CARBONIC ANHYDRASE IN NEWBORN INFANTS [1]

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Carbonic anhydrase was described first in 1932 (1) as an enzyme which accelerates the reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$. Meldrum and Roughton (2) found that goat fetuses were low in carbonic anhydrase and stated, "In the very young fetuses, there is extraordinarily little enzyme and the amount does not begin to rise appreciably until very near the end of term." It was shown, further, that the enzyme is present with hemoglobin in the red blood cells (3) but is separable from these and gives the reactions of a zinc protein (4). The enzyme is inhibited by serum (5).

It has been our impression for some time that premature and full-term newborn infants who are not thriving—*e.g.*, showing poor color, feeble respirations, little or no gain in weight, low vitality—are benefited by transfusions of whole blood from a compatible donor. With the above findings in mind, determinations of carbonic anhydrase were done on newborn infants in order to determine whether infants show a significant deficiency of carbonic anhydrase which can be restored by blood transfusions.

METHOD

The glass boat method, described by Meldrum and Roughton (2), was used. Two cc. of 0.2 M phosphate buffer (pH 6.8) were shaken vigorously with 2.0 cc. of 0.2 M sodium bicarbonate in an air tight chamber connected to a water manometer. The velocity of carbon dioxide evolution was noted. With an identical set-up, a small amount of blood was added and the increment in the velocity of the evolution of carbon dioxide was utilized as a measure of the carbonic anhydrase present in the blood. Enzyme unitage was calculated as described by these authors, with the exception that the present experiments were run at 0° C. and no correction for temperature has been made. Throughout the present work, the same machine has been used.

While for some purposes, the changes in the concentration of the blood cells would have to be taken into consideration, the changes in the enzyme concentrations in the present observations are of such magnitude as to permit one to disregard this factor. Furthermore, in the early work, we determined the number of red cells, the concentration of hemoglobin, and the volume of red cells by hematocrit, at the same time as carbonic anhydrase determinations and found no marked correlation.

Blood was obtained by an incision of the toe or heel, adequate to insure a free flow without recourse to squeezing the tissues. It was collected in a 0.1 cc. capillary pipette and mixed immediately with 20 cc. of distilled water with a resulting 1 : 200 dilution. Five-tenths cc. of this dilution was added to the bicarbonate solution when running a determination.

RESULTS

Table I gives average blood levels of carbonic anhydrase for 4 categories of normal individuals. The adults were healthy medical students and nurses. The premature infants, weighing less than 2500 grams, were in the first week of life and were progressing satisfactorily. The newborn infants were selected at random from the nursery and are representative of children during the first 7 days of life. It will be seen that newborn infants possess less than half the adult concentration of carbonic anhydrase in their blood and that premature infants possess about one quarter of the adult value. These differences are statistically significant.

TABLE I

Normal blood levels of carbonic anhydrase

Classification	Number of cases	E per c. mm.*	Standard deviation
Adult males	11	3.51	0.360
Adult females	17	3.28	0.339
Prematures	39	0.79	0.470
Normal newborns †	56	1.41	0.371

* In all tables, "E per c. mm." denotes units of carbonic anhydrase per cubic millimeter of blood.

† Includes first 7 days of life. Standard deviation of averages for each of these days: 0.194.

Table II demonstrates that large, spontaneous changes in the level of enzyme in the blood do not usually occur within a period of several days.

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TABLE II

Constancy of blood level of carbonic anhydrase over a period of days

(*a*) *Levels obtained on newborn infants*

E per c. mm.	Interval	E per c. mm.
1.20	4 days	1.02
1.30	11 days	1.14
0.40	11 days	0.45
0.10	11 days	0.00
0.76	10 days	0.00
1.87	10 days	1.50
1.70	3 days	1.82
0.53	3 days	0.55

(*b*) *Levels obtained on premature infants*

Initial value	Intervals in days after initial reading with enzyme value
E per c. mm.	
0.28	3 days: 0.27— 5 days: 0.29
0.00	1 day: 0.00
0.40	23 days: 0.45—33 days: 0.63
0.10	11 days: 0.00
0.50	6 days: 0.45
0.75	39 days: 0.85
0.33	28 days: 0.55—45 days: 0.37
0.53	1 day: 0.50— 3 days: 0.54
0.40	1 day: 0.40
0.40	18 days: 0.51—20 days: 0.43—27 days: 0.48
0.31	20 days: 0.76
0.54	20 days: 0.43
0.93	7 days: 1.17
0.76	7 days: 0.89
0.41	7 days: 0.50
0.58	9 days: 0.51

The small variations probably represent errors inherent in the sampling and the method. The only full-term infant showing a gross change (from 0.76 to 0.00 E per c. mm.) was carefully checked and the second value was confirmed. This inexplicably low finding in an infant who was clinically quite well at the time is at variance with the data to be given below. In the case of the only premature infant showing a gross change (from 0.31 to 0.76 E per c. mm.), a period of 20 days elapsed between the 2 enzyme determinations.

TABLE III

Rise in blood level of carbonic anhydrase following transfusion (20 cc. per kilogram)

Before blood	Interval	After blood
E per c. mm.		*E per c. mm.*
0.00	4 days	1.0
0.82	9 days	1.37
0.55	1 day	0.92
0.85	2 days	1.77
0.66	5 days	1.67
0.43	10 days	0.74
0.27	1 day	1.52
0.40	1 day	1.92 (2 transfusions)
0.37	1 day	2.35
0.51	2 days	1.60
1.02	1 day	1.60
0.63	2 days	1.92
0.15	2 days	2.05 (2 transfusions)
0.23	1 day	1.02
0.34	1 day	0.93

Table III demonstrates that transfusions of adult blood raise the level of carbonic anhydrase when the initial value is low. The data were obtained from both well and sick newborn infants. The blood used for transfusion was either drawn freshly or taken from the hospital blood bank, but the blood was never more than 1 week old. Twenty cc. per kilogram were given intravenously by the indirect, citrate method. While there is no precise correlation, it will be seen that the increase approximates the expected value when it is remembered that the average adult blood level of enzyme is 3.39 E per c. mm. and that 20 cc. per kilogram of adult blood are added to an infant blood volume of approximately 60 cc. per kilogram.

Table IV presents data which show that plasma infusions do not raise the carbonic anhydrase level of the recipient's blood. Such a rise is not to be expected, since it will be recalled that the enzyme is present within the red blood cells (3) and is inhibited by serum (5).

TABLE IV

No rise in blood level of carbonic anhydrase following plasma infusion

Before plasma	Interval	After plasma
E per c. mm.		*E per c. mm.*
0.76	7 days	0.89
0.00	1 day	0.00
0.56	5 days	0.37

The plasma was a pooled mixture of plasma from several bloods, obtained from the blood bank, and was given in amounts of 20 cc. per kilogram of body weight. The age of the plasma presumably should make no difference in the results, since we have observed that whole blood, on standing, loses no measurable amount of its carbonic anhydrase content over a period of several days, and Lambie (6) has shown that laked blood may be stored for 5 days without loss in its carbonic anhydrase potency.

Table V shows that the rise in carbonic anhydrase content of the recipient's blood is not a transitory phenomenon and probably is sustained as long as the donor's red blood cells remain in-

TABLE V

Sustained rise in blood level of carbonic anhydrase following transfusion

Initial value	Intervals after transfusion with enzyme value
E per c. mm.	*E per c. mm.*
0.00	2 days: 1.00— 3 days: 1.40
0.40	1 day : 1.92—10 days: 1.52
0.37	1 day: 2.35— 3 days: 1.80—16 days: 1.43
0.85	2 days: 1.77— 9 days: 1.77

tact in their new environment. Not shown in the table is the only patient whose enzyme level, raised by 2 blood transfusions from 0.12 E per c. mm. to 2.05 E per c. mm., declined to 0.43 E per c. mm. within the next 2 days. This fall was coextant with an increase in cyanosis and dyspnea and the development of jaundice. The bady died just after the last enzyme determination and autopsy showed extensive kernikterus. Unfortunately, the blood typing and cross-matching were not checked but it was felt that this infant suffered from a transfusion reaction, with hemolysis of the recipient's cells and resultant loss of carbonic anhydrase complement.

Table VI shows normal carbonic anhydrase levels obtained from infants exhibiting cyanosis. In each case, the cyanosis is adequately explained by an accompanying morbid condition. Since it will be shown below that certain types of cyanosis of the newborn are associated with a low blood level of carbonic anhydrase, it seems advisable to call attention to the fact that Table VI shows that cyanosis, *per se,* does not lower the enzyme value. This is in agreement with Hodgson (7) who found increased enzyme concentration in cyanotic adults whose blood showed high hematocrit readings (congenital heart disease, bronchitis, cardiac failure).

Table VII shows that morphine is without effect on the enzyme potency of the patient's blood. It was thought desirable to demonstrate this, since the mothers of some of the babies discussed below had been given morphine at variable intervals before delivery. Furthermore, it was found that morphine sulfate added to blood *in vitro,* in concentrations equal to a 10 grain dose given by hypodermic injection, did not significantly reduce the enzyme activity of the blood.

TABLE VII

Failure of morphine to inhibit carbonic anhydrase

Before	After
E per c. mm.	*E per c. mm.*
3.57	3.57
3.47	3.26
3.26	3.52
3.00	3.26

Carbonic anhydrase determinations on 4 adults before and one-half hour after gr. ¼ of morphine sulfate.

Table VIII presents certain clinical data in connection with the studies on carbonic anhydrase. The cases were selected because they showed cyanosis and were doing poorly at a time when no recognized explanations for these findings were discovered. Two of the infants were full-term while 11 were premature. Attention is directed to the fact that when cyanosis and failure to thrive was found unassociated with recognized cause, transfusion of adult blood led to clinical improvement. Usually the initial level of the enzyme was below 0.5 E per c. mm. but this was not invariably

TABLE VI

Cyanosis from known cause, accompanied by normal blood levels of carbonic anhydrase

Weight		Clinical note
grams	*E per c. mm.*	
9225	2.65	Markedly cyanotic; diagnosis: congenital heart disease.
3200	1.67	Markedly cyanotic; difficult resuscitation; bulging fontanel; bloody cerebrospinal fluid; died after 3 days; no autopsy; diagnosis: probable intracranial hemorrhage.
3600	1.67	Severe cyanotic spells; diagnosis: low calcium tetany (blood calcium: 6.4 mgm. per cent).
1655	1.02	Markedly cyanotic; died after 2 days; autopsy: tracheo-esophageal fistula.
1600	1.43	Markedly cyanotic; died after 1 day; autopsy: intracranial and intraperitoneal hemorrhage.

TABLE VIII

Infants with unexplained cyanosis: their subsequent course

Weight	1st day of life	Clinical note
grams	*E per c. mm.*	
1. 2935	0.73	Very cyanotic; died in 4 hours; no cause apparent; no autopsy.
2. 2770	0.85	Seemed well but persistently cyanotic without demonstrable cause; at 2 days, transfused, E: 1.77; cyanosis cleared within 24 hours of transfusion; at 5 days, E: 1.77; continued to do well.
3. 1040	0.38	Very cyanotic and feeble; died in 2 hours; complete autopsy showed only minimal atelectasis.
4. 2490	0.26	Cesarean section; mother had gr. ¼ morphine 1 hour before delivery; infant feeble, cyanotic; no apparent cause; died in 3 hours; no autopsy.
5. 2330	0.28	Cyanotic for first 3 days of life without apparent cause; enzyme level unchanged at the end of this period (E: 0.27); the following day, color good and E: 0.43 (infant had not been transfused); continued to thrive.
6. 1110	0.00	Cyanotic and feeble without apparent cause for 3 days; after plasma infusion, no improvement and E: 0.00; on fourth day of life, transfused; immediate disappearance of cyanosis and E: 1.0; did well thereafter.
7. 1030	0.27	Very cyanotic and seemed moribund; transfused at 2 days and seemed better immediately with E: 1.52; died suddenly next day; autopsy: subtentorial hemorrhage.
8. 1300	0.40	Color only fair; eating and gaining poorly; transfused with immediate improvement in appetite, color, and weight gain which was sustained thereafter; E: 1.92.
9. 1270	0.12	Cyanotic, sucking poorly, minimal atelectasis; after 2 transfusions, E: 2.05 but baby did poorly, became increasingly cyanotic and died after 2 days, with E: 0.43 just before death; autopsy: marked kernikterus; question of transfusion reaction.
10. 745	0.23	Very feeble, weak cry, poor respirations, cyanotic even in oxygen; after 1 day and transfusion, E: 0.93; immediate sustained improvement in general status; after 4 days, E: 1.18; after 11 days, E: 1.17; continued to thrive.
11. 905	0.34	Very low vitality, weak cry, cyanotic, apneic periods; after 1 day and transfusion, E: 1.02; seemed much stronger, sucked well, color better, more vigorous cry; after 4 days, doing well, out of oxygen, E: 1.07; continued to thrive.
12. 1405	0.43	Severe spells of cyanosis, poor appetite, low vitality; immediate, sustained improvement after transfusion and E: 0.74.
13. 1775	0.82	Seemed well but persistently cyanotic until transfused on fifth day; immediate disappearance of cyanosis; E: 1.37; continued improvement thereafter.

the case. In the 2 full-term infants (cases 1 and 2), although the levels are almost 2 standard deviations below the average newborn level, the initial values are not low by premature standards. One of these patients died without a transfusion or autopsy so no conclusion can be made except that the enzyme was somewhat low. In the other case, transfusion led to striking and prompt clinical improvement and increase in the level of enzyme.

Of the premature infants, 2 (cases 3 and 4) died before transfusions could be given. In one case, autopsy failed to reveal adequate anatomical cause for cyanosis while in the other case, autopsy was not permitted. In this case, morphine poisoning was suspected but, if present, it cannot explain the low level of carbonic anhydrase since it has been shown that morphine is without measurable effect on the activity of carbonic anhydrase (Table VII). In the case of number 5, the cyanosis disappeared at the same time that a spontaneous rise in the blood level of the enzyme developed. The case probably would not have been included here except for the fact that it was one of the group

of newborn infants who exhibited unexplained cyanosis. Even though the increase in carbonic anhydrase is minimal, the correlation is suggestive. In spite of a normal enzyme level, number 13 continued to exhibit cyanosis until transfusion almost doubled the carbonic anhydrase value. The remaining infants all showed a great clinical improvement after their low levels of enzyme had been raised by transfusion of whole blood. Following the transfusion, number 7 improved from a moribund condition to such an extent that recovery was anticipated. The sudden death, due to subtentorial hemorrhage, is felt to represent an additional complication. Number 9 has been commented upon previously (Table V); it is felt that the failure to sustain the rise in enzyme concentration and the poor course following transfusion, when considered with the autopsy finding of kernikterus, suggest a transfusion reaction with hemolysis of the donor's red cells. Although Rh studies were not carried out in this case, it was felt that erythroblastosis fetalis could be excluded by the absence of clinical or pathological evidences of this disease.

While the transfusions undoubtedly had other effects than raising the level of the carbonic anhydrase, none of the usual indications for transfusion was present. None of the infants was dehydrated or exhibited good evidences of shock. There were no clinical evidences of anemia and the hemoglobins were demonstrated to be over 15 grams in those cases on which initial values were estimated. No reactions occurred except as noted in case 9.

In Figure 1, the levels of carbonic anhydrase are plotted against birth weight. The babies showing unexplained cyanosis are designated by crosses and those with cyanosis from recognized causes are marked by circles. It will be seen that low levels of carbonic anhydrase are found in newborn infants of all weights including occasional full-term infants (not shown in the Figure). However, none of the 10 infants weighing less than 1500 grams at birth showed levels of carbonic anhydrase greater than 0.5 units. Thus, a certain degree of maturity is necessary for the development of high levels of carbonic anhydrase, but occasionally, the enzyme fails to develop in otherwise mature, newborn infants.

The figure also shows that, with one exception, the instances of unexplained cyanosis occur in infants with levels of carbonic anhydrase below 0.5

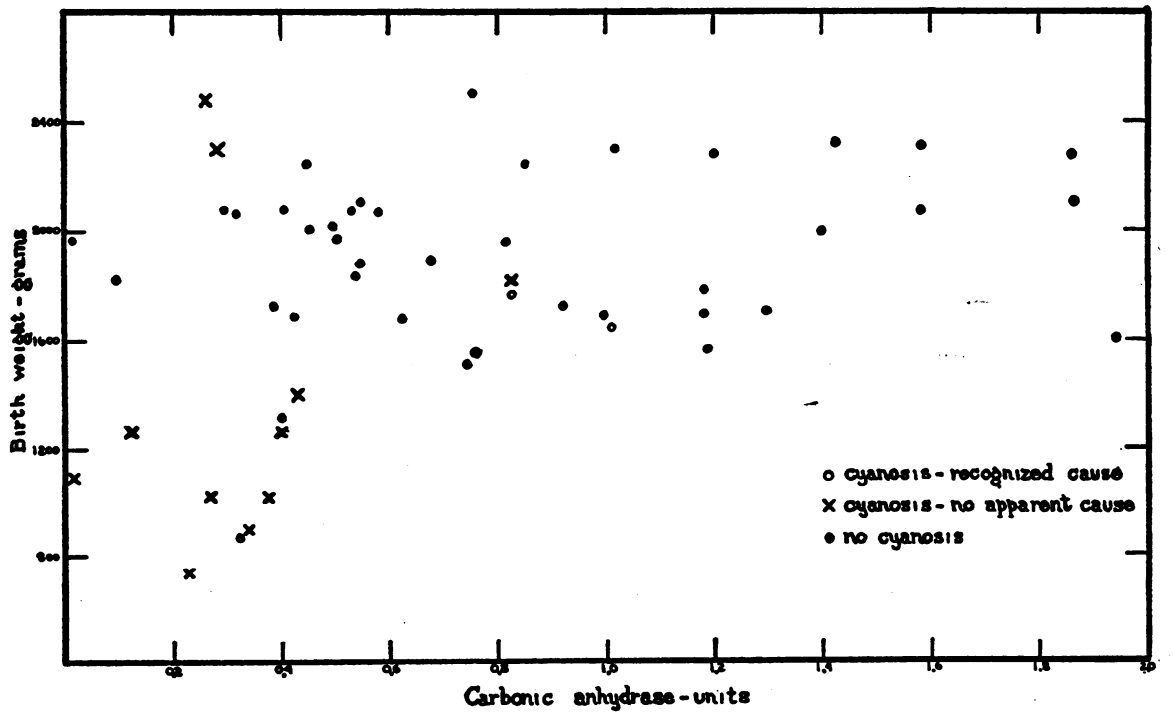

FIG. 1. THE RELATION BETWEEN BIRTH WEIGHT AND ENZYME LEVEL IN THE BLOOD

units. However, as was pointed out in connection with Table VIII, 2 full-term infants showed unexplained cyanosis with levels of carbonic anhydrase of about 0.8 units. Of the 21 infants with levels below 0.5 units, 10 showed cyanosis. Of these 10, with unexplained cyanosis, 8 weighed less than 1600 grams.

Since the Figure shows very low levels in thriving infants, low levels of carbonic anhydrase do not directly lead to cyanosis nor necessarily interfere with apparent well-being.

DISCUSSION

Although no precise relationship between birth weight and the level of carbonic anhydrase can be demonstrated, small premature infants (1500 grams or less) tend to show the lowest levels of enzyme. It will be recalled that Meldrum and Roughton (2) demonstrated that the level of enzyme in goat fetuses does not begin to rise until very near term. For this reason the level of carbonic anhydrase may be considered one of the measures of maturity in the newborn. Hall (8) has suggested that there exists a fetal form of hemoglobin, with a greater than normal affinity for oxygen, which is adapted to intrauterine existence with its low oxygen tension. It is possible that the low level of carbonic anhydrase in premature infants is a characteristic of this fetal hemoglobin.

Roughton (9) has demonstrated mathematically that conversion of bicarbonate to carbon dioxide without carbonic anhydrase would be so slow as to be the limiting factor in respiratory exchange. However, he has also shown that the levels of enzyme in adults accelerate the reaction 100 times as much as is necessary, but this margin of safety decreases when maximum activity is undertaken. Lambie (6) has shown that the blood level of the enzyme can be reduced to 22 per cent of the normal without demonstrable ill effects in adults. Since there is this margin of safety and newborn infants are relatively inactive, the level of carbonic anhydrase in premature and full-term newborn infants is theoretically and practically adequate. This does not mean that difficulties due to low carbonic anhydrase may not develop in active babies. Indeed, the frequency of cyanotic spells in premature infants after feeding and exertion or during infections suggests that such is the case. However, it must be recalled that one baby showed no symptoms in spite of a blood carbonic anhydrase level of 0.00. While measurements of pH and carbon dioxide tension would be necessary to prove that there were no physiological disturbances due to the low carbonic anhydrase, the observation proves that very low levels are compatible with life in a premature infant.

There are theoretical reasons why a low blood level of carbonic anhydrase might interfere with oxygenation of the blood. Oxygenation of hemoglobin is physiologically speeded when the blood gives up carbon dioxide in the lungs (10). Also, the hemoglobin dissociation curve is shifted to the left in proportion to the lowering of carbon dioxide tension. It is possible that a low blood level of carbonic anhydrase delays the lowering of the carbon dioxide tension in the lung capillaries, resulting in a slow and incomplete oxygenation of hemoglobin. In this connection, it is interesting to note that Smith and Kaplan (11) have shown that the arterial blood of premature infants generally possesses a lower oxygen saturation than that of adults or full-term infants of comparable age.

It is well known that patients being treated with sulfanilamide are prone to develop cyanosis and it is interesting to note that sulfanilamide (alone, of all the sulfonamides) inhibits the action of carbonic anhydrase (12, 13). We realize that this neglects the observations that the cyanosis of sulfanilamide therapy may be due to methemoglobin (14, 15).

The poor appetite of many of the infants with low carbonic anhydrase quite possibly may be related to a lessened secretion of gastric juice. Davenport (16) has shown that the adequate function of the parietal cells of the gastric mucosa is dependent on the presence of carbonic anhydrase and it has been suggested previously (13) that the anorexia accompanying sulfanilamide therapy may be related to inhibition of the enzyme.

SUMMARY AND CONCLUSIONS

Because Meldrum and Roughton (2) had found goat fetuses low in carbonic anhydrase, because carbonic anhydrase is found with hemoglobin in the red blood cells (3), because of a clinical impression that some premature and full-term new-

born infants, who were doing poorly and who were exhibiting cyanosis, improved after whole blood transfusions, an attempt was made to correlate unexplained cyanosis in newborn infants with low concentrations of carbonic anhydrase in the blood.

The concentration of carbonic anhydrase in the blood of newborn infants is less than one-half and the concentration in premature infants is only one quarter of that found in the blood of adults. Spontaneous changes in the blood enzyme level do not usually occur but significant increase in the level can be accomplished by whole blood transfusions.

Thirteen infants were studied who exhibited cyanosis, unexplained by recognized physical causes, and who were doing poorly. In many instances, these infants showed levels of carbonic anhydrase which were significantly low. Improvement in respect to cyanosis and general condition was accompanied by a rise in the blood concentration of carbonic anhydrase. The rise occurred in one infant spontaneously and was minimal. In the remaining infants, the rise followed transfusion and was significant in most instances.

The findings show that many premature infants who are doing poorly and exhibit cyanosis have low levels of carbonic anhydrase. Following transfusions of adult blood, the level of carbonic anhydrase increases and clinical improvement, accompanied by a disappearance of cyanosis, follows.

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