RED CELL CHANGES IN CHRONIC PULMONARY INSUFFICIENCY [1]

By JOSEPH L. GRANT, ALASTAIR MACDONALD, JOHN ROBERT EDWARDS, RICHARD R. STACEY, AND GEORGE H. STUECK, JR.

(From the Medical and Research Services, Veterans Administration Hospital, White River Junction, Vt., and Dartmouth Medical School, Hanover, N. H.)

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It is well known that in chronic pulmonary disease there is often an increase in packed red blood cells. It is less widely recognized that while the hematocrit value rises, the hemoglobin concentration may remain normal. This is well shown in reports where both values are given (Table I). In 71 cases the mean hematocrit was 54.4 per cent, the mean hemoglobin was 15.8 Gm. per 100 ml., and the mean value for mean corpuscular hemoglobin concentration was 28.8 per cent (normal, 32 to 36 per cent). This is a study of the nature of the resulting hypochromia, which we have also consistently observed.

Methods for hemoglobin and hematocrit, when reported, varied in the papers cited. Figures for both venous and arterial blood are included. The inaccuracy of results of hemoglobinometry by acid or alkaline hematin conversion is well known, and has led to the recent widespread use of the cyanmethemoglobin method, while the venous hematocrit value may vary depending on the duration of tourniquet application as well as on the tube, centrifuge speed, and axis of rotation (1). It is hoped that in this study such inaccuracies have been avoided by the use of arterial blood oxygen capacity and uniform standard hematocrits. Blood oxygen capacity is a standard method for active hemoglobin; it does not include any inactive pigment which may be present. Comparison of the oxygen capacity (in ml. of oxygen per 100 ml. whole blood) to the hematocrit (in ml. of packed cells per 100 ml. whole blood) gives a ratio, the "$O_{2/Vc}$ ratio," of ml. of oxygen uptake per ml. of packed cells, which is the mean corpuscular *active* hemoglobin concentration. We use it in this study in place of the usual mean corpuscular hemoglobin concentration (MCHC) to emphasize that hemoglobin values are obtained gasometrically rather than colorimetrically, and because the question of inactive hemoglobin is part of the study. If inactive hemoglobin is neglected, MCHC can be derived from any $O_{2/Vc}$ ratio by dividing by 1.34 (ml. of O_2 taken up per gram of hemoglobin at standard temperature and pressure conditions). The mean normal value for oxygen capacity in our laboratory is 20.6 ± 0.95 volumes per 100 ml. and the mean normal hematocrit is 43.9 ± 1.87 per cent, giving an $O_{2/Vc}$ ratio of 0.469 ± 0.011. These values were found in eight healthy medical students.

The $O_{2/Vc}$ ratio in pulmonary disease has been found to be consistently reduced, and this appears to be due to an increase in red cell water.

TABLE I

Hematocrit and hemoglobin values from reported cases of chronic pulmonary disease

Source	No. of cases	Mean hematocrit	Mean hemoglobin	Mean corpuscular hemoglobin concentration
Normal values, Wintrobe (1)	28	47	16	34
Harvey (28)	28	51.1	14.1	27.6
Hammarsten (29)	16	54	16.8	32
Wilson (30)	14	52	15.2	29.6
Taquini (19)	6	69	20.1	29.3
Austen (31)	4	58	15.3	26.5
Carroll (32)	1	56	16	28.6
Loman and Dameshek (33)	1	80	22	27.5
Pare and Lowenstein (34)	1	68	19	27.9
Mean	71	54.4	15.8	28.8

METHODS

Arterial blood, collected anaerobically into heparinized syringes with mercury seals, was used for all measurements except for hematocrits. Oxygen and carbon dioxide content and oxygen capacity were determined by the method of Van Slyke and Neill. (2). The oxygen capacity is the volume of oxygen that can be liberated from blood after systematic equilibration with air for seven minutes in a tonometer, expressed in ml. per 100 ml. of whole blood. Arterial blood for hematocrits was ex-

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pelled from dry syringes into mixed-oxalate tubes where it was gently and thoroughly agitated to prevent clotting. The hematocrit value was determined either by the standard method of Wintrobe (1) or by that of Strumia, Sample, and Hart (3); whole blood pH was determined anaerobically either by the glass electrode method of Behrmann and Fay (4) or by using a Cambridge research model pH meter with a jacketed electrode at 37° C. These methods have given interchangeable values in our hands. The partial pressure of carbon dioxide was determined from the carbon dioxide content, pH and hematocrit of the whole blood using the nomogram of Singer and Hastings (5). The partial pressure of oxygen was determined from the oxygen dissociation curves using the saturation and pH as given by Clark (6). Serum and whole blood water were determined according to Eisenman, Mackenzie, and Peters (7). Two ml. of whole blood or serum is weighed in a pyrex dish, then kept at 93 to 97° C. for 72 hours. Water content is taken as the original weight minus the final weight. Cyanmethemoglobin determinations were by the method of Drabkin (8) and those for carbon monoxide capacity were by that of Van Slyke, Hiller, Weisiger, and Cruz (9). Electrophoresis of hemoglobin solutions was performed by the method of Smith and Conley (10). Determinations of fetal hemoglobin were carried out on a Beckman D. U. apparatus by a modification of the method of White and Beaven (11). Solutions were denatured with 0.2 per cent NaOH, and the optical density at 575 millimicrons measured at 10 minute intervals. By this method mixtures of adult and umbilical cord blood give characteristic curves, depending on the proportion of cord blood.

Red cell diameter was measured on dried films photographed on Kodak metallographic plates projected to a magnification of 1,400; 200 cells were measured in two diameters at right angles to each other, each to the nearest 0.2 micron. The measuring error as determined by remeasurement of 100 cells was 0.0095 micron. Measurements in both patients and control subjects were by the same individual, who did not know the identity of the slides until after the results were reported. Statistical

TABLE II

Observed values for hematocrit and oxygen capacity in 31 males with chronic pulmonary disease

Patient	Hematocrit	O_2 Capacity	O_2/V_c	Age	Diagnosis
				yrs.	
Normal	43.9	20.6	0.469*	21	Eight healthy medical students
A. P.	58.0	20.8	0.359	47	†Bullous emphysema, fibrosis
H. M.	48.5	18.9	0.390	51	†Sarcoidosis, silicosis
J. G.	52.0	21.5	0.413	53	Emphysema, fibrosis
H. W.	43.4	17.7	0.408	62	†Emphysema, fibrosis
H. S.	51.7	23.0	0.445	51	†Emphysema, cor pulmonale
H. P.	60.5	23.6	0.390	54	†Bullous emphysema, fibrosis, cor pulmonale
G. LaR.	52.3	21.5	0.411	56	†Asthma, fibrosis, emphysema
B. S.	46.6	18.8	0.403	59	Emphysema, obesity
R. B.	53.9	23.2	0.430	59	Asthma, emphysema, fibrosis
F. S.	49.9	20.9	0.419	45	†Emphysema, fibrosis, cor pulmonale
C. McK.	53.1	21.3	0.401	33	Fibrosis, emphysema
L. G.	46.1	21.0	0.456	53	Emphysema, fibrosis
P. Y.	48.1	20.0	0.416	55	Asthma, emphysema
C. M.	49.1	21.2	0.432	48	Asthma, emphysema
C. L.	54.2	20.4	0.376	53	†Emphysema
E. W.	49.5	19.7	0.398	36	Fibrosis
R. R.	49.9	19.8	0.397	61	†Silicosis, emphysema, cor pulmonale
J. C.	45.2	19.0	0.420	37	Silicosis
E. S.	47.0	19.6	0.417	61	Asthma, emphysema, old thoracotomy with flail chest
R. W.	49.0	20.9	0.427	52	Bronchitis, emphysema, obesity
A. B.	56.8	23.1	0.407	41	Obesity, hypoventilation, cyanosis
R. H.	58.7	20.7	0.353	60	†Asthma, emphysema, cor pulmonale
F. T.	50.5	17.4	0.345	64	†Bronchitis, bronchiectasis, fibrosis
E. S.	54.1	16.9	0.312	55	Bullous emphysema, fibrosis, bronchitis
H. W.	60.0	22.7	0.378	63	†Emphysema, cor pulmonale
J. G.	54.6	21.4	0.392	55	Obesity, hypoventilation, cyanosis
J. C.	49.8	18.5	0.371	59	†Emphysema, cor pulmonale
A. S.	50.5	18.3	0.363	60	†Kyphoscoliosis, emphysema, cor pulmonale
J. C.	56.0	19.7	0.351	58	†Emphysema, fibrosis
L. J.	63.6	25.1	0.395	61	Bronchitis, fibrosis
B. W.	48.9	18.1	0.371	55	Emphysema, fibrosis
Mean	52.0	20.5	0.394‡	54	

* Range, 0.458 to 0.488.
† Confirmed by autopsy.
‡ Range, 0.312 to 0.456; S.D., 0.031; S.E., 0.006.

TABLE III

Hermatocrit and oxygen capacity in 22 patients without pulmonary disease

Name	Hematocrit	O_2 Capacity	O_{2/V_c}	Age	Diagnosis
				yrs.	
W. C.	40.8	18.1	0.444	36	Hypothyroidism
R. P.	43.0	19.5	0.452	23	Peptic ulcer
E. K.	46.8	20.9	0.447	29	Bullet wound in liver
W. S.	40.5	19.0	0.470	28	Infectious hepatitis
J. C.	44.6	19.8	0.444	40	Chronic lymphadenitis
J. M.	41.6	18.7	0.450	27	Infectious mononucleosis
R. B.	44.7	20.5	0.460	26	Conversion reaction
W. C.	44.6	19.2	0.430	39	Low back strain
L. N.	50.5	21.7	0.430	23	Flat feet
B. F.	46.6	20.4	0.438	40	Lipoma, right scapula
C. R.	41.6	18.9	0.453	26	Gastric neurosis
H. M.	46.9	21.9	0.466	29	Glomerulonephritis, latent
A. J. P.	28.0	12.8	0.457	59	Periarteritis nodosa
S. J. McC.	47.5	20.9	0.440	64	Nephrosis
G. T. S.	38.0	16.4	0.432	64	Carcinoma prostate
S. P.	42.0	16.8	0.424	66	Carcinoma colon
A. H.	36.0	15.9	0.442	70	Carcinoma prostate
F. S.	29.5	13.3	0.451	50	Glomerulonephritis
S. G.	45.5	21.1	0.469	45	Aortic stenosis
E. V.	40.5	17.6	0.435	70	Hiatus hernia
F. T.	40.0	17.8	0.445	63	Thoracic goiter
A. T.	50.5	22.5	0.446	46	ASHD; ? failure
Mean	42.3	18.8	0.447*	43	

* Range, 0.424 to 0.470; S.D., 0.013.

analyses were performed according to Bradford Hill (12) and Mainland (13).

RESULTS

The arterial hematocrit, oxygen capacity, and O_{2/V_c} ratio in 31 patients with chronic pulmonary disease are given in Table II, and contrasted to those obtained in eight healthy, 21 year old, male medical students. Usual clinical criteria for the diagnosis were supported by pulmonary function studies and in many instances by autopsy. The mean oxygen capacity was 20.5 ± 1.97 volume per cent in the patients and 20.6 ± 0.95 volume per cent in the controls; the mean hematocrit level was 52.0 ± 4.8 per cent in the patients and 43.9 ± 1.8 per cent in the controls. The mean O_{2/V_c} ratio in patients with pulmonary disease was 0.394 ± 0.031 and was 16 per cent below the normal value of 0.469. By the "t" test (13) p is less than 0.01, and the result is highly significant. Since these patients were on an average 33 years older than the normal controls, a group of 22 patients without pulmonary disorders (average age, 43) was contrasted to them (Table III). The ratio in patients with pulmonary disease is 12 per cent below the mean of 0.447 found in the control patients (p is again less than 0.01).

Attempts at correlation between measurements of O_{2/V_c} ratio and the arterial oxygen saturation, oxygen tension, pH, carbon dioxide content, and carbon dioxide tension (all of which varied from normal to grossly abnormal) were all unsuccessful. pH, carbon dioxide tension and oxygen tension are presented with the O_{2/V_c} ratios in Table IV. In general it was found that the ratio was consistently low when arterial oxygen unsaturation and respiratory acidosis (whether compensated or not) were of long standing.

Possible causes for the low O_{2/V_c} ratio that we investigated included the presence of inactive hemoglobin, abnormal hemoglobins, and rise in cell water concentration.

Inactive hemoglobin

In three cases we investigated inactive hemoglobin by the carbon monoxide capacity method of Van Slyke and co-workers (9) (Table V). Values found were less than 5 per cent and cannot account for the mean 12 per cent drop in O_{2/V_c} ratio. Comparison of the cyanmethemoglobin values with the hemoglobin values from oxygen capacity determinations also showed agreement within 5 per cent (Table VI). It is concluded that

TABLE IV

pH, carbon dioxide tension, oxygen tension, and O_{2}/V_{c} ratio in 30 males with chronic pulmonary disease

Name	pH	P_{CO_2}	P_{O_2}	O_{2}/V_{c}
		mm. Hg	*mm. Hg*	
"Normal"	7.43	41	100	0.469
A. P.	7.37	71	51	0.359
H. M.	7.35	42	40	0.390
J. G.	7.35	54	76	0.413
H. W.	7.41	58	39	0.408
H. S.	7.48	35	76	0.445
H. P.	7.47	49	47	0.390
G. LaR.	7.36	70	94	0.411
B. S.	7.36	51	42	0.403
R. B.	7.52	38	37	0.430
F. S.	7.43	49	54	0.419
C. McK.	7.48	45	37	0.401
L. G.	7.43	47	47	0.456
P. Y.	7.39	46	68	0.416
C. M.	7.42	48	51	0.432
E. W.	7.43	43	98	0.398
R. R.	7.50	55	34	0.397
J. C.	7.48	35	62	0.420
E. H. S.	7.44	44	59	0.417
R. W.	7.41	50	56	0.427
A. B.	7.42	42	56	0.407
R. H.	7.42	61	44	0.353
F. T.	7.42	58	35	0.345
E. S.	7.42	47	38	0.312
H. W.	7.32	69	22	0.378
J. G.	7.41	47	55	0.392
J. C.	7.41	64	23	0.371
A. S.	7.45	45	62	0.363
J. C.	7.33	61	49	0.351
L. J.	7.21	96	24	0.395
B. W.	7.31	69	22	0.371

the presence of inactive hemoglobin was not the explanation for the decreased oxygen capacity in the patients studied.

Abnormal hemoglobin

If fetal hemoglobin is present in a pigment solution, a characteristic notch at 289.8 millimicrons is produced in the curve of optical density. In hemoglobin solutions from eight of our patients with chronic pulmonary insufficiency, this notch was searched for but none was found. In the same eight cases denaturation of the pigment with 0.2 per cent NaOH gave curves which were uniformly typical of adult pigment (Figure 1). Abnormal pigments of types S, F, and C through I were searched for by filter paper electrophoresis

TABLE V

Inactive hemoglobin in chronic pulmonary disease, determined by carbon monoxide capacity

Subject	O_{2}/V_{c} ratio	Active hemoglobin	Total hemoglobin	Inactive hemoglobin
				%
F. T.	0.375	14.01	14.31	2.1
C. McK.	0.399	13.37	13.72	2.5
R. W.	0.429	13.88	14.51	4.3

TABLE VI

Inactive hemoglobin (fourth column) expressed as the difference between the values obtained by oxygen capacity and by cyanmethemoglobin conversion

Patient	Hemoglobin from cyanmet. Hgb.	Hemoglobin from O_2 cap.	Diff.
			%
R. H.	18.1	18.5	2
J. G.	12.5	12.6	1
G. A.	17.3	17.9	4
R. A.	14.7	15.5	5
H. C.	13.7	14.1	3
D. S.	15.5	16.0	3
R. G.	15.1	14.9	−1
E. G.	12.4	12.6	2

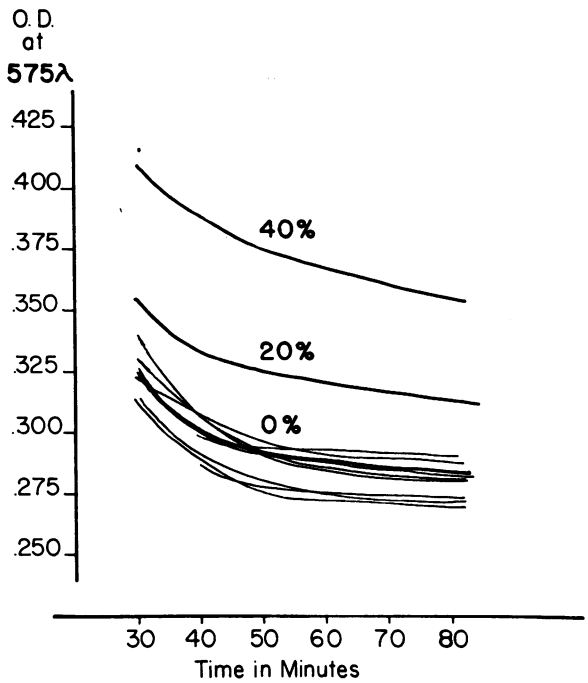

FIG. 1. ALKALI DENATURATION OF SOLUTIONS OF HEMOGLOBIN FROM EIGHT PATIENTS WITH CHRONIC PULMONARY INSUFFICIENCY (THIN LINES), CONTRASTED TO CURVES FROM CONCENTRATIONS OF UMBILICAL CORD BLOOD OF 40, 20 AND 0 PER CENT IN ADULT BLOOD

Negligible amounts of fetal hemoglobin are shown in patients by this method.

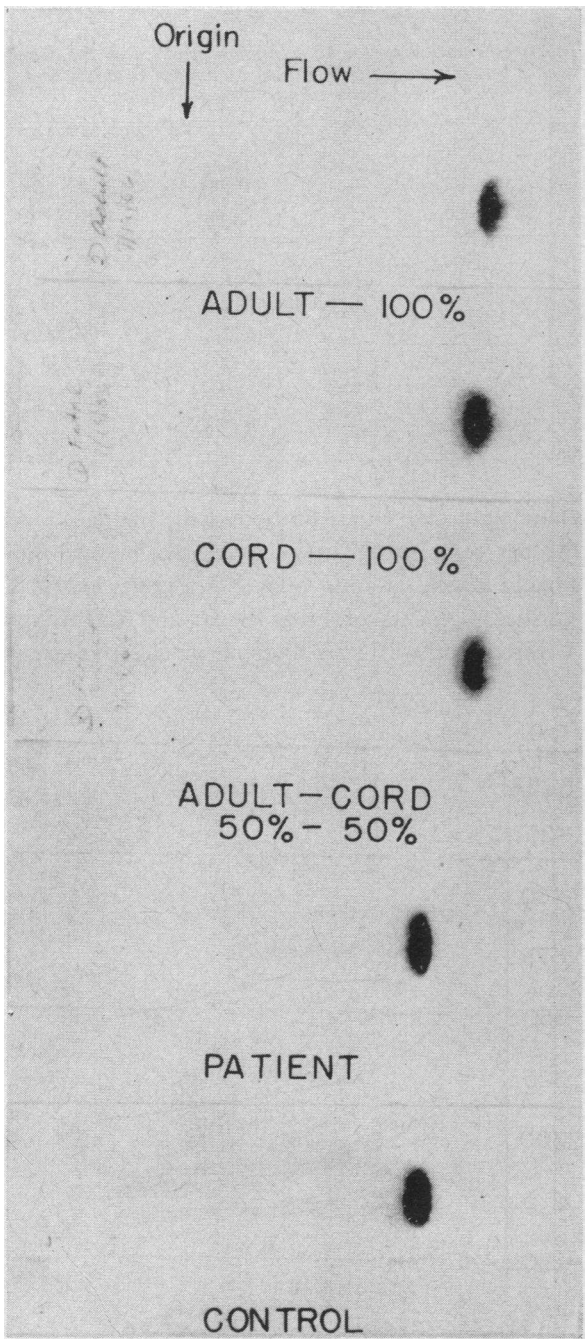

FIG. 2. ELECTROPHORETIC PATTERN OF PIGMENT MIGRATION IN PULMONARY DISEASE (FOURTH FROM TOP), IDENTICAL TO THE ADULT CONTROLS (BOTTOM)

Top patterns are those of umbilical cord and adult bloods as labeled.

after the method of Smith and Conley (10), and a typical pattern is shown in Figure 2. No abnormal pigment was found by the procedure.

Cell water

Decrease in hemoglobin concentration suggests a relative increase in other cell constituents, especially in water which constitutes about 72 per cent of the cell. Results of cell water determinations are shown in Figure 3, and it can be seen that in the 24 patients studied, cell water increase correlated well with the reduced O_{2/V_c} ratio. The coefficient of correlation was -0.95; standard error, 0.18. The extra water may be said to dilute rather than displace hemoglobin in the cell, since hemoglobin is not believed to be lost from intact human erythrocytes (14).

Increased packed cell water suggests but does not require an increase in cell size; the number of cells could vary independently. The mean red cell diameter for 9 healthy volunteers between ages 35 and 60 was 7.84 microns (S.D., 0.169) and for 10 patients with chronic pulmonary disease was 8.04 microns (S.D., 0.168; $p < 0.05$). The coefficients of variation are about 2 per cent. This shows a definite increase in red cell diameter in the patients with pulmonary disease. Mean frequency distribution curves of mean cell diameters are shown in Figure 4. The curve for the patients lies as a whole to the right, and shows a bulge or skew to the right in its shape. This was due to the presence of a double peak of frequency in six of the patients. The curves for all average diameter values to the right of the mean are shown in Figure 5. There are two peaks of frequency in the curves from patients with pulmonary insufficiency, one at 8.06 microns and the other at 8.16 microns, while the control curves show a single peak at 7.86 microns and a smooth descending limb.

DISCUSSION

In our patients the decrease in oxygen capacity compared to hematocrit was of the same order as that reported in the literature cited. The first explanation we considered was that plasma trapping, which has been reported to be relatively greater with high hematocrits (15), might be responsible for the increase in the hematocrit, with consequent reduction in the O_{2/V_c} ratio. The amount of trapped plasma in the red cell mass of the hematocrit tube has been carefully studied by different methods by Ebaugh, Levine, and Emerson (15) and by Chaplin and Mollison (16), and

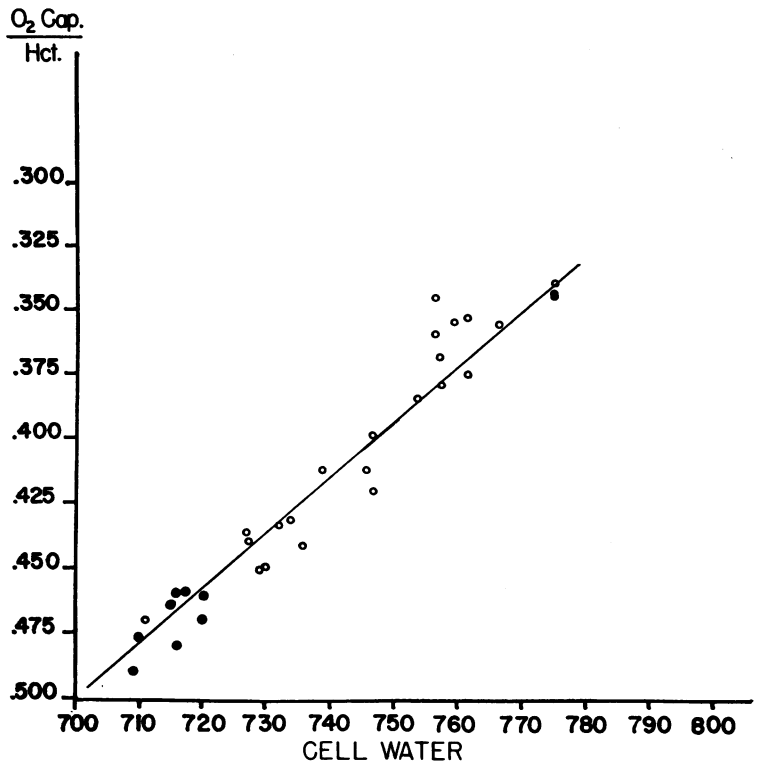

FIG. 3. CELL WATER (GRAM PER LITER OF PACKED CELLS) COMPARED TO $O_{2/vc}$ RATIO

Black dots are normal males; open dots, patients with pulmonary disease. Coefficient of correlation, -0.95; S.E., 0.18. Regression equation: (H_2O) $c = 915 - 422\ O_{2/v}$.

they agree that at hematocrit levels of 50 per cent there is only about 2 to 2.5 per cent trapped plasma, not enough to explain the 16 per cent drop in O_{2/v_c} ratio. Furthermore, if trapped plasma were the explanation the ratio should be decreased in direct and close proportion as the hematocrit rises, since plasma trapping varies directly with the hematocrit. This was not the case in our patients, in whom correlation between hematocrit and the O_{2/v_c} ratio was only approximate, with a coefficient of correlation of -0.41 and a standard error of -0.18 (Figure 6). Finally, with the microhematocrit of Strumia, trapped plasma is negligible; yet the mean O_{2/v_c} ratio in our cases studied with this apparatus was 0.381, compared to a mean of 0.403 in those whose hematocrit was determined by the method of Wintrobe. We conclude that plasma trapping is not the cause of the lowered ratio.

The next explanation to be considered was that the change in packed cell hemoglobin concentration might be a normal attribute of age, since the patients averaged over thirty years older than the medical students. Wintrobe (1), in his discussion of the erythrocyte, mentions no such phenomenon, stating only that with advanced age there may be a decrease in both hemoglobin and hematocrit. Robinson (17) found constant values for mean normal whole blood oxygen capacity from age 17 to 70 in males. Our own additional control data (Table III) are on 22 patients selected because of the absence of clinical pulmonary disease. There was a wide distribution of the O_{2/v_c} ratio at all ages, ranging from age 23 to age 70. There was a poor negative correlation between age and the ratio, which decreased slightly as age increased (coefficient of correlation, -0.406; S.E., 0.22). The mean ratio in a group of 10 of the control patients of average age 60 was 0.444 ± 0.013, again highly significantly dif-

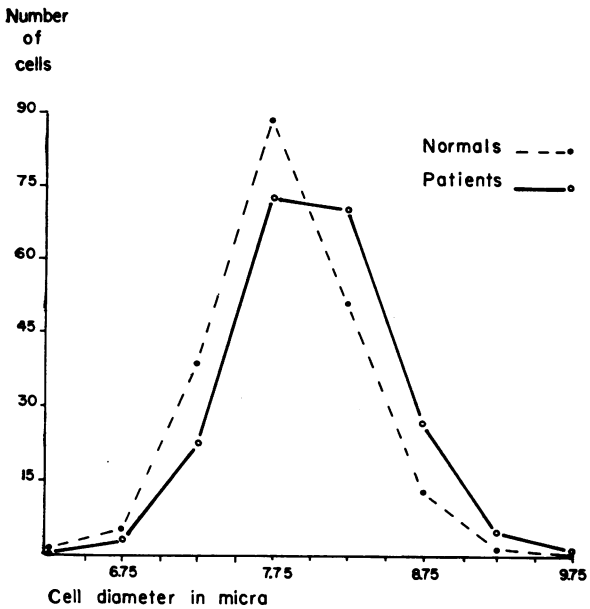

FIG. 4. MEAN FREQUENCY DISTRIBUTION CURVES OF CELL DIAMETERS, GROUPED AT 0.5 MICRON INTERVALS, FOR 10 PATIENTS WITH PULMONARY INSUFFICIENCY AND 9 CONTROLS OF COMPARABLE AGE

The curve for the patients lies as a whole to the right and shows a skew to the right.

ferent from that in the patients with pulmonary insufficiency ($p < 0.01$). Age alone, therefore, does not appear to explain the findings.

The possibility that a portion of the hemoglobin in these patients with chronic pulmonary disease was physiologically inactive appeared worth investigation. In a state characterized by chronic arterial oxygen unsaturation, hypercapnia and acidosis, the ability of red cell enzymes to reduce oxidized (ferric) hemoglobin to the ferrous functional form might be expected to be impaired, with consequent accumulation of methemoglobin and lowering of the numerator of the $O_{2/Vc}$ ratio. Besides this possible alteration of the porphyrin complex of the hemoglobin molecule, the possibility that the globin portion might be abnormal was considered worth investigation. Several different types of pigment with abnormal globin moieties have now been reported (10) (S, F, and C through I), and denaturation of the globin may alter the reversible reaction between heme and oxygen. Though Meakins and Davies (18) and Taquini, Fasciolo, Suarez, and Chiodi (19) had found normal oxyhemoglobin dissociation curves in a few cases, the possible presence of abnormal pigment was considered worth further investigation. Fetal hemoglobin was of special interest since bone marrow in the fetus, as in patients with chronic pulmonary disease, functions at a low oxygen tension (20). Methods for fetal hemoglobin are of limited precision, but the failure of filter paper electrophoresis to yield any evidence of abnormal pigment is supported by the finding of no fetal pigment by spectrophotometry (Figure 1). The closeness of the results of hemoglobin determination by oxygen capacity to those of cyanmethemoglobin conversion and carbon monoxide capacity excludes the possibility of significant amounts of methemoglobin. (Table VI, incidentally, in emphasizing the close agreement between results of cyanmethemoglobin conversion and oxygen capacity, supports the value of the former for routine laboratory use.) An inactive pigment moiety, therefore, does not appear to explain the low $O_{2/Vc}$ ratio.

Since water constitutes 72 per cent of red cells, and other constituents besides hemoglobin only 2 per cent, an increase in water would be the logical expectation if dilution of hemoglobin ex-

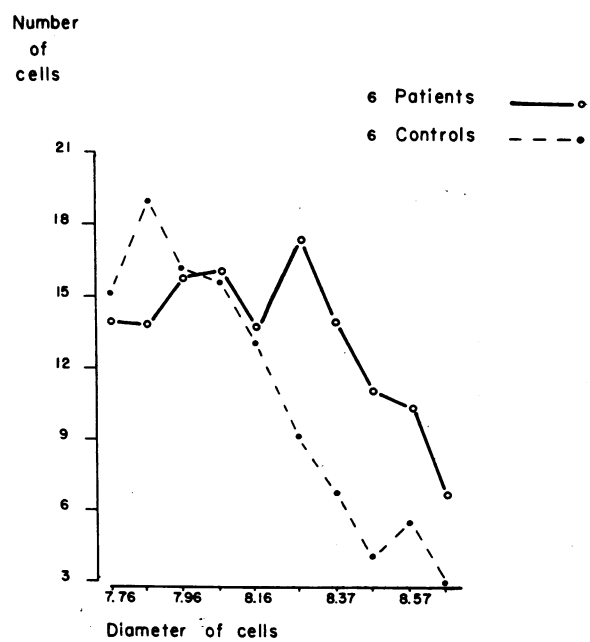

FIG. 5. FREQUENCY DISTRIBUTION CURVES FOR ALL CELL DIAMETERS 7.76 MICRONS AND ABOVE

The curve for the patients shows two peaks of frequency at 8.06 and 8.26 microns.

plains the $O_{2/Vc}$. Taquini and co-workers (19) found cell water per Gm. of packed cells to be elevated in three cases, with values of 0.740, 0.742, and 0.745 Gm. per ml. compared with a normal mean of 0.720, and related it to the decreased hemoglobin concentration. Our own studies on 32 individuals show good correlation between the increase in cell water above our normal value of 715 Gm. per L., and the decrease in $O_{2/Vc}$ ratio, both in healthy males and in patients (Figure 3). It is hard to escape the conclusion that the decrease in hemoglobin concentration results from dilution by the increase in cell water. The serum water, which Taquini found reduced, varied in our cases (range, 927 to 948 Gm. per L.).

Increased "cell water" values mean that the concentration of water in the packed cell column rises. If the number of cells remains constant, this would imply a rise in the individual cell water content, and consequently in cell volume. Berkson (21) has shown that hemocytometer cell counts, and hence values for mean cell volume that are derived from them, are determined significantly only to within plus or minus 12 per cent. One cannot therefore measure mean corpuscular volume within closer limits of accuracy. The most direct and accurate, although rather tedious, method of measuring cell size is that of Price-Jones (22). By photographing dried blood films, he found an increase in mean cell diameter from 7.20 in controls to 7.69 microns in 22 cases of emphysema. Frequency distribution curves were simply displaced to the right, retaining their shape, and he concluded that the enlarging effect was exerted on all circulating cells at once, and not on the hemopoietic organs.

Our own measurements confirm those of Price-Jones, in that the mean cell diameter was 0.2 micron greater in the 10 patients studied than in 9 controls. No correlation was found between individual mean cell diameter and the mean corpuscular hemoglobin concentration or the $O_{2/Vc}$ ratio, nor between cell diameter and the pO_2, oxygen saturation, oxygen capacity, pH, pCO_2, CO_2 content, or cyanmethemoglobin values. The overall mean increase appears nevertheless to be significant, both statistically and in the light of the small measuring error per 100 cells (0.0095 micron). The mean $O_{2/Vc}$ ratio in these 10 patients was 0.428, a 9 per cent decrease from normal.

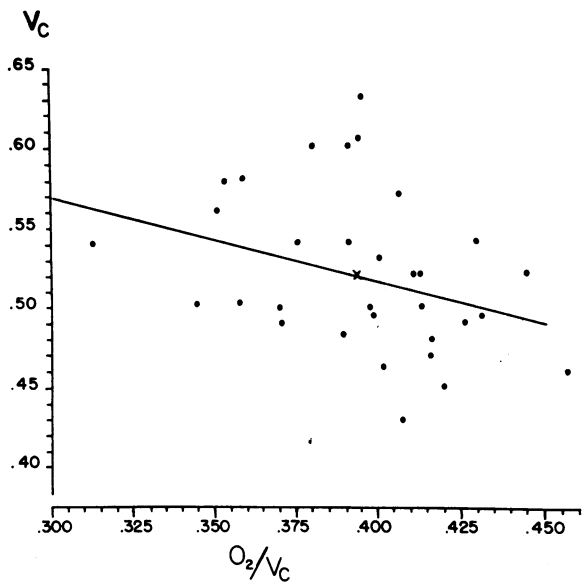

FIG. 6. CORRELATION BETWEEN $O_{2/Vc}$ RATIO AND HEMATOCRIT

The relationship is only approximate; even though the correlation coefficient is more than twice its standard error, the data show a wide range of the ratio for any given hematocrit value.

According to Figure 3, this would correspond to a 3 per cent cell water rise. If the number of cells was constant, this would result in a mean 2 per cent volume increase, since the cell is 70 per cent water. While we have made no measurements of the thickness of the cells, it is usually taken as about 2 microns, and the increase that would result in cell diameter with such a volume change would be 0.08 micron, considering the cell as a short cylinder with a radius of 4 microns and a height of 2, and assuming no change in thickness. If such a cell with a diameter of 7.84 microns increases its volume 2 per cent by increasing its diameter alone, the new diameter can be calculated to be 7.92, while the diameter found was actually 8.04. It must be remembered that only rough agreement can be expected when one is comparing measurements from dried blood films with those from gravimetric and volumetric methods, and that no information is available as to changes in red cell thickness either in these patients, or in any disease states.

Our results differ from those of Price-Jones in that we recorded a double peak of cell diameter frequency in 6 of the 10 patients with emphysema.

No methodologic reason for this is apparent; the data for the controls were grouped by exactly the same process and no niches in the curves were found, and the choice of measurement intervals for cell grouping is shown not to be at fault since there is still a double peak when all the data are plotted (Figure 5). There appear to be two populations of cells in 6 of the 10 patients studied. Either they are produced by a change in only part of the normal adult population, or *de novo* by the marrow. We cannot tell from the curves which is correct. There was no relation between the presence of two frequency peaks and the O_{2/V_c} ratio, or any of the other physiologic variables measured.

In vitro, it has been known since the early studies of Hamburger (23), von Limbeck (24), Henderson (25), and Van Slyke, Wu, and McLean (26) that increases in hematocrit, cell water and cell volume result when blood is exposed to increased carbon dioxide and low oxygen tensions. This is ascribed to the osmolar effects of increases in the concentration of cations and of bicarbonate and chloride in the cells when carbonic acid is buffered. The methods used in this study to determine oxygen capacity, hematocrit, cell water, and cell diameter all allow or encourage the free access of air to the blood under study, and allow roughly equal escape of carbon dioxide. (See Methods. We have measured the hematocrit and pH before and after air equilibration of seven blood specimens during the measurement of oxygen capacity; there was a mean drop of only 0.3 hematocrit unit with a rise in pH of 0.511 unit.) There does not therefore seem to be any methodologic cause for increased cell size. Also, since the bloods from the normal controls were handled in exactly the same manner, any such effect of carbon dioxide must have been applied equally to them.

We have failed to find correlation between the fall in O_{2/V_c} ratio or the rise in cell water and the *in vivo* pO_2, pH or pCO_2. Fluctuations in the clinical state of the patients may perhaps explain this lack of correlation. Platts and Greaves (27), who have recently made careful measurements on the red cells in acute and chronic respiratory acidosis, have also found decreased concentrations of hemoglobin per volume of cell water. Their patients with chronic acidosis (pCO_2 over 52 mm. Hg) had somewhat increased red cell water concentration (741 Gm. per L. compared to their mean normal value of 722 Gm. per L.) and a mean cell pH of 7.21 compared to their normal 7.24. By contrast, normal subjects made acutely acidotic by breathing 7 per cent CO_2 showed no change in cell water despite a drop in cell pH from 7.24 to 7.12. *In vivo* findings in these studies, as in our own, failed to follow what would be predicted from the known *in vitro* behavior of blood, and suggest that the red cell response to acute CO_2 retention may differ from that in chronic acidosis. We have been unable to gauge accurately, in retrospect, the acuteness of the clinical state of pulmonary failure at the time of these measurements, but have the impression that the O_{2/V_c} ratio was generally lowest in cases of longest standing.

SUMMARY

Patients with chronic pulmonary insufficiency show a rise in hematocrit but not in hemoglobin levels. This results in a lowered mean corpuscular hemoglobin concentration.

Studies of the arterial oxygen capacity and standard hematocrit values in 31 patients confirm this phenomenon in that the mean oxygen capacity: hematocrit ratio ("O_{2/V_c} ratio") was 0.394, compared to the control value of 0.469.

The lowered ratio could not be correlated with pH, oxygen tension or saturation, or carbon dioxide tension or content.

Plasma trapping is not the explanation for the lowered ratio.

No inactive or abnormal hemoglobin was found in the blood to explain the decreased oxygen capacity.

A close inverse correlation was found between the ratio and the red cell water concentration, and it is concluded that entrance of water into the cells best explains the phenomenon.

The mean red cell diameter in 10 patients was found to be increased by 0.2 micron, which agrees roughly with the rise expected from the increased cell water.

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