Since rubber tubing and stoppers contain large amounts of sulfides the solutions must not come in contact with them.

Hydrogen Sulfide in Air. Hydrogen sulfide is not absorbed in one M sulfuric acid so that in analyzing samples of air, hydrogen sulfide is not titrated. It may be removed from acid solution by boiling for several minutes. Old sulfide solutions, however, cannot be freed of reducing substances in this way.

Summary. Mustard (I), ethyldichlorarsine (II), Lewisite (III), and phenyldichlorarsine (IV) may be titrated with 10^{-4} M bromine using methyl red as indicator. The method is accurate to about $\pm$ 0.02 ml 10^{-4} M bromine per ml of solution titrated. The reaction of these compounds is that of one mole of bromine per mole of gas.

16189

Carbonic Anhydrase in the Pallium of Primates Compared with that of Lower Mammals.*†

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Carbonic anhydrase reversibly catalyzes the change of carbon dioxide and water to carbonic acid. It occurs in the central nervous system in amounts ranging from a doubtful positive in the cord of the hog[1] to a content in either the cerebellum or cerebrum approximately equal to one-tenth of that found in the blood.[2] None has been found in peripheral nerve of man and dog (unpublished). The content in the spinal cord of mature animals in which it occurs in any considerable amount is well below that of the medulla, pons and higher centers.[3]

Previously reported data indicated a well defined difference in the pattern of the quantitative distribution of carbonic anhydrase in the pallium of the lower animals studied, the dog, the hog and the cat, from that found in man. In the lower animals the cortex held the greater content. In man the pattern was reversed; the white matter immediately below the cortex had a markedly greater enzyme content than the cortex. Exception was found in the motor cortex where more definite approximation to the animal pattern appeared in the leg area.[4]

The present study is an extension of work previously reported. The brains of 4 Rhesus monkeys were examined to see whether the pattern of man is found also in these primates; and additional data on other of the lower mammals, the horse, the steer, the sheep and the rabbit are given. Data on the guinea pig are not included as the subcortical white matter of the brain was too small in amount to be separated successfully from the cortex.

Technique. The technique employed for the determination of carbonic anhydrase was essentially that of Philpot and Philpot[5] as modified by Keilen and Mann.[6] Specific procedures employed[4] and the method used to determine the enzyme activity attributable to the blood in the tissue[7] are given elsewhere.

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† I am indebted to Dr. Anna Dean Dulaney of the Pathological Institute of the University of Tennessee for monkey brains.

1 Ashby, W., *J. Biol. Chem.*, 1944, **152**, 235.

2 Ashby, W., *J. Biol. Chem.*, 1943, **151**, 521.

3 Ashby, W., *J. Biol. Chem.*, 1944, **155**, 671.

4 Ashby, W., *J. Biol. Chem.*, 1944, **156**, 323.

5 Philpot, F. J., and Philpot, J. St. L., *Biochem. J.*, 1936, **30**, 2191.

6 Keilin, D., and Mann, T., *Biochem. J.*, 1940, **34**, 1165.

7 Ashby, W., and Chan, D. V., *J. Biol. Chem.*, 1943, **151**, 515.

TABLE I.
Comparison of Carbonic Anhydrase Content of Tissue from Exsanguinated and Non-exsanguinated Guinea Pigs.

	Carbonic anhydrase : Units per gram					
	Control			Exsanguinated		
Tissue	Total	Correction for blood content	Net	Total	Correction for blood content	Net
Cerebrum	(Pig A) 26.7	3.4	*23.3*	(Pig B) 25.0	0.02	*25.0*
Cerebellum	(" C) 40.4	3.0	*37.4*	(" D) 49.3	0.8	*48.5*
Frontal Pole	(" C) 20.0	3.4	*16.6*	(" D) 18.7	0.3	*18.4*

The brain was sampled while fresh—preferably unfrozen. Part of a gyrus was dissected from the pallium and pure white matter beneath the gyrus was taken. This was called "D." The gyrus was then sliced vertically to expose the layer of cortex surrounding the white matter within the gyrus. Approximately the outer half of the cortex was removed with a sharp blade. The remaining inner portion was separated from the white matter by cutting or scraping. These three latter samplings were designated "A," "B" and "C" respectively. (Fig. 1)

Effect of Perfusion. Since the first 3 monkeys received had been used to produce malarial antitoxin and had been bled and perfused when sacrificed until there was little evidence of blood in the brain, it was considered desirable to control the effect of perfusion by using guinea pigs. The degree of anemia produced in these animals was such that the carbonic anhydrase attributable to the blood in the tissue was reduced from 3.0 units or more in the control animals to only 0.8 to 0.02 units in the perfused. The enzyme content of the brain tissue, however, was within the same range in the two series. The results are given in Table I. There was no indication that carbonic anhydrase activity of the brain was lost by perfusion.

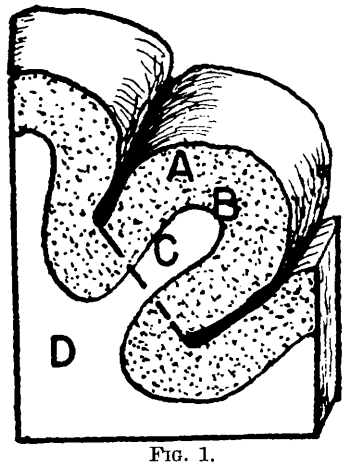

FIG. 1.
Illustrating the method used in dissecting the gyrus into the samples "A," "B," "C," and "D."

Man vs Rhesus Monkey. The carbonic anhydrase content found in the cerebrum of the 4 monkeys studied was low. The content in the cerebellum was 2 to 3 times greater and within the range found in man.

Although the difference between the carbonic anhydrase content of the white and grey matter in the pallium was less in monkeys than in humans, especially in the brains with the smaller amount of enzyme, the pattern of distribution was the one typically found in the human brain. No attempt was made to determine whether, as in the human, the motor cortex of these monkeys gave the reverse distribution typical of the dog, cat and hog. Results obtained by the same technician, from one human and 4 monkey brains are given in Fig. 2 in which the gyrus in the region from which the test material was taken is magnified.

Horse vs Man. Comparisons, in the brain of a horse and of a man, of the carbonic anhydrase content of the cortex with that of the white matter immediately below it, are given in Fig. 3.

In 3 samplings, taken from the parietal area and from the frontal and occipital poles of the brain of the horse, there was definitely less carbonic anhydrase in the white matter

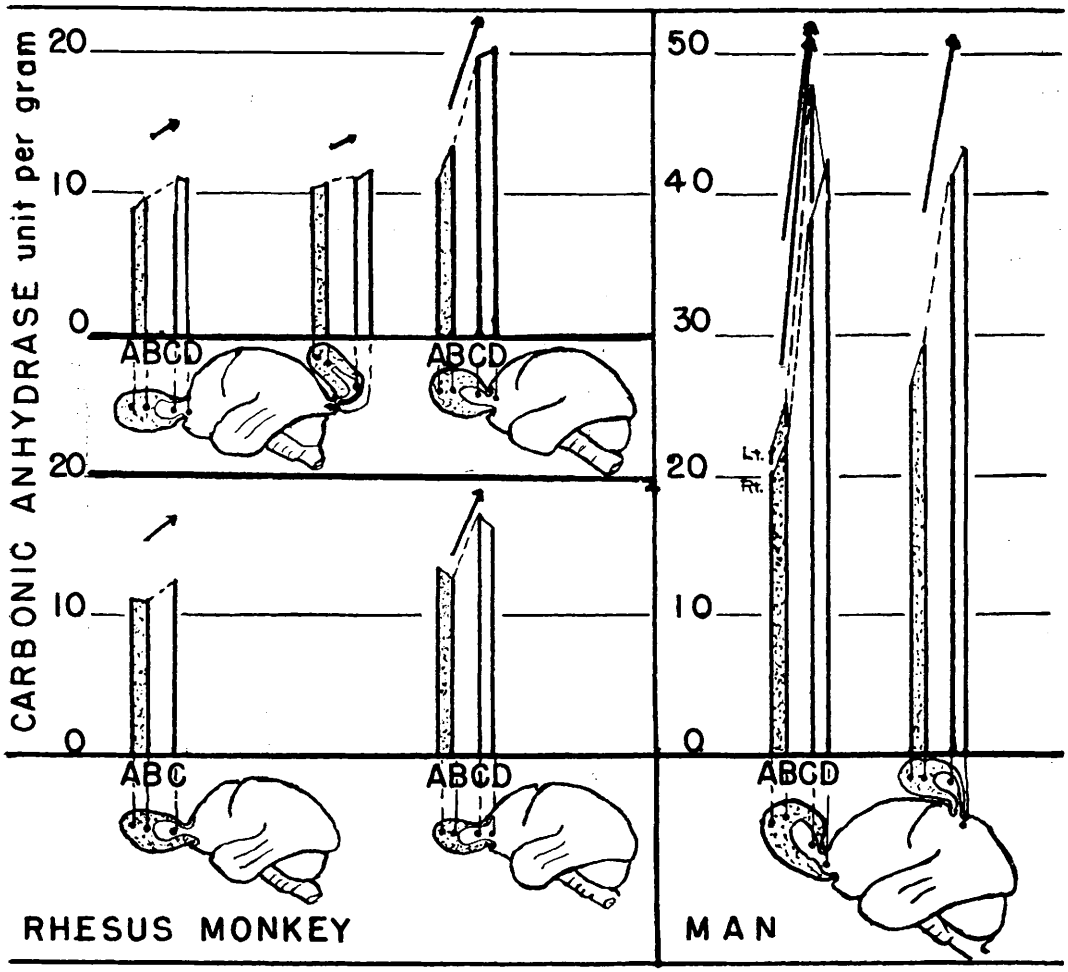

FIG. 2.

Pattern of distribution of carbonic anhydrase between cortex and subcortical white matter, in the cerebrum of a man and Rhesus monkeys.

"A," "B," "C," "D" have the significance indicated in Fig. 1. In the areas from which specimens were taken a gyrus is drawn as though greatly magnified and the "A," "B," "C" samplings are indicated thereon.

below the cortex than in the cortex. In the frontal pole the findings tended to be approximately equal in this brain, in marked contrast with the pattern found in man where, in the frontal pole, there was a great excess of enzyme in the white matter over that of the cortex and in the occipital pole, an approach to equality.[8] The pattern of distribution found in the motor cortex of the human brain was as previously reported, like that found in the horse and in other non-primates studied.

Studies on Sheep, Rabbits and Cattle. Data on the relationship between the carbonic anhydrase content of the cortex and of the white matter below it, in the brains of 2 sheep, 3 rabbits, a calf and a steer, are given in Table II. Sheep I was sacrificed because of its extreme age. Sheep 2 was comparatively young. The rabbit brains were too small to make more than one series of determinations per brain. The calf had been exsanguinated. The steer was an experimental animal and had not been bled. Since its blood was not available, the blood of a cow was used to determine the correction to be made for

[8] Ashby, W., *J. Biol. Chem.*, 1944, **156**, 331.

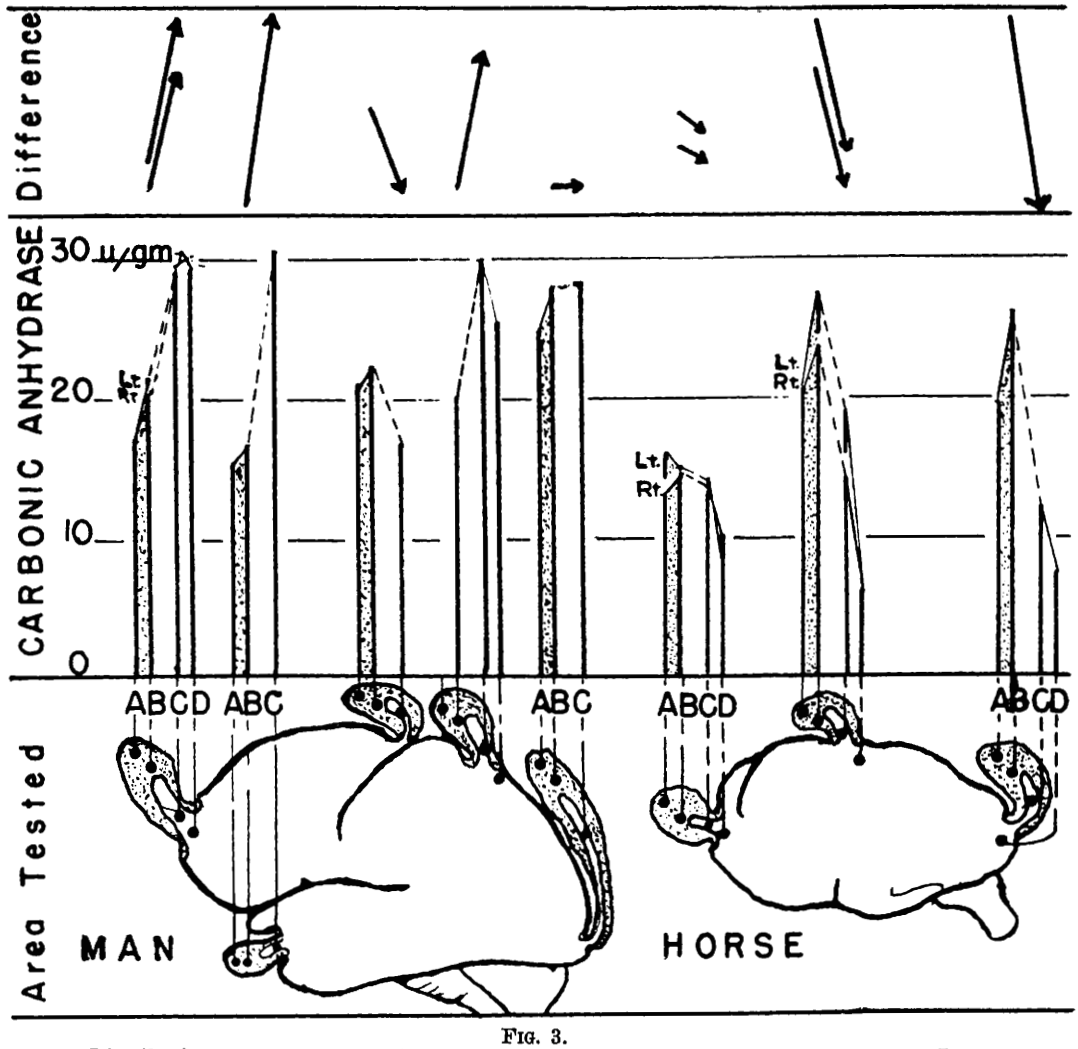

FIG. 3.

Distribution pattern of carbonic anhydrase in the pallium of a man and a horse. In man data on the leg motor area is included. "A," "B," "C," and "D" have the significance indicated in Fig. 1.

blood content. It is probable that the correction in the cortical specimens was too great (Table II).

Summation of Average Results. The data on the relative carbonic anhydrase contents of the cortex and the subcortical white matter are summarized in Table III and compared with findings from 10 frontal and 10 occipital poles from the same human brains. Averaged results from motor areas are also given. Subtraction of the average carbonic anhydrase content of the white matter immediately below the grey matter from that of the grey matter gives a positive number in all species below the primates. In man and Rhesus monkey, on the other hand, a negative quantity is found except in the motor area.

Discussion. It would seem probable that the quantitative pattern of distribution of carbonic anhydrase found in the pallium of man and Rhesus monkey and, so far, not in other animals studied (namely, the dog, hog, cat, horse, cattle, sheep, rabbit) may be part of an evolutionary development peculiar to the primates.

TABLE II.
Comparison of Carbonic Anhydrase Content of Cortex with That of Sub-cortical White Matter in Sheep, Rabbit, and Cattle.

Animal	Area	Carbonic anhydrase, units per gram, corrected for blood content: correction in parentheses			
		Cortex		Sub-cortical white	
		A	B	C	D
Sheep 1.	Frontal	17.1 (1.1)		16.3 (0.7)	
	Occipital	18.2 (0.9)		12.2 (0.7)	
" 2.	Frontal	38.2 (0.9)		19.0 (1.4)	15.8 (0.7)
	Precentral	34.0 (4.5)		18.0 (2.4)	
	Postcentral	39.0 (2.7)		16.6 (1.4)	
	Occipital	39.0 (4.5)		25.2 (1.8)	14.7 (1.4)
Rabbit 1.		16.8 (1.3)		13.5 (1.8)	
" 2.		35.1 (2.0)	24.1 (1.5)	22.3 (0.5)	
" 3.		37.9 (5.6)	32.9 (5.6)	24.7 (3.5)	
Calf		12.5 (0.2)	12.4 (0.1)	8.5 (0.1)	7.0 (0.1)
"		11.1 (0.1)	11.4 (0.1)	8.1 (0.1)	
Steer		12.7 (8.0)	12.9 (6.5)	11.8 (5.0)	12.5 (1.4)
"		18.2 (5.1)	16.0 (4.9)	12.9 (4.0)	12.0 (1.6)
"		11.6 (6.7)	9.4 (5.0)	9.9 (1.6)	8.7 (1.3)

TABLE III.
Comparison of Carbonic Anhydrase Contents of the Cortex and the Sub-cortical White Matter in Primates and Lower Animals.

Animal	No. of comparisons	No. of animals	Average findings Units per gram		Difference %
			Cortex	White matter	
Rabbit	3	3	27.3	20.2	26
Steer	5	2	12.8	10.2	20
Sheep	4	2	30.9	18.0	42
Horse	6	1	20.5	14.9	27
Cat	6	4	39.2	24.9	36
Dog	6	2	33.7	14.4	57
Hog	9	5	34.8	26.4	24
Rhesus monkey	5	4	11.2	14.9	—33
Man					
Frontal pole	10		19.2	29.7	—55
Occipital pole	10		25.3	29.2	—15
Motor cortex	11		33.1	26.2	21

A tendency toward an inverse relationship among species between the size of the animal and the rate of metabolism of its brain has been reported.[9] In the series studied, the brain of the horse, which most nearly approaches that of man in size, shows a cortical content of carbonic anhydrase somewhat below that in the human brain. Therefore the larger amount of enzyme found in the subcortical white matter of man as compared with that found in the cortex might be regarded as an increase of the enzyme in the white matter beneath the cortex, rather than as a decrease within the cortex.

In the occipital pole in man, where there is a point to point projection from the geniculate nucleus, there is a decreased difference between the enzyme content of the cortex and the white matter beneath it. In the motor cortex, where the white matter consists largely of efferent fibers, the usual pattern of distribution peculiar to man and the Rhesus monkey is not present. Here, especially in the leg area, the white matter below the cortex

[9] Page, I. H., *Chemistry of the Brain*, Springfield, Ill., Thomas, 1937.

contains definitely less enzyme than the cortex and the pattern of distribution is like that in the lower animals.

In this region below the cortex, which in the primates shows the increased enzyme content, are found fibers which make extrinsic cortico-cortical connections. The coincidence of this pattern of distribution with the development in the primates of the capacity for wider mental association, as found in man, may be of significance. If this difference in the pattern of distribution proves to be tenable after further study, it would differentiate qualitatively between the brain of primates and that of the lower animals.

Summary. Within the pallium a quantitative distribution pattern of carbonic anhydrase, not found in the dog, cat, hog, horse, sheep, cattle and rabbit, has been found in man and the Rhesus monkey. Its possible significance is discussed.

16190

Purification of the Resin Amberlite IR-100 for Blood Coagulation Studies.*

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The phenol-formaldehyde resin, Amberlite IR-100, which can remove calcium from blood completely by virtue of its ion exchange property[1] has become a valuable agent for quantitative studies of calcium in the coagulation mechanism.[2,3] Since certain lots of Amberlite have been found to contain impurities which interfere with coagulation, an improved method of purification had to be devised. Several studies have been carried out with the purified product to determine further whether Amberlite induces any change in blood other than the removal of calcium.

Experimental. 1. *Purification of Amberlite.*‡ Twenty grams of Amberlite were covered with 100 cc of 5% (by volume) sulfuric acid and the mixture boiled and stirred vigorously for 5 minutes. The resin was washed with distilled water by decantation until the wash water was no longer acid. The material was then treated with 100 cc of 5% sodium carbonate solution, heated to approximately 70°C and thoroughly stirred. The deeply colored supernatant fluid was poured off and the process repeated until little coloring matter could be extracted. The resin was finally washed with warm distilled water until all traces of sodium carbonate were removed.

The cation-exchange resin was prepared for reaction by adding to it 250 cc of 5% solution of sodium chloride, stirring vigorously for 30 minutes and allowing the mixture to stand for 60 minutes more. The resin was then washed with distilled water and filtered by suction until the wash water no longer contained chloride ions. The solid was dried at 37°C.

2. *Decalcification with Amberlite.* To prevent incipient coagulation, blood obtained by venipuncture with minimal trauma was drawn into a syringe coated with Silicone and immediately passed through a column of 3 g of Amberlite, an amount sufficient for complete decalcification of 10 cc of blood. The

* This work was supported by a grant from the United States Public Health Service.

† Department of Internal Medicine, University of Roma; at present, Senior Research Fellow, National Institute of Health.

1 Steinberg, A., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 124.

2 Quick, A. J., *Am. J. Physiol.*, 1947, **148**, 211.

3 Stefanini, M., and Quick, A. J., *Am. J. Physiol.*, in press.

‡ Mr. James C. Winters of the Resinous Products and Chemical Company offered valuable suggestions.