BLOOD CARBONIC ANHYDRASE ACTIVITY IN ANEMIA, WITH A NOTE ON POLYCYTHEMIA VERA

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THE ERYTHROCYTES of the blood contain a number of respiratory enzymes, one of which, carbonic anhydrase, is important in the transport of carbon dioxide. The conversion of carbon dioxide derived from the tissues to bicarbonate in the blood, and the breakdown of bicarbonate to release carbon dioxide in the lungs could not proceed at a rate compatible with health if it were not for the presence of carbonic anhydrase in the red blood cells. This enzyme, widely distributed in nature, is especially abundant in mammalian erythrocytes. Since the enzyme in the blood is contained wholly within the erythrocytes, it is apparent that the carbonic anhydrase activity of the blood might be abnormal in anemia. Although earlier workers have investigated the blood carbonic anhydrase activity in anemic blood,[1–3] their studies were carried out by means of methods which were not strictly quantitative and which had no significance for respiratory function at body temperature. Accordingly it was considered desirable to study this matter again, using a new method.

Materials and Methods

One hundred and twelve observations were made on 85 patients; the latter for the most part were patients with anemias of various types and degrees. Some patients, not anemic, were also included in order to control phenomena associated with anemia, such as icterus, bone marrow disease, etc. Five of the patients studied had polycythemia vera. The ages and diagnoses are in the tables. All studies were made on venous blood at 37 C by means of a method described elsewhere;[4] the method is a modification of that of Mitchell et al.[5] in that the Warburg apparatus is used, observations are made at 37 C, and calculations of activity are made by extrapolation to undiluted blood from measurements made on three dilutions. In each instance, measurements were made in duplicate, using three different dilutions of blood, so that six measurements were made on each sample. Erythrocyte counts and measurements of hemoglobin content and hematocrit were made on each sample of venous blood used for the estimation of carbonic anhydrase activity. The findings in normal subjects by means of this method[4] are summarized in table 1.

Observations

Pernicious Anemia

Of the 10 patients studied, 8 were anemic when first seen, the other 2 having responded completely to treatment given previously. All patients studied while anemic showed levels of carbonic anhydrase which were in the normal absolute range, i.e., they lay between 1.1 and 2.4 units per ml. of blood (table 2, fig. 1).

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In all of the anemic subjects the carbonic anhydrase activity of the blood was high relative to the hemoglobin concentration and erythrocyte counts; in all but 2 the carbonic anhydrase per unit RBC was also above the upper limit of normal, i.e., 5.8, at some time (fig. 2). During the course of treatment, absolute values for blood carbonic anhydrase activity rose; however, since hematocrits, hemoglobin levels and erythrocyte counts increased several times, the ratio between carbonic anhydrase activity and these measurements decreased toward normal (table 2, fig. 2). Normal relationships between enzyme activity and hematologic measurements did not regularly obtain, however, until the latter had returned to or almost to normal (table 2, fig. 2).

Blood Loss

Anemia consequent to hemorrhage from peptic ulcer or from carcinoma of the stomach, colon or bladder was associated with a decrease in blood carbonic anhydrase activity (table 3, fig. 3). The fall in activity of the enzyme paralleled decreases in hematocrits, hemoglobin levels and erythrocyte counts, so that the ratio of carbonic anhydrase activity to these measurements was in the normal range in every instance (table 3, fig. 4). Neither the cause of the bleeding nor its chronicity influenced the level of activity of the blood carbonic anhydrase; the 3 patients (Cases 15, 18 and 20, table 3) in whom the anemia had been present for months with a resultant decrease in cell size, showed relationships between enzyme activity and the various other measurements made on the blood which were similar to those found in patients with bleeding of recent onset; changes in cell size had no significant effect.

TABLE 1.—*Observations in Forty-two Normal Subjects*

	Range	Average
Hematocrit (per cent erythrocytes)	38.0–56.0	45.2
Hemoglobin (grams per ml. blood)	0.133–0.199	0.138
Erythrocytes (billions per ml. blood)	3.82–5.93	4.87
Carbonic Anhydrase (units per ml. blood)	1.2–2.6	1.8
Carbonic Anhydrase (units per ml. erythrocytes)	2.6–5.8	4.05
Carbonic Anhydrase (units per gram of hemoglobin)	8–17	13
Carbonic Anhydrase (units per billion erythrocytes)	0.25–0.56	0.37

Infection

Slight anemia was encountered in 10 patients in association with chronic febrile diseases; these included rheumatoid arthritis, rheumatic fever, pyelonephritis, pulmonary tuberculosis and ulcerative colitis. The carbonic anhydrase activity was slightly lowered but in every case lay within the range of normal in keeping with the mildness of the anemia (table 3, figs. 4 and 5); the presence of persistent diarrhea (Cases 30 and 31, Table 3) did not influence the findings.

Uremia

The anemia of uremia likewise was found to be accompanied by a decrease in blood carbonic anhydrase level; the diminution in activity of the enzyme in the blood paralleled the severity of the anemia so that the ratio between carbonic

TABLE 2.—*Observations in Patients with Pernicious Anemia*

Case	Age	Sex	Date	*Hematocrit* per cent erythrocytes	*Carbonic Anhydrase* units per ml. blood	*Carbonic Anhydrase* units per ml. erythrocytes
1	75	M	4-18-47	17.5	1.3	7.4
			4-30	30.3	1.8	5.9
			5-6	32.3	2.1	6.5
			5-15	34.1	2.3	6.8
2	70	F	4-24-47	27.2	1.6	5.9
			5-8	37.0	1.8	4.9
			5-28	35.1	1.6	4.6
3	59	F	5-21-47	41.8	2.3	5.5
4	66	M	8-5-47	15.0	1.9	12.7
			8-19	26.2	1.9	7.2
			8-27	31.5	2.4	7.6
			3-2-48	44.8	1.5	
			4-20-48	43.0	1.5	
5	56	F	11-17-47	43.0	1.4	3.3
6	40	F	12-16-47	22.0	1.1	5.0
			1-13-48	41.0	2.2	5.3
7	81	F	1-21-48	14.3	1.8	12.7
			1-27	14.7	1.8	12.2
			2-11	26.5	2.2	8.3
			4-16	41.0	1.3	
8	85	F	1-28-48	15.6	1.4	9.0
9	64	F	2-11-48	23.3	1.3	5.7
			2-18	26.5	1.4	5.3
10	82	M	7-7-48	21.0	2.2	10.6

anhydrase activity and the hematocrits, hemoglobin levels and erythrocyte counts remained normal (table 3, figs. 4 and 5). The severity of acidosis did not influence the findings.

Hepatic Disease

Of the eight patients with cirrhosis studied, four were anemic and the others not. In all of them but one the ratios of carbonic anhydrase activity to hematocrits,

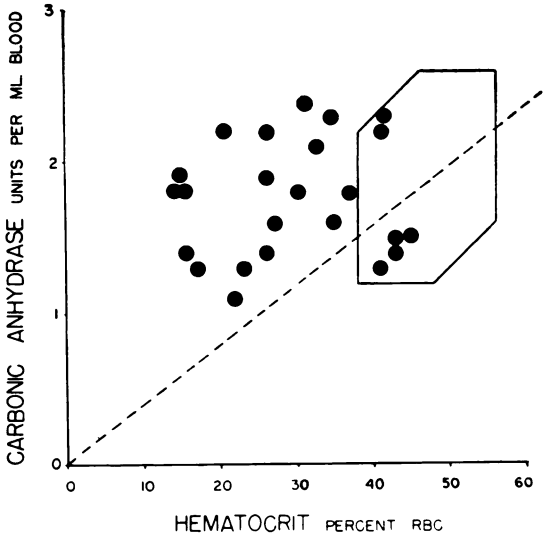

FIG. 1.—PERNICIOUS ANEMIA. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF WHOLE BLOOD AND HEMATOCRIT. The parallelogram indicates the normal range; the dotted line is drawn from the origin through the average normal value.

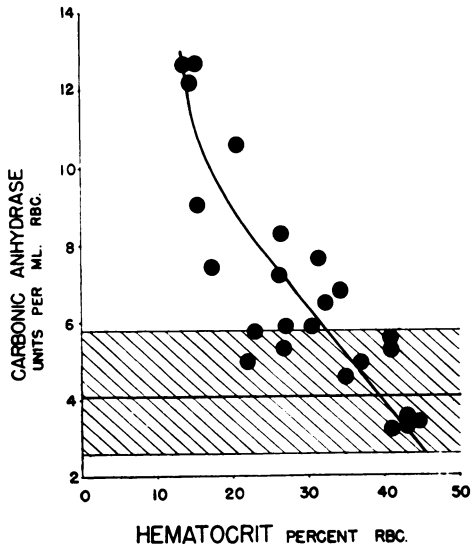

FIG. 2.—PERNICIOUS ANEMIA. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF ERYTHROCYTES AND HEMATOCRIT. The cross-hatched area is the normal range for carbonic anhydrase activity per ml. erythrocytes; the heavy line through it is the level of the normal mean value.

hemoglobin levels and erythrocyte counts were normal (table 3, fig. 6); the one exception (table 3, Case 42) had an excessive amount of the enzyme for the degree of anemia he presented and one of those with normal ratios (Case 37) was at the

TABLE 3.—*Observations in Patients with Various Conditions*

Case	Age	Sex	*Hematocrit* per cent erythrocytes	*Carbonic Anhydrase* units per ml. blood	*Carbonic Anhydrase* units per ml. erythrocytes	
11	52	M	19.0	0.88	4.6	Hemorrhage
			28.0	1.1	3.9	1 week later
12	66	M	11.8	0.46	3.9	Hemorrhage
13	72	M	38.0	1.7	4.5	Hemorrhage
14	26	F	33.0	1.0	3.0	Hemorrhage
			36.5	1.6	4.4	2 weeks later
15	67	M	36.2	1.5	4.1	Hemorrhage
16	89	F	30.0	1.2	4.0	Hemorrhage
17	53	M	15.1	0.7	4.7	Hemorrhage
18	43	M	29.5	1.5	5.1	Hemorrhage
19	54	F	41.0	1.5	3.8	Hemorrhage
20	78	M	39.5	1.3	3.3	Hemorrhage
21	75	M	25.5	1.3	5.1	Hemorrhage
22	56	F	26.5	1.2	4.5	Hemorrhage
23	28	F	38.0	1.4	3.7	Chronic Infection
24	32	M	37.0	1.9	5.1	Chronic Infection
25	57	M	36.9	1.3	3.5	Chronic Infection
26	69	F	42.0	1.3	3.1	Chronic Infection
27	45	F	39.2	1.3	3.3	Chronic Infection
28	62	F	39.0	1.8	4.6	Chronic Infection
29	70	F	35.0	1.4	4.0	Chronic Infection
30	47	F	32.5	1.6	4.9	Chronic Infection
31	35	F	32.0	1.2	3.7	Chronic Infection
32	31	F	38.0	1.4	3.7	Chronic Infection
33	55	M	23.0	1.0	4.3	Uremia

TABLE 3.—*Continued*

Case	Age	Sex	*Hematocrit* per cent erythrocytes	*Carbonic Anhydrase* units per ml. blood	*Carbonic Anhydrase* units per ml. erythrocytes	
34	65	M	39.0	1.3	3.2	Uremia
			20.5	0.81	3.9	5 weeks later
35	72	M	32.0	1.4	4.4	Uremia
36	50	M	34.0	1.2	3.5	Uremia
37	36	F	36.0	2.0	5.6	Portal Cirrhosis
38	45	F	44.7	1.3	2.9	Portal Cirrhosis
39	68	M	40.5	1.3	3.2	Portal Cirrhosis
40	55	M	38.5	1.5	4.0	Portal Cirrhosis
41	38	M	29.5	1.3	4.4	Portal Cirrhosis
42	66	M	33.5	2.0	6.0	Portal Cirrhosis, hemorrhage
			42.8	1.6	3.7	After 1500 blood I.V.
43	49	M	43.5	1.4	3.2	Biliary cirrhosis, severe icterus.
44	52	M	51.0	1.7	3.3	Hemachromatosis
45	67	M	40.3	1.3	3.5	Cancer of pancreas, severe icterus.
46	78	F	34.0	1.4	4.1	Cancer of liver, severe icterus.
47	68	M	20.4	0.70	3.4	Chronic myelogenous leukemia.
			24.2	1.0	4.1	After 3 weeks.
			23.0	1.1	4.8	After 6 weeks.
			27.0	2.0	7.4	After 7 months.
48	54	F	28.2	1.0	3.5	Chronic myelogenous leukemia.
49	49	F	26.7	1.2	4.5	Chronic meylogenous leukemia.
			35.4	1.7	4.8	After 1 week.
			31.8	1.7	5.3	After 3 weeks.
50	45	M	29.9	1.8	6.0	Chronic myelogenous leukemia.
51	48	F	35.1	2.3	6.5	Chronic myelogenous leukemia.
52	35	F	35.0	2.1	6.0	Chronic meylogenous leukemia.
53	47	M	31.0	1.5	4.8	Chronic myelogenous leukemia.
54	47	F	29.4	1.6	5.3	Chronic myelogenous leukemia.
			35.5	1.7	4.8	After 3 months.

TABLE 3.—*Continued*

Case	Age	Sex	*Hematocrit* per cent erythrocytes	*Carbonic Anhydrase* units per ml. blood	*Carbonic Anhydrase* units per ml. erythrocytes	
55	37	M	51.3	1.5	2.9	Acute meylogenous leukemia.
56	62	M	21.2	0.86	4.1	Chronic lymphatic leukemia.
			27.0	1.1	4.1	After 3 months.
57	61	M	35.0	1.1	3.1	Chronic lymphatic leukemia.
58	40	F	37.4	1.6	4.3	Lymphoma.
59	56	M	40.0	1.5	3.7	Lymphoma.
60	19	M	36.0	1.3	3.6	Lymphoma.
61	68	F	35.5	1.4	3.9	Hodgkin's Disease.
62	63	F	33.7	1.5	4.5	Multiple Myeloma.
63	54	F	26.2	0.9	3.4	Plasma cell leukemia.
64	57	F	30.0	1.8	6.0	Refractory anemia.
			30.5	1.9	6.2	After 3 months.
65	56	F	36.7	2.0	5.4	Refractory anemia.
66	55	F	27.2	2.1	7.7	Refractory anemia.
67	48	M	37.5	1.5	4.0	Refractory anemia.
68	63	M	17.9	1.2	6.4	Multiple deficiencies.
69	46	F	39.5	1.8	4.6	Scurvy.
70	58	F	36.6	1.5	4.1	Aplastic anemia.
71	54	F	31.6	1.1	3.5	Aplastic anemia.
72	22	F	28.0	1.8	6.4	Sickle cell anemia.
			34.0	1.5	4.4	After 1 month.
73	20	M	30.2	1.4	4.6	Sickle cell anemia.
74	24	F	35.0	1.7	4.9	Infectious mono-nucleosis.
75	30	F	36.0	1.7	4.7	Cooley's anemia.
76	55	M	22.0	1.1	5.0	Acute Hemolytic Anemia.
77	43	M	25.1	1.2	4.6	Paroxysmal Nocturnal Hemoglobinuria.

TABLE 3.—*Concluded*

Case	Age	Sex	*Hematocrit* per cent erythrocytes	*Carbonic Anhydrase* units per ml. blood	*Carbonic Anhydrase* units per ml. erythrocytes	
78	32	F	39.0	1.6	4.1	Familial Hemolytic Icterus.
79	34	F	36.0	1.7	4.7	Anorexia nervosa.
80	28	F	33.0	2.0	6.1	Diabetes mellitus, malnutrition.
81	56	M	69.2	2.7	3.9	Polycythemia Vera
82	60	M	69.2	3.1	4.5	Polycythemia Vera
83	80	M	56.5	1.8	3.2	Polycythemia Vera
84	55	F	60.0	2.1	3.5	Polycythemia Vera
85	55	F	67.5	2.6	3.9	Polycythemia Vera.

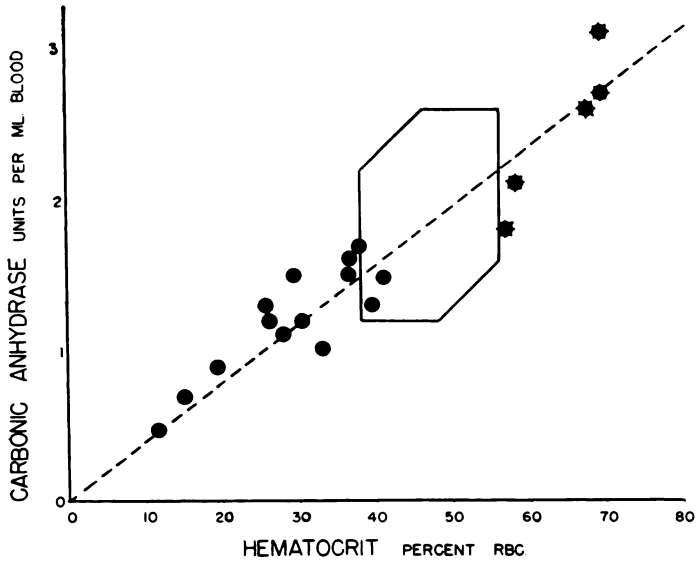

FIG. 3.—ANEMIA OF BLOOD LOSS; POLYCYTHEMIA VERA. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF WHOLE BLOOD AND HEMATOCRIT. The crenellated dots indicate polycythemia vera. The parallelogram indicates the normal range; the dotted line is drawn from the origin through the average normal value.

upper range of normal. The presence of icterus caused no deviation from the carbonic anhydrase activity expected on the basis of the hematological findings.

Leukemia and Allied Conditions

Anemia was present in all of the 17 patients studied, with the exception of one, who had acute myelogenous leukemia (table 3, Case 53). When anemia was present,

the blood carbonic anhydrase activity was reduced as a rule to or below the lower range of normal, the ratio between enzyme activity and hematocrits, hemoglobin levels and erythrocyte counts remaining normal (table 3, fig. 7). In 4 patients, however, (table 3, Cases 45, 48, 49, 50) who comprised half of the patients with

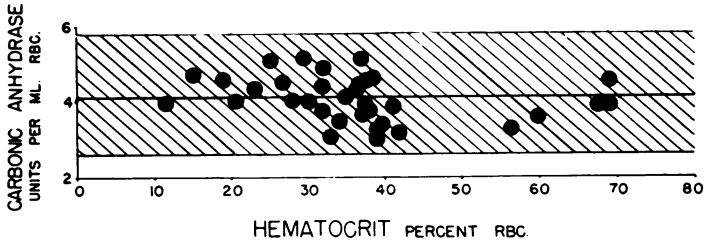

FIG. 4.—ANEMIAS OF BLOOD LOSS, INFECTION AND UREMIA; POLYCYTHEMIA VERA. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF ERYTHROCYTES AND HEMATOCRIT. The cross-hatched area is the normal range for carbonic anhydrase activity per ml. erythrocytes; the heavy line through it is the level of the normal mean value.

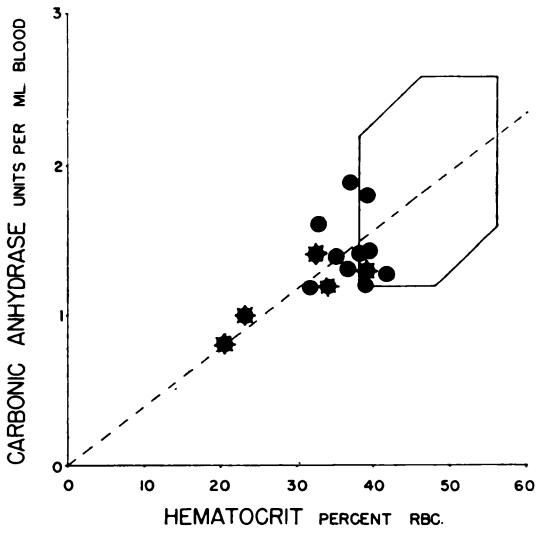

FIG. 5.—ANEMIAS OF INFECTION AND OF UREMIA. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF WHOLE BLOOD AND HEMATOCRIT. The crenellated dots indicate uremia. The parallelogram indicates the normal range; the dotted line is drawn from the origin through the average normal value.

chronic myelogenous leukemia, the blood carbonic anhydrase level was high in the normal range in spite of the presence of anemia, so that the ratio between enzyme activity and hematocrits, hemoglobin levels and erythrocyte counts was abnormally high.

Miscellaneous Anemias

Studies on instances of various uncommon types of anemia revealed, with a few exceptions, blood carbonic anhydrase levels in or below the lower normal range;

decreases in enzyme activity paralleled the severity of anemia so that the ratio between carbonic anhydrase level and the hematocrits, hemoglobin levels and

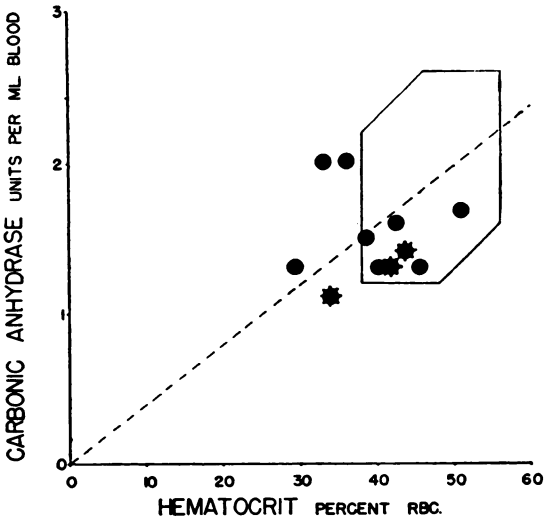

FIG. 6.—HEPATIC DISEASE. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF WHOLE BLOOD AND HEMATOCRIT. The crenellated dots indicate jaundice; the parallelogram indicates the normal range; the dotted line is drawn from the origin through the average normal value.

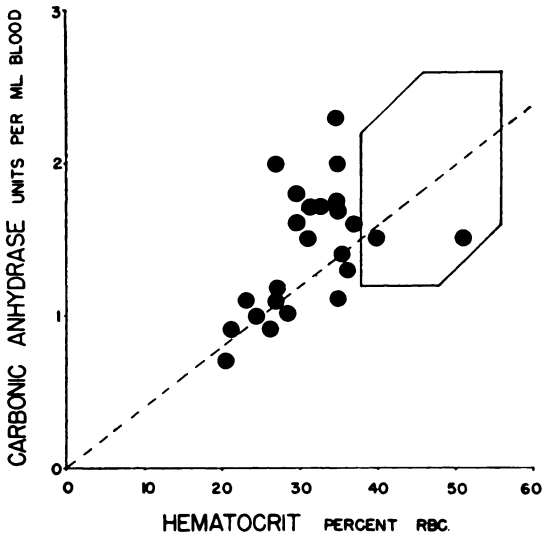

FIG. 7.—LEUKEMIA. RELATION BETWEEN CARBONIC ANHYDRASE ACTIVITY OF WHOLE BLOOD AND HEMATOCRIT. The parallelogram indicates the normal range; the dotted line is drawn from the origin through the average normal value.

erythrocyte counts were normal. Exceptions to this finding were encountered in 2 patients with dietary malnutrition (table 3, Case 77) and 2 of the 4 instances of

"refractory" anemia (table 3, Cases 62 and 64); the 2 other cases of refractory anemia (table 3, Cases 63 and 65) revealed ratios in the normal range.

Polycythemia Vera

Patients with polycythemia vera showed values for blood carbonic anhydrase activity high in or above the normal range, depending on the severity of the condition (table 3, fig. 3). The ratios between enzyme activity and the hematocrits, hemoglobin levels and erythrocyte counts were normal (fig. 4).

Discussion

The present study for the most part is in qualitative agreement with the earlier observations of Lambie[2,3] on anemia. Different types of anemia vary in regard to the relation between red cell mass and carbonic anhydrase activity of the blood. In the commonly encountered anemias consequent to loss of blood, infection and uremia and probably also in the less common aplastic and hemolytic anemias, a decrease in erythrocytes signifies not only a parallel loss of hemoglobin, but also a corresponding diminution in carbonic anhydrase activity of the blood. The same condition also obtains in most patients with anemia associated with hepatic disease and with leukemia and allied conditions. On the other hand, in patients with pernicious anemia and in some instances of "refractory" anemia, of hepatic disease and of myelogenous leukemia, the blood carbonic anhydrase activity remains in or only slightly below the normal range in spite of marked decreases in hematocrit, hemoglobin level and erythrocyte count. Patients with pernicious anemia have extremely high blood carbonic anhydrase activity relative to erythrocyte count and exhibit levels of enzyme activity which may be several times as high as that shown by patients with comparable hemoglobin or hematocrit levels associated with anemia of blood loss, infection or uremia. The reason for this difference is not known. In contradistinction to earlier workers[1–3] no evidence was found to support the concept that icterus increases blood carbonic anhydrase activity.

The precise significance of the findings of the present study cannot be stated in the absence of complete information as to the physiological function of the blood carbonic anhydrase. Theoretical considerations indicate that its property of accelerating the reaction $H_2O + CO_2 \rightleftarrows HCO_3$ is essential for the prevention of accumulation of carbon dioxide in the body. It appears, therefore, that under the conditions of accelerated blood flow through the tissues and lungs which obtain in anemia,[6] the need for carbonic anhydrase is greater than normal.

There is no experimental evidence available at present which proves that loss of blood carbonic anhydrase activity definitely causes dyspnea. Sulfanilamide inhibits carbonic anhydrase and therefore observations on sulfanilamide intoxication are pertinent to the problem of dyspnea. The clinical observation that the administration of sulfanilamide causes increased respiratory activity[7–9] and intolerance to exercise[10] and to inhalation of carbon dioxide,[11] is difficult to interpret; in clinical conditions of sulfonamide intoxication not only is the activity of the carbonic anhydrase of the blood depressed, but that of the renal tubular carbonic anhydrase probably is also, with the consequent development of acidosis

due to loss of base. Changes in blood and urinary chemistry over a period of time after administration of sulfanilamide are so complicated as to suggest the effects of the action of several factors.[7–9] The work of Wood and Favour[9] showed, however, that the injection of sulfanilamide intravenously rapidly causes inhibition of the enzyme in the blood and that immediately thereafter lowering of the arterial blood carbon dioxide content occurs. This observation suggests that decreased carbonic anhydrase activity in the blood may cause or contribute to dyspnea through impaired removal of carbon dioxide from the tissues; accumulation of carbon dioxide in the brain causes stimulation of respiration with consequent hyperventilation and immediate lowering of arterial blood carbon dioxide tension. Apparently accumulation of carbon dioxide in the blood through retardation of its excretion in the lungs is not a factor. The fall in arterial blood carbon dioxide level which occurs as a consequence of inhibition of carbonic anhydrase activity by administration of sulfanilamide resembles the decrease in blood carbon dioxide usually found in patients with anemia[6]; however, in these patients additional factors, such as anoxia and also impaired heat dispersal consequent to cutaneous vasoconstriction, also cause hyperventilation. Data now available do not permit distinction between hyperventilation possibly due to lack of carbonic anhydrase and that consequent to other factors in patients with anemia.

The numerous and complex cardiovascular and respiratory compensations in anemia have been discussed elsewhere.[6] The importance of erythrocytes in carbon dioxide transport is established. Although the red blood cells hold less carbon dioxide than plasma, they take up approximately 40 per cent of the carbon dioxide added to the blood as it circulates through the tissues. The mechanisms whereby erythrocytes are able to hold so much carbon dioxide at a pH of 7.1 in competition with plasma whose pH is 7.4 have not been delineated completely. Several factors have been studied. Hemoglobin is a buffer; change in its acidity when it is reduced accounts for an important part of the carbon dioxide carrying power of erythrocytes; carbonic anhydrase is important in this phenomenon, for without the enzyme bicarbonate cannot enter the red cells in a normal fashion.[12, 13] Similarly the chloride shift cannot occur at a normal speed in the absence of adequate amounts of active carbonic anhydrase in erythrocytes.[13–15] Another possible factor, not completely studied, is the transport of carbon dioxide in the form of carbamates in combination with hemoglobin and possibly other substances; it is probable that still other factors also operate. Whether deficiency of blood carbonic anhydrase interferes with carbon dioxide transport solely through impairment of mechanisms involving hemoglobin or whether other factors also play a part is not known; the lack of complete knowledge as to the precise function of carbonic anhydrase in blood gas transport makes it impossible at the present time to examine critically the role of the enzyme in the production or prevention of symptoms.

Although hemoglobin is essential for oxygen transport and very important in carbon dioxide transport, the fact remains that patients with pernicious anemia have long been known to tolerate exertion without the development of severe dyspnea even when the blood hemoglobin level is as low as that which is associated with dyspnea in patients with some other chronic anemias, such as those consequent to slow loss of blood. This fact indicates the importance of factors

other than hemoglobin level in the genesis of the dyspnea of anemia and suggests that the observed differences in blood carbonic anhydrase activity might be significant in this regard.

In polycythemia vera, the increases in blood carbonic anhydrase activity which parallel increases in hematocrit apparently have no vital importance in the altered cardiorespiratory function which occurs in this disease.

Summary and Conclusions

Measurements of blood carbonic anhydrase activity were made in patients with a variety of blood dyscrasias, using a new method. In patients with anemia due to loss of blood, infection and uremia, and in most of those with anemia associated with liver disease and leukemia, a constant relation was found between blood carbonic anhydrase activity and the hematocrit; the same holds in polycythemia vera. In patients with pernicious anemia, and in some with "refractory" anemia, and anemias associated with hepatic disease and with myelogenous leukemia, blood carbonic anhydrase activity was in or near the normal range in spite of lowered hematocrit values. The possible relation between these differences among anemias and the tolerance of patients with various anemias to exercise is discussed.

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