos. 7-14). The averages and the ranges of the findings in this group are as follows: colour index 0.52 (0.42-0.64), mean cell volume $66 \mu^3$ (57-73), Hb per litre cells 216 g. (180-250), cell K 112 m.eq. (106-122), cell Na 13 m.eq. (11-14), cell Cl 53 m.eq. (48-57), cell water 75 % (70-79). Plasma K averaged 4.0 m.eq. (3.7-4.5), Na 141 m.eq. (139-144), HCO_3 25 m.eq. (23-26), Cl 102 m.eq. (99-107), and the ratio of cell to plasma chloride concentrations was 0.64.

Thus the mean cell volume and amount of Hb per unit volume of cells are decreased. In the plasma, K, Na, Cl and HCO_3 are about normal while the contents of cell anion and base are greater than normal. As cell H_2O is also increased however the concentrations of cell acid and base are only slightly raised.

In three of the cases of microcytic anaemia the reticulocyte count was in the neighbourhood of 3 %, but this appeared to make little difference to the concentration of cell base. Indeed the latter remained fairly constant in spite of great variations in cell water and Hb in all anaemias with the single exception of acholuric jaundice. It seems as if the concentration of cell base were of the first importance and that the other cell constituents were modified so as to maintain this constancy. Thus in case 9 although, as a result of treatment, Hb rose from 28 to 70 % and Hb per unit volume of cells by 20 %, the concentration of cell base remained practically unaltered. The same is true of case 10.

The macrocytic anaemias (Table I, Nos. 16-21). One of these cases contained less Hb per unit volume of cells than normal (No. 18), a case of sprue; the others contained about the normal amount.

Colour index averaged 1.36 with a range from 1.24 to 1.56. Mean cell volume was 137 (122-149). The concentration of cell base was about normal, whilst that of anion was slightly decreased. The ratio of cell and plasma chloride concentrations was decreased, being 0.6 against the normal 0.65. Since this ratio varies inversely with cell $[\text{Hb}^-]$ and since the latter is not increased, the decrease in R is difficult to explain, unless some other non-permeating anion be present in the cell besides Hb.

As a result of treatment cell base decreased and water increased, as observed by Henderson in a case described by him. The increase in plasma base noted by Henderson, however, is not observed in this series.

The chloride ratio rises as a result of treatment, but since the concentration of cell Hb does not decrease, the increase in this ratio may perhaps be attributed to a decrease in some other non-permeating anion.

In three cases of the present series the apparent excess of osmotically active substances in the plasma over those in the cell was greater than normal, being 29 m.eq. compared with 21. Henderson [1928] had previously noted this increased discrepancy between plasma and cell osmotic pressures in pernicious anaemia and suggested that it might be due to the presence of reticulated cells

or cells which have recently passed through this stage. In such cells it is possible that "water and electrolytes are associated with nuclear material in proportions quite different from those existing in the cell solution". Equally, it is possible that the reticulocyte has a greater proportion of bound water than has the mature erythrocyte.

But whatever deviation the immature erythrocyte may present, it may be said that cell K remains fairly constant and that cell destruction as shown by increased bilirubin in the blood or cell regeneration as shown by reticulocytosis has very little influence on the potassium level.

Myelosclerosis (Table I, Nos. 21 and 22). The two cases of this rare disease are of interest because of certain findings which sometimes compare and at others contrast with those present in acholuric family jaundice. Features in common are: an anaemia with colour index in the neighbourhood of unity; a marked excess of haemobilirubin in the blood and the presence of nucleated and reticulated erythrocytes.

Myelosclerosis differs from acholuric jaundice in that the amount of haemoglobin per unit volume of cells is normal and not increased and that cell fragility is not increased. In both conditions the spleen is enlarged, but in myelosclerosis splenectomy is not followed by improvement in the anaemia, reticulocytosis or jaundice.

The various chemical findings, including cell potassium are not abnormal in myelosclerosis.

Acholuric family jaundice (Table I, Nos. 23-29). The erythrocyte of acholuric family jaundice (congenital haemolytic icterus) differs from the normal in several respects; it tends to be less biconcave and even biconvex; it contains more haemoglobin per unit volume of cells (345 g. compared with the normal 290). In agreement with this observation cell water is decreased, being 65-69 % compared with the normal 69-72 %. Mean cell volume is normal or slightly decreased; the decrease in cell diameter is more marked and hence it follows that the cell tends to be more convex than normal.

Cell K is decreased by 20-30 %, cell Na is normal; the other constituents of the cell show no abnormality. The decrease of cell K is not related to the intensity of the Van den Bergh reaction or to the reticulocytosis. It would not indeed have been expected to be so related in view of the findings in the anaemias and in myelosclerosis where marked jaundice and noteworthy reticulocytosis are unaccompanied by any significant change in cell K. Nor can cell K be correlated with the cell fragility in saline solutions for in case 25, where haemolysis was seen in 0.7 % NaCl, cell K was 87 m.eq., whilst in case 23 with slight haemolysis only in 0.6 % NaCl cell K was 72 m.eq. Further the same case may show considerable variations in cell K without corresponding changes in fragility (cases 23 and 24). The findings are summarised in Table II.

The more complete data of cases 23, 24 and 29 show certain other abnormalities. The concentration of cell base (K + Na) is less than the concentration of plasma base, averaging 144 m.eq. against 157, whilst the apparent excess of plasma osmotic pressure is much greater than normal, being over 40 osmolar millimols in each case, compared with the normal 21. Now unless the p_H of the acholuric cell is less than 6.6, the isoelectric point of haemoglobin, cell base concentration must be greater than plasma base. On general grounds the p_H of the acholuric erythrocyte is not likely to be less than p_H 7, in fact although it is more acid than normal its p_H is definitely greater than 7 [Hampson and Maizels, 1927-28]. Here again it may be that, as Henderson suggests, the distribution of electrolytes in the immature erythrocyte is different from that in

Table II.

Case No.	Date	Cell K content m.eq.	Van den Bergh units	Reticulocytosis %	Haemolysis commencing NaCl, %
23	9. x. 34	72	—	—	0.6
	17. xi. 34	72	—	—	—
	16. i. 35	85	9	6	0.6
	30. i. 35	86	—	—	—
	11. xi. 35	77	6	5	0.6
24	16. x. 34	84	4	12	0.57
	22. iii. 35	73	3	10	0.57
	11. iv. 35	82	2.5	6	0.57
	26. ii. 36	87	3.5	4	0.57
25	—	87	3.5	12	0.70
26	16. v. 34	88	4	12	0.51
	3. xii. 35*	92	0.4	0.1	0.48
27	—	82	5	18	0.6
28	26. v. 35	86	9	13	0.57
	26. i. 36*	87	0.25	1.4	0.57
29	27. v. 35	84	12	18	0.6
	20. i. 36*	88	1.5	0.4	0.54

* Post-operative record.

the mature cell. It is however more probable that the increased proportion of bound water in the acholuric cell explains the apparent anomalies. It has been remarked previously that in normal blood, plasma osmotic pressure is apparently greater than cell osmotic pressure, and in order to explain this it was suggested that about 8% of cell water (6 ml. per 100 ml. cells) was bound. If the same amount of bound water were present in the acholuric cell, cell base concentration would be 150 m.eq. against 157 in the plasma. It is further possible that the amount of bound water in the acholuric cell is greater than that assumed to be present in the normal. This is in fact quite likely, since the absolute amount of haemoglobin in the acholuric cell is about 20% greater than that of the normal cell, corresponding to the binding of about 10% of total water. Further the concentration of Hb is also greater and under these circumstances the quantity of water bound is relatively increased. Thus Kunitz *et al.* [1933-34] have shown that 1 g. Hb in 6.3% solution binds 0.14 g. water, whilst in 10.45% solution it binds 0.22 g.

It is of course possible that this deficiency in cell K+Na might be compensated by the presence of Ca or Mg, but estimation of total base in one case showed that this was not so.

After removal of the spleen the condition of the patient shows a notable improvement: reticulocytosis and excess of bilirubin disappear from the blood, cell volume increases slightly. The haemoglobin content of the cells falls by 10%, the water content increases slightly and fragility becomes slightly less marked. Cell K shows a very slight increase.

DISCUSSION.

There remain for discussion two aspects of the preceding observations; firstly, the relation of cell anion to cell cation, and secondly, the nature of the acholuric erythrocyte.

Relation of cell cation to cell anion. The concentration of cell cation $[K^+] + [Na^+]$ should equal that of $[Cl^-] + [HCO_3^-] + [Hb^-]$. Any excess of cation over the

latter value must presumably be due to the combination of base with some undetermined acid. Such an excess has been demonstrated by Henderson [1928] who refers to the acid as HX. $[X^-]$ equals $[K^+] + [Na^+] - [Cl^-] - [HCO_3^-] - [Hb^-]$. In the present investigations, K, Na, Cl and HCO_3^- are known. To determine $[Hb^-]$ it is necessary to know the concentration of Hb and also the cell p_H . The latter value, unfortunately, is not known. It is however possible to assign certain probable limiting values to cell p_H , and hence to $[Hb^-]$ and so to $[X^-]$.

Thus in normals cell p_H will certainly be between 0.05 and 0.2 less than that of the plasma [Hampson and Maizels, 1927-28]. In the present experiments, plasma p_H was between 7.7 and 7.8 and it is very probable that cell p_H was between 7.5 and 7.7. The average concentration of cell Hb was 25.5 m.eq. corresponding to between 52 and 65 m.eq. base bound [Adair, 1925]. The excess of cell base concentration over $[Cl^-] + [HCO_3^-]$ was 72 m.eq. so that the concentration of base uncombined with $[Cl^-] + [HCO_3^-] + [Hb^-]$ was between 7 and 20 m.eq. The latter figures are obviously approximate and it must be interpreted to mean that a small excess of base is present in the normal erythrocyte over and above that combined with $[Cl^-]$, $[HCO_3^-]$ and $[Hb^-]$; it is presumably combined with some other anion of simple or complex nature.

In the microcytic anaemias the value of $[Hb]$ averaged 18 m.eq. The excess of cell base over $[Cl^-] + [HCO_3^-]$ was 75 m.eq. and in the absence of X^- it follows that 4.2 m.eq. base are bound per m.eq. Hb^- corresponding to a cell p_H of 8.8. Such a low hydrogen ion concentration is quite impossible and it is certain that the anion X^- must be present in the anaemic cell and in greater amounts than in normal cells. It is in fact very probable that p_H 7.5-7.75 will cover the cell p_H range in anaemia and this corresponds to between 38 and 48 m.eq. bound; under these circumstances the concentration of X^- must be between 27 and 37 m.eq. It is clear therefore that when all the various approximations are taken into account, the value of $[X^-]$ in anaemic cells is much higher than in normals. It varied directly with the degree of anaemia and decreased when Hb rose as a result of treatment.

In the macrocytic anaemias, the concentration of base not bound by $[Cl^-]$ and $[HCO_3^-]$ averaged 85 m.eq. and that of $[Hb]$ was 24.6. As the p_H of the macrocytic erythrocyte is less than normal [Hampson and Maizels, 1927-28; Henderson, 1928] it is clear that the base bound by Hb^- will also be less than normal and that X^- will be greater than normal. Thus the p_H difference in pernicious anaemia ranged between 0.2 and 0.5. Assuming then that under the experimental conditions cell p_H lay between 7.25 and 7.55, the concentration of base bound by Hb would lie between 38 and 55 m.eq. and the concentration of X between 30 and 47 m.eq.

As a result of treatment the concentration of cell base combined with $[Hb^-] + [X^-]$ fell to 73 m.eq. while the concentration of Hb remained unchanged. Even if one assumes that cell p_H were unaltered (although it had probably in fact actually increased) then it is certain that the amount of base combined with $[X^-]$ had decreased. Such a decrease in the value of X^- in treated cases had been previously noted by Henderson [1928].

The chloride ratio rose in treated macrocytic anaemia. This ratio varies inversely with the concentration of ionised haemoglobin. But as $[Hb^-]$ had not decreased but rather increased, one must ascribe the increase in the chloride ratio to a decrease in some other non-permeating anion, possibly X itself.

In acholuric jaundice $[Hb]$ averaged 31.5 m.eq. From the data of Hampson and Maizels, cell p_H may be taken to be between 0.3 and 0.5 less than that of the plasma, namely between 7.25 and 7.45. The concentration of base bound by

[Hb⁻] under these conditions lies between 49 and 63 m.eq. Base combined with [Hb⁻] + [X⁻] equals 55 m.eq. and therefore the concentration of cell X⁻ lies between +6 and -8 m.eq. From which it follows that the concentration of X⁻ in this malady is very low.

Regarding the nature of the erythrocyte in acholuric jaundice, it will have been realised that this cell is very distinctive. It tends to be more globular than normal, contains more haemoglobin and less water than the normal cell and is more prone to haemolysis. Its Na content is normal, but K and therefore total base are low. It might be thought that the globular shape of the acholuric cell and its fragility depended on its distension by the increased quantity of the contained Hb but this is probably not the case; thus No. 25 which has the lowest Hb per litre of cells has the highest fragility.

It is probable that the low level of the cell K in acholuric jaundice is significant, although it does not parallel cell destruction, regeneration or fragility and is therefore not dependent on these; like them, however, it may arise from some primary defect in the cell which affects these manifestations in different degrees.

Considerations of such a defect must necessarily be speculative; it might lie in the structure or composition of the cell membrane and might act in one of two ways: (1) accelerated loss of K from the mature cell; (2) delayed permeation of K into the immature cell.

(1) Cell base is chiefly K; plasma base Na. It is possible that the originally impervious cell suspended in plasma, gradually becomes effete and sheds a proportion of its K. Indeed it is possible that haemolysis may occur when cell K reaches a certain low level. There are, however, certain objections to this theory. If K diffuses out of the cell into the plasma, Na should pass from plasma to cell; there is no evidence that this occurs. Further, there is no evidence that the acholuric cell suspended even in simple salt solutions is more pervious than normal. Thus the gain of K in KCl or loss in NaCl solutions (after suspension for 1 hour at 37°) is not greater than normal, although acholuric cells are specially liable to damage by foreign solutions. Finally, it has already been noted that the excessive haemolysis of pernicious anaemia and myelosclerosis is not accompanied by a decrease in cell K. It seems improbable therefore that the low level of cell K in acholuric jaundice depends on accelerated loss.

(2) The level of K in the normal erythrocyte is higher than that of most other cells. It seems probable that the primitive erythrocyte contains no more K than other cells, but that at an early stage an inflow of K occurs, determined by unknown factors. Later, the erythrocyte becomes impervious to all cations, and the entry or exit of K and Na ceases.

This may be part of the process of maturation and if the process occurs too early the content of K achieved will be less than normal so that a number of cells will be left in a more or less immature condition with deficient K and a persistent reticulum in relation to which there exists an excessive proportion of bound water.

SUMMARY.

1. The concentration of anions and cations in erythrocytes is less than the concentration of anions and cations in plasma. An apparent deficiency in cell osmotic pressure thus exists, which makes it necessary to assume that about 8% of cell water is bound.

2. An amount of base is present in the erythrocyte which is greater than that required to combine with cell Cl⁻ + HCO₃⁻ + Hb⁻. It is suggested that this excess of base is combined with some other anion present in the cell. This unknown anion is increased in most anaemias, but not in acholuric family jaundice.

3. Anion, cation and water are about normal in the macrocytic anaemias and in myelosclerosis. In microcytic anaemias, the cation content of the cell and also its water content are increased, but the cation concentration is only slightly raised. The concentration of cell cation is thus relatively constant in spite of great variations in the concentration of cell Hb and notwithstanding wide variations in blood regeneration and destruction.

4. In acholuric family jaundice, cell K and water are low; cell Na is normal and Hb per unit volume of cells is increased. Cell base concentration is less than plasma base concentration in spite of the p_H of the cell being greater than the p_H at which oxyhaemoglobin is isoelectric. This can only be explained on the assumption that more bound water is present in the acholuric cell than in the normal.

5. The level of cell K in acholuric jaundice is not related to cell destruction, regeneration or fragility. It is suggested that it may be due to failure of K to penetrate during an early stage of the cell's development.

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