Isolation and Properties of Carbonic Anhydrase from Dog Kidney and Erythrocytes

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SUMMARY

DEAE-cellulose chromatography of dog red cell carbonic anhydrase (CA) yielded a single main fraction, whereas under identical conditions human CA showed two distinct activity peaks corresponding with types B and C. When subjected to zone electrophoresis at pH 9.0 or 6.0, the main dog CA fraction moved as a single band with the same mobility as human CA type B. Its K_m was found to be approximately 30 mM CO_2 at 0°, 50% inhibitory concentrations for acetazolamide and sulfanilamide were similar to those of human CA type C. Structurally the enzyme appeared to be similar to other mammalian CA varieties. Preliminary experiments with CA obtained from the cytoplasmic fraction of dog kidney showed this enzyme to be similar to the red cell enzyme with respect to electrophoretic mobility and inhibition kinetics.

INTRODUCTION

At present human red cell carbonic anhydrase (CA) has been investigated fairly extensively, and various authors (1–5) have reported on the existence of two or three isoenzymes which differ to some degree in structure, kinetics, and response to inhibition by sulfonamides.

Investigation of carbonic anhydrase from other species, however, has lagged behind, and in view of the large body of information available on the physiology, kinetics, and inhibition of dog red cell CA (5), it seemed important to isolate and purify the enzyme in order to compare its properties with that of the human enzymes. Moreover, in the dog it is possible to compare the CA from erythrocytes with that from other organs, for example, the kidney.

METHODS

Carbonic anhydrase activity and inhibition was determined by the changing pH method of Philpot and Philpot (6) according to Maren *et al.* (7).

DEAE-cellulose columns were prepared from coarse DEAE (diethylaminoethylcellulose, Sigma Chemical Company). The resin was first washed with 1 N NaOH, followed by 0.5 N HCl and H_2O on a Büchner funnel. Suspensions of the resin were poured into glass columns and were packed under a pressure of 2–4 psi. The columns were equilibrated against 0.01 M Tris buffer pH 8.0 (8), and the solutions to be chromatographed were dialyzed extensively against the same buffer. Elution was generally carried out by means of a linear or slightly concave gradient using a multichamber mixing device (Varigrad, Technicon).

Absorption at 280 mμ was measured in a Beckman model DU spectrophotometer.

Gel electrophoresis was carried out in a vertical gel electrophoresis apparatus (E-C Apparatus Corporation). After completion of the runs the proteins were stained with amidoblack and the gels were destained with a special electrophoretic destainer (E-C Apparatus Corporation). Acrylamide gel electrophoresis was generally carried out in 0.01 M Tris buffer pH 9.0 and 8–10% cyanogum gel for 4–5 hr at approximately

10 volts cm^{-1}. Agar gel electrophoresis was done in 1% agar and 0.03 M sodium citrate–citric acid buffer, pH 6.0.

Preparation of hemolyzates. Venous dog blood was obtained with heparinized syringes and spun down at 2000 *g* for 30 min. The erythrocytes were washed 4–5 times with isotonic saline and dialyzed against distilled water for 2 × 24 hr after the addition of one volume of distilled water. The resulting hemolyzate was centrifuged in a Spinco Model L preparative ultracentrifuge at 100,000 *g* for 1 hr. All procedures were carried out at about 4°.

Preparation of crude carbonic anhydrase from hemolyzates. (1) Chloroform-ethanol treatment: To the hemolyzate were added 0.8 volume 40% ethanol and 0.4 volume chloroform under constant stirring. After incubation of the mixture for approximately 1 hr at 4°, the precipitated hemoglobin was centrifuged down at 2000 *g* for 30 min. The pink supernatant was dialyzed against distilled water and concentrated by means of ultrafiltration.

(2) DEAE chromatography: The lysate was charged to a 4 × 50 cm column under the conditions described above, and eluted with 1.5–2.0 liters of a gradient from 0.01 to 0.1 M Tris buffer pH 8.0. Flow rates were in general approximately 0.5 ml/min. Fractions of 5–8 ml were collected, and their CA activity as well as their absorbance at 280 mμ were measured. A single peak was obtained, which was eluted just prior to the hemoglobin. In general the CA and hemoglobin peaks overlapped slightly, giving rise to a pink color in the last CA fractions.

In a number of the chromatograms some CA was eluted in the region of the hemoglobin peak, giving rise to a second front of CA activity. As the activity of pure CA was found to be 2–4 times higher if determined in the presence of hemoglobin (see below), the amount of enzyme protein present in the hemoglobin front must have been comparatively small. If the second CA peak was subjected to gel electrophoresis, it appeared to contain two bands with positions similar to CA and hemoglobin, respectively.

Chromatography of crude carbonic anhydrase on DEAE-cellulose. The fractions obtained from the above described crude separations were pooled and concentrated by ultrafiltration to a volume of approximately 50 ml. The concentrate was dialyzed against 0.01 M Tris buffer and charged on to a 2 × 40 cm DEAE-cellulose column as described above. After washing through 100–200 ml 0.01 M Tris buffer, pH 8.0, the column was eluted with a linear gradient of 0.01–0.1 M Tris buffer, pH 8.0, with a total volume of 0.6–1.5 liters.

Zinc analyses. The zinc content of a purified sample of dog erythrocyte CA (main chromatographic fraction) was determined with dithizone according to Malmström (9). The volumes recommended were reduced, however, and the extractions were carried out in test tubes with the aid of a Vortex mixer, instead of in separatory funnels. A number of dilutions were made from a stock solution containing 1 mg CA per milliliter and their zinc contents were determined in triplicate.

Amino acid analyses. Purified samples of dog erythrocyte CA were hydrolyzed in 6 N HCl at 110° for 18 hr in evacuated tubes. The hydrolyzates were taken to dryness by means of vacuum distillation and dissolved in 0.01 N HCl. Chromatography was carried out on a Technicon amino acid analyzer. The tryptophan content was determined according to the method of Spies and Chambers (10).

Ultracentrifugation experiments. Purified samples of dog erythrocyte CA were dissolved in and dialyzed against 0.1 M sodium phosphate buffer pH 6.8, and subjected to sedimentation analyses in a Spinco Model E analytical ultracentrifuge at 56,100 rpm and 20.0°. Diffusion coefficients were determined by runs in a double sector synthetic boundary cell at 10,589 rpm.

N-terminal amino end group determinations. Batches of 10 mg each of the pure dog erythrocyte enzyme (main chromatographic fraction) were coupled with 1-fluoro-2,4-dinitrobenzene (2.5%) in 5% $NaHCO_3$ for 18 hr. The mixture was acidified by the addition of a large amount of 1 N HCl and washed repeatedly with 1 N HCl, ethanol, acetone, and ether. The dried

powder was weighed and hydrolyzed in sealed tubes in 11.1 N HCl during 4 hr and 6 N HCl for 16 hr. Two-dimensional chromatography was performed on the ether-soluble amino acids in the hydrolyzate, on Whatman No. 7 paper sprayed with 0.05 M phthalate buffer pH 6.0. For the first and second direction were used, respectively: *sec*-butanol saturated with 0.05 M phthalate buffer pH 6.0 and 1.5 M sodium phosphate buffer pH 6.0 (11, 12).

Preparation of carbonic anhydrase from dog kidney. Fresh dog kidneys were thoroughly perfused with isotonic saline in the cold (about 4°), until the perfusate was clear. The cortex of the kidneys were isolated, and only absolutely bloodless tissue was selected. The tissue was blended in 5 volumes of 0.25 M sucrose and the homogenate was centrifuged at 600 *g* for 10 min. At the bottom of the resulting sediment, a small pellet of red blood cells could be observed. The supernatant was clarified by centrifugation at 100,000 *g* for 1 hr and extensively dialyzed against 0.01 M Tris buffer pH 8.0. After concentration by ultrafiltration it was chromatographed on a 4 × 50 cm DEAE-cellulose column.

RESULTS

Chromatography and Electrophoresis of Human Carbonic Anhydrase

CA from human erythrocytes, purified on G-75 Sephadex as described by Rickli *et al.* (4) was chromatographed on an analytical DEAE column under the same conditions as described above for crude dog CA.[1] The resulting chromatogram shown in Fig. 1 indicates the presence of three distinct fractions; of these—in the order of their elution from the column—the first one had a high, the second and third ones low specific activities. The third peak was only a minor fraction of the total product. From what is presently known about the various human carbonic anhydrase types, the first, second, and third peak can be identified as human carbonic anhydrase types C, B, and A, respectively. This was confirmed by comparing the fractions obtained with the three human types (generously supplied by Dr. Nymann, University of Uppsala, Sweden) by means of acrylamide gel electrophoresis (Fig. 2).

Chromatography of Dog Erythrocyte Carbonic Anhydrase

For comparison of the two methods used to obtain the crude CA preparations, a hemolyzate was divided into two portions, of which one was subjected to the chloroform-ethanol treatment, whereas the other was chromatographed on a preparative DEAE-cellulose column. The CA preparations obtained were dialyzed against 0.01 M Tris buffer and concentrated. They were charged to an analytical DEAE-cellulose column and eluted according to the conditions described above. In both cases single peaks were eluted at similar positions, of which the CA activity and the absorbance at 280 mμ coincided very well. In other chromatograms a discrepancy existed of varying degree between the maxima of the curves indicating absorbance and enzyme activity. The variability in the chromatographic separation of the two positions of these maxima is probably due to individual differences in the CA preparations applied to the columns. An example of a chromatogram in which this discrepancy was very conspicuous is shown in Fig. 3. It can be seen that there is a single major peak, which seems to consist of two components, differing in their specific activity. In most chromatograms one to three minor peaks trailing behind the major peak were obtained. Acrylamide gel electrophoresis at pH 9.0 of the isolated fractions indicated that the electrophoretic mobilities of the peaks shown in Fig. 3 increased in the order of their appearance from the column (Fig. 4). The mobility of the main fraction, which appeared to be homogeneous under these conditions, corresponded to that of human erythrocyte CA type B (Fig. 8), that of the first minor peak was similar to that of

[1] It was found that if 10–15 ml of human red cell hemolyzate (prepared as described above) was charged to a large 4 × 50 cm DEAE column, excellent separation was obtained of components B and C, and hemoglobin, using very slow flow rates of 8 ml/hr.

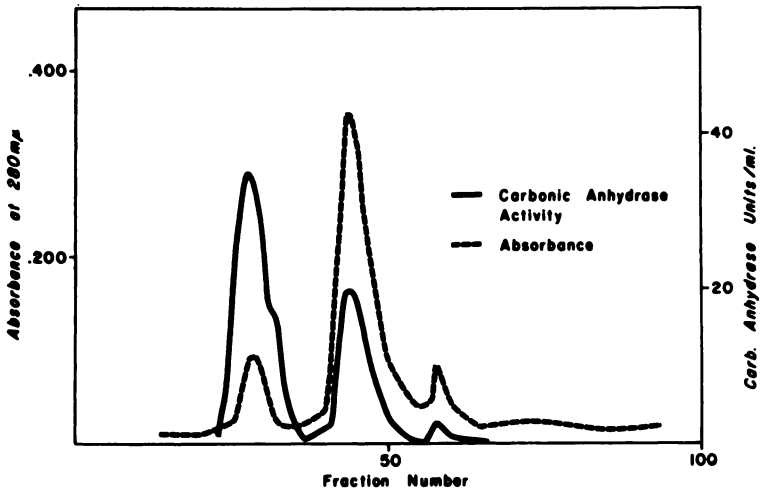

FIG. 1. *Chromatography of human carbonic anhydrase on DEAE-cellulose using gradient elution*

Before chromatography, the enzyme was purified on Sephadex G-75, as described by Rickli *et al.* (4). The CA-containing fractions were pooled, dialyzed against 0.01 M Tris buffer pH 8.0 and concentrated by ultrafiltration. The concentrate was charged to a 2 × 40 cm DEAE-cellulose column. Elution was effected by a Tris buffer gradient of 0.01–0.1 M, pH 8.0. Ultraviolet absorbance and CA activity were determined as described under Methods.

human CA type A. Also in other experiments the main fraction eluted from DEAE showed only a single band when run in acrylamide gel at pH 9.0 or in agar gel at pH 6.0.

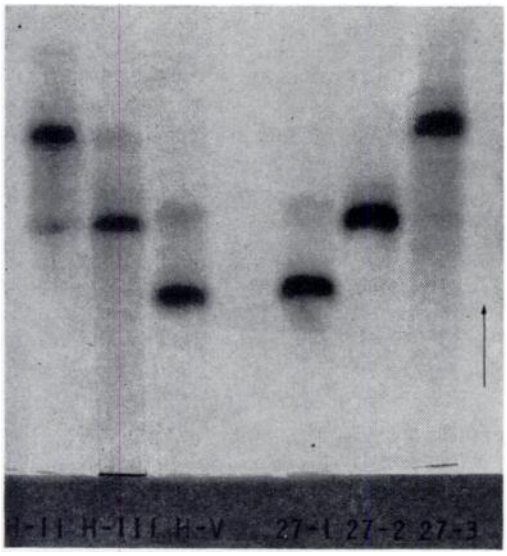

FIG. 2. *Acrylamide gel electrophoresis at pH 9.0 of human erythrocyte CA type A (H-II), type B (H-III), type C (H-V), peak 1 (27-1), 2 (27-2) and 3 (27-3) of the chromatogram shown in Fig. 1*

Details are presented under Methods.

Attempts to separate the two components present in the main chromatographic peak failed consistently, except for one case, in which the chromatogram was obtained shown in Fig. 5. In this experiment a 6-month-old mongrel dog received 1 mC $^{65}ZnCl_2$ intravenously, and after 48 hr the blood was collected by heart puncture. Crude erythrocyte CA was prepared with chloroform-ethanol and chromatographed on DEAE as described. This time the two components were eluted as two separate fractions, of which the first one was present in the greatest amount. The second component, however, had a much higher specific activity than the first one.[2] The two main and three minor peaks were separately pooled, dialyzed against distilled water and lyophilized.

Acrylamide gel electrophoresis at pH 9.0 of the five peaks showed the stepwise in-

[2] Upon rechromatography of a mixture of approximately equal amounts of fractions 1 and 2, a single peak was obtained which only showed a slight discrepancy between the absorbance and enzyme activity maxima.

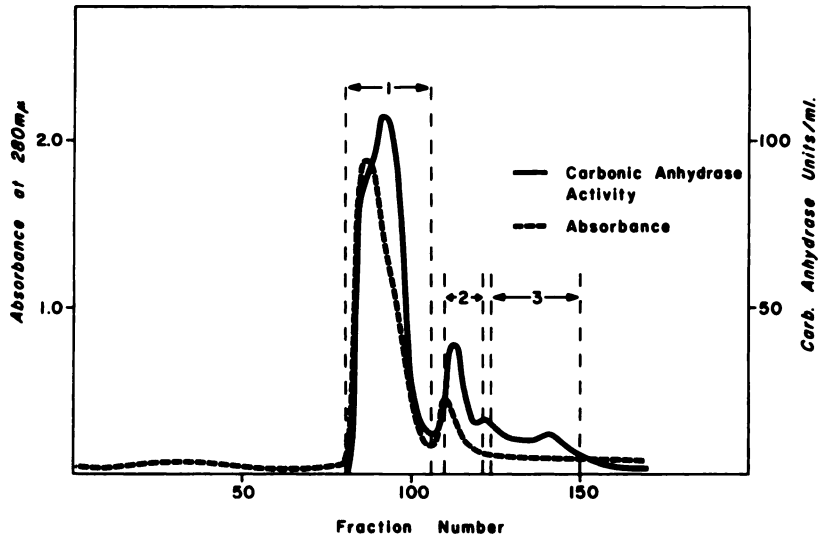

FIG. 3. *DEAE-column chromatography of dog erythrocyte carbonic anhydrase*

Prior to chromatography the enzyme was purified by means of chloroform-ethanol. The dialyzed and concentrated preparation was charged to a DEAE-cellulose column and chromatographed as described (see Fig. 1). Fractions 1, 2, and 3 were pooled, dialyzed, and lyophilized. The acrylamide gel electrophoresis patterns of these fractions are shown in Fig. 4.

FIG. 4. *Acrylamide gel electrophoresis at pH 9.0 of the various chromatographic fractions shown in Fig. 3*

Patterns designated 13-1, 13-2, and 13-3 correspond with peaks 1, 2, and 3, respectively.

crease in mobilities mentioned previously (inset, Fig. 5). Peak 1 and 2 both had the same mobility as human type B (H-III), peak 3 was identical with human type A (H-II).

The results of some determinations carried out on the different fractions are recorded in Table 1. They indicate that although the first peak had a lower specific activity than the others, the I_{50} (50% inhibitory concentration) for methazolamide (14) was the same for all fractions. Also in other experiments, in which the first two components were not as well separated, the I_{50} was always the same for any area under the first peak. The uniformity of the specific radioactivities of the various peaks, indicates that their Zn content was approximately similar, every peak, therefore, representing more or less pure CA. This point was further established by determining the radioactivity of each band after acrylamide gel electrophoresis of the various peaks, as shown in Fig. 6. These data indicate that the lower specific enzyme activity of the first peak was not due to contamination with non-CA protein.

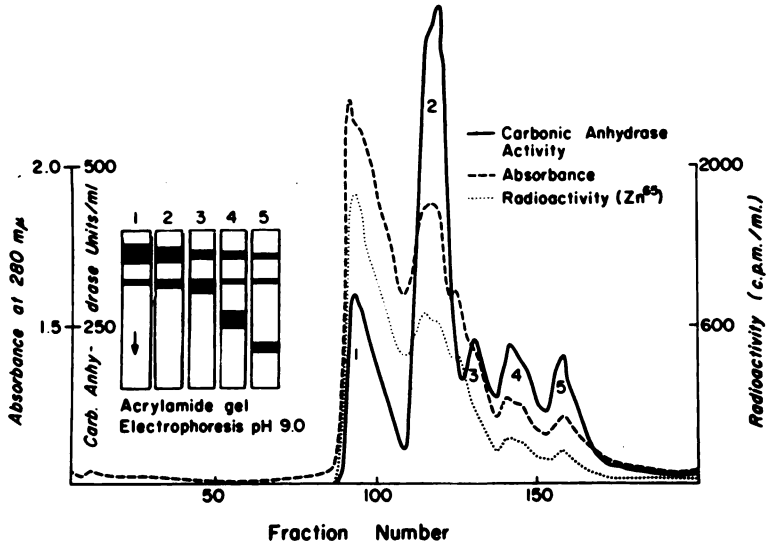

FIG. 5. *Chromatography of dog erythrocyte carbonic anhydrase labeled with ^{65}Zn on DEAE-cellulose*

Purification and chromatography of the enzyme were carried out in exactly the same manner as outlined in Fig. 3. Inset: acrylamide gel electrophoresis of the various peaks at pH 9.0.

Another possibility is that the two portions of the main fraction, represent two different enzymes, similar to human CA type B and C. The enzyme kinetic properties of the two portions recorded in Table 2 indicate that although the specific activity of the two portions differed to a large degree, their substrate affinities were practically equal, in contrast to the human isoenzymes. It seems, therefore, that the very

TABLE 1

Characteristics of the various peaks shown in the chromatogram recorded in Fig. 5

The relative radio- and enzyme activities of the various fractions were measured by dissolving portions of the lyophilized protein in distilled water and determining protein concentration and enzyme activity according to Lowry *et al.* (13) and Maren *et al.* (7), respectively. Radioactivity was measured by counting in a Nuclear Chicago crystal scintillation counter.

Peak	Specific radioactivity (cpm/mg protein)	Molar conc.[a] of enzyme/e.u. (E/e.u.) $\times 10^6$	$I_{50} \times 10^8$ for methazolamide
1	913	10.1	4.3
2	950	2.9	2.7
3	813	3.3	3.6
4	881	2.2	3.2
5	772	2.0	—

[a] The molar concentration was calculated from the protein concentration on the assumption that the molecular weight was 30,000 and the solution contained pure carbonic anhydrase.

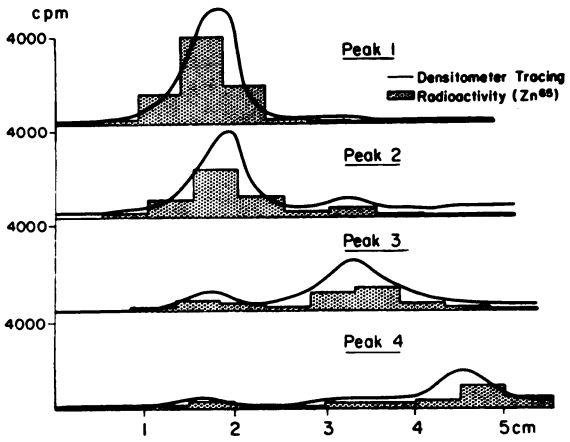

FIG. 6. *Densitometer tracings of the acrylamide gel patterns of the different peaks shown in Fig. 5*

The shaded areas represent the radioactivity due to ^{65}Zn present in the corresponding 0.5 cm gel strips, obtained from an unstained, unfixed duplicate sample run alongside and cut into 0.5 cm pieces which were then counted in a Crystal Scintillation Counter.

first portion of the main peak which is eluted from the DEAE-cellulose columns was originally similar to the other portion but has been deteriorated in some way. The electrophoretic homogeneity of the main fraction indicates that the structural change which causes the fall in specific activity must be slight. In view of the difficulty of separating the low activity portion from the more active part of the main fraction chromatographically, it seems possible that this deterioration occurred on the column during chromatography. This view is supported by the fact that generally 30–60% of enzyme activity was recovered, whereas experiments with ^{65}Zn-labeled CA indicated 90% recovery of all radioactivity.

As shown in Table 2, the E_0/e.u. value of the active portion appeared to be approximately twice that found for dog hemolyzate (2.4×10^{-9} M) (14). As the enzyme activities of the various dog red cell CA preparations could be increased about 1.5–2-fold by the addition of 1–2 mg/ml peptone (17–19), and 2–4-fold by the addition of 0.1–0.3 mg/ml hemoglobin to the reaction mixture, we may assume that the active portion of the main chromatographic peak and crude dog hemolyzate have approximately equal specific activities.[3]

TABLE 2

Kinetic constants of human and dog red cell CA[a]

Dog red cell CA preparation was purified with chloroform-ethanol, and subjected to DEAE-cellulose column chromatography. The fractions collected under the areas with low and high specific activity of the main peak were pooled separately, dialyzed and concentrated in a rotating flash evaporator. The K_m for the hydration reaction was determined with the changing pH method at 0° using the barbital buffer system (15). The E_0/e.u. (moles of enzyme per enzyme unit) was determined in carbonate buffer with the Easson-Stedman procedure according to Maren *et al.* (14).

Source	K_m (mM)	E/e.u. ($\times 10^9$ M)
Dog hemolyzate	23 (0°) (15)	2.4 (5)
Dog main CA fraction Part I	36.0 (0°)	14.8
Dog main CA fraction Part II	27.6 (0°)	5.0
Human hemolyzate	5 (0°) (16)	8 (5)
Human CA type B	2.6 (25°) (17)	80 (5)
Human CA type C	14 (25°) (17)	2 (5)

[a] Hydration reaction.

Zinc Content

The Zn content of the main chromatographic fraction of CA was 2.13 ± 0.03 μg/mg or one atom zinc per molecule of protein (see Methods), with a molecular weight of 28,100, which agrees closely with the value of 29,000 obtained by sedimentation and diffusion studies (see below). Similar zinc contents have been reported for human CA components (1, 3, 4) and bovine CA (8, 20, 21).

Amino Acid Analyses

The average amino acid composition from three different determinations (see Methods) are recorded in Table 3. For comparison the amino acid compositions of bovine and human CA, as reported in the literature, are included. In the case of human erythrocyte CA types B and C, the averages were computed of the amino acid contents reported by Rickli *et al.* (4), Nymann and Lindskog (20), and Laurent *et al.* (22). As can be seen all CA varieties appear to be rather similar in their amino acid content. The only outstanding features of the dog erythrocyte CA is the absence of methionine. This, and the presence of one cysteine residue, were verified by means of chromatography of a hydrolyzate of CA which was pretreated with performic acid as described by Moore (23).

Molecular Weight

The sedimentation coefficients obtained in three different runs were calculated and

[3] The hemoglobin used in these studies was obtained from a hemolyzate which had been heated at 55° for 20 min. This treatment destroys the CA activity present, without affecting the protective effect of hemoglobin on CA during the determination of its activity by the changing pH method. The hemoglobin was then separated further from CA by DEAE-cellulose column chromatography. The CA activity of hemoglobin prepared in this way was negligible.

corrected to give the values in water at 20° ($s_{20,w}$) (24) (see Methods). The average value for $s_{20,w}$ resulting from these experiments was 2.82. The diffusion coefficient $D_{20,w}$ was found to be 8.99 (see Methods).

TABLE 3
Residues per molecule erythrocyte carbonic anhydrase

Amino acid	Dog	Human type B (4, 20, 22)	Human type C (20, 22)	Bovine type B (20, 21)
Lysine	22.3	17.8	25.5	18.7
Histidine	11.9	11.2	12.8	11.3
Arginine	6.9	7.4	7.5	9.0
Tryptophan	6.4	5.9	7.0	7.0
Asparagine	33.6	30.9	29.9	32.1
Threonine	11.2	13.7	12.5	14.5
Serine	24.9	28.8	18.9	16.1
Glutamine	26.3	22.6	26.5	23.7
Proline	19.0	16.9	16.8	19.5
Glycine	24.4	15.4	23.1	20.2
Alanine	17.5	18.7	13.6	16.9
Valine	14.8	17.0	16.7	19.9
Methionine	—	1.97	1.1	3.1
Isoleucine	12.7	9.18	8.9	5.0
Leucine	24.5	19.8	27.0	26.2
Tyrosine	7.8	8.0	8.4	7.9
Phenylalanine	11.5	11.0	12.5	11.3
Cysteine[a]	1.2	1.14	1.3	—

[a] Determined as cysteic acid.

Assuming a partial specific volume of 0.735 (20, 21), the calculated molecular weight of dog erythrocyte CA was 29,000, which is similar to that reported for human CA types B and C (20, 25) and bovine CA type B (8, 20).

N-Terminal Amino Acids

Ether extracts of hydrolyzates of FDNB-treated CA exhibited only two yellow compounds, which were identified as dinitroaniline and dinitrophenol by two-dimensional chromatography (see Methods). These results indicate that similar to human CA (4), no N-terminal end group can be detected in dog erythrocyte CA by the use of the Sanger procedure.

Dog Kidney Carbonic Anhydrase

A typical example of the chromatograms obtained by DEAE chromatography of the cytoplasmic fraction of dog kidney (see Methods) is shown in Fig. 7. As can be seen, a large main peak with a small minor component was obtained among a large complex of other proteins.

Acrylamide gel electrophoresis of the first and second peak, designated 11-1 and 11-2, resulted in the patterns recorded in Fig. 8. As can be seen, the first main peak contained mainly protein with electrophoretic mobility similar to dog erythrocyte CA (10-1, 7-1, 4B, C) and human type B. Traces of other proteins present in the cytoplasmic sap of the kidney can also be observed. The gross contamination of peak 2 with these proteins did not permit a clearly discernible CA band in the picture shown, although it was visible with the naked eye. These results seem to indicate that at least electrophoretically, dog kidney CA is similar to erythrocyte CA. Because of the small amounts of CA which could be obtained from the kidney, purification by means of rechromatography did not appear feasible.

Enzyme-Inhibition Studies

The kinetics of inhibition of purified, chloroform-ethanol treated dog erythrocyte CA and crude kidney CA were compared with those of purified human erythrocyte CA types B and C, using the methods described by Maren *et al.* (14). The concentrations needed to obtain 50% inhibition with sulfanilamide and acetazolamide, which are examples of a weak and a moderately strong inhibitor, are recorded in

TABLE 4
In vitro inhibition of different varieties of carbonic anhydrase

Carbonic anhydrase	$I_{50} \times 10^8$ M[a] for: Sulfanilamide	Acetazolamide
Dog hemolyzate	533	3.1
Dog kidney	529	4.4
Human erythrocyte type B	2570	29
Human erythrocyte type C	245	3.3
Human hemolyzate	360	3.0

[a] As determined according to Maren *et al.* (14) using the bicarbonate-carbonate buffer system.

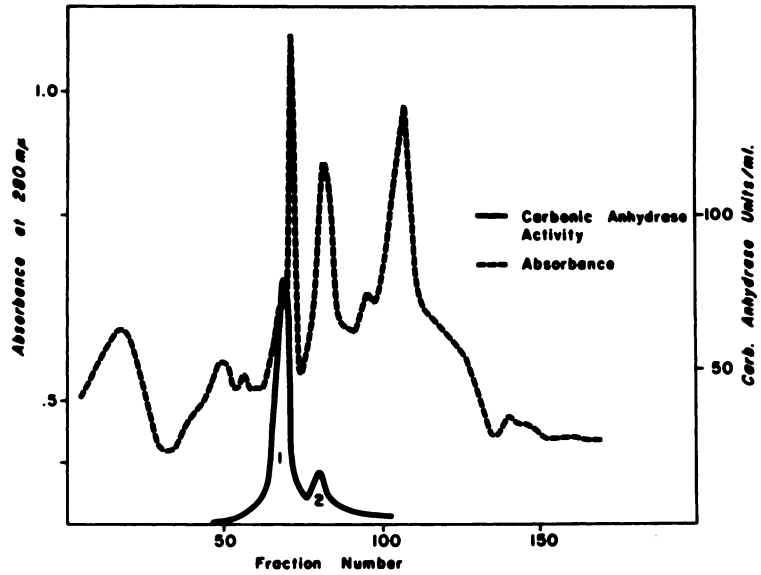

FIG. 7. *Chromatography of the 100,000 g supernatant fraction of dog kidney homogenate on DEAE-cellulose*

The supernatant was dialyzed against 0.01 M Tris buffer pH 8.0 and chromatographed in the same manner as outlined in Fig. 1.

Table 4. It appears that the I_{50}'s for both enzymes are similar to those of human erythrocyte CA type C. These data indicate that the carbonic anhydrases in the dog resemble the active human type C variety.

DISCUSSION

Human erythrocyte CA has been found to consist of two main fractions which differ greatly with respect to their affinity for substrate and sulfonamides. The data re-

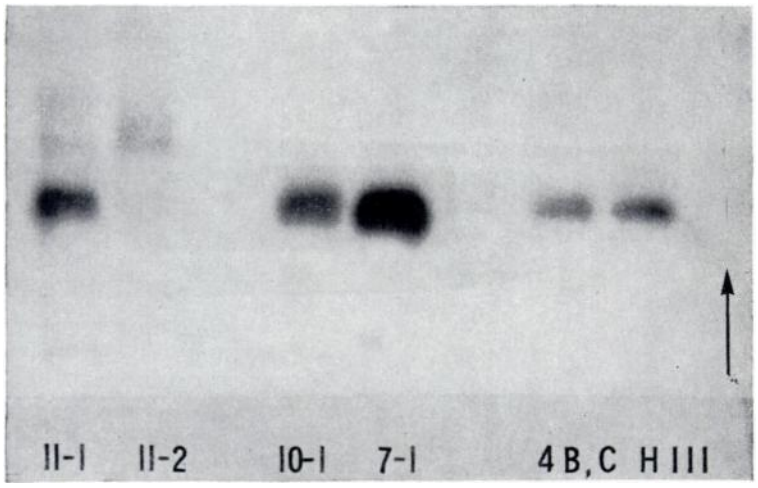

FIG. 8. *Acrylamide gel electrophoresis at pH 9.0 of various chromatographic fractions*

Patterns 11-1 and 11-2 correspond with peaks 1 and 2 of the chromatogram obtained from dog kidney shown in Fig. 7. Patterns 10-1 and 7-1 were obtained from DEAE-purified dog erythrocyte CA, prepurified with chloroform-ethanol. Pattern 4B, C represents purified dog erythrocyte CA, prepurified by means of DEAE; and H-III is human erythrocyte CA type B.

corded in Tables 2 and 4 indicate, however, that the crude hemolyzate yielded approximately the same values as fraction C. Only fractionation revealed the existence of an isoenzyme which in spite of its higher concentration in the original hemolyzate was almost completely masked by the much more active fraction C. The present studies indicate that, in contrast, dog red cells do not contain any isoenzymes of which the enzyme kinetics or affinity for sulfonamides differ from that of the crude hemolyzate.

DEAE column chromatography of crude dog red cell CA revealed a single peak, whereas under identical conditions human blood showed two distinct activity peaks. When subjected to acrylamide gel electrophoresis at pH 9.0, dog CA showed one main band, accompanied by a number of faster moving minor bands. Under the same conditions the major human CA fraction B had the same mobility as the main dog CA fraction, whereas human type C moved slightly slower. Apart from those produced by fractions B and C, human CA also contained some additional faster moving bands, including human type A. No dog red cell or kidney CA preparations ever showed bands with mobilities lower than that of the main fraction.

The question whether the presence of two components with different specific activities in the main fraction, observed in some chromatograms, is an artifact or not, remains unanswered. If not, the situation would have some similarity with that in the human red cell which also contains two main fractions with different specific activities. In the latter, however, the two isoenzymes have distinctly different enzyme kinetics, which does not seem to be the case in the dog red cell. Chromatographically there is also a difference, as the active human fraction is the first fraction eluted from DEAE, whereas the active part of the main dog CA fraction is eluted immediately following the less active fraction. Since the two fractions do not differ with respect to inhibition kinetics, Zn content, or electrophoretic mobility at different pH's, it seems most likely that the early eluted low specific activity fraction is the result of deterioration, which possibly occurs on the column during chromatography.

The molecular weight, Zn- and amino-acid content of the main dog CA fraction classify it as another typical example of the very special class of proteins to which the mammalian carbonic anhydrases belong. Electrophoretically, the dog kidney CA appeared to be identical to the red cell enzyme.

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