ble of neutralizing antibodies *in vitro*, is not antigenic when injected into animals or into human subjects, known to be capable of producing Rh antibodies. The failure to produce a rise in antibody titer in 2 human subjects who later responded to injections of whole red cells is a more convincing demonstration of lack of antigenicity than the failure to produce Rh antibodies in animals. It is interesting in this connection that one subject (W.M.) who showed a response to the injection of whole Rh positive cells failed to show a rise in antibody titer after the injection of freshly hemolyzed cells. Further observations are necessary to determine if the apparent loss of Rh antigenicity following hemolysis is partial or complete. The failure of the human subjects to show a drop in antibody titer following injections of elinin from Rh positive cells is not surprising. Calculations from serum inhibition studies indicate that 3 to 5 g of elinin will be needed to neutralize the circulating antibodies in an individual with an antibody titer of 1 to 64 if the relationship is stoechiometric *in vivo*. As yet the greatest amount given by I.M. injection is 1 to 1.5 g. In addition to the lack of quantity given we have no information as to the rate of absorption of elinin from an intramuscular depot or its antibody neutralizing activity when absorbed after exposure to tissue enzymes. The possibility that 3 to 5 g of elinin given intravenously would neutralize the circulating antibodies has not as yet been explored largely because of the difficulty of obtaining this amount of material and free from pyrogens. As stated above, we have not been able to obtain an active fraction of crude preparations of elinin by chemical or enzymatic means. This has also been true of the purified preparations of elinin. We have not been able to repeat the work of Carter(5), in extracting an active substance from whole red cells by alcohol and ether. The preparations obtained by her method of extraction yielded uniformly negative results with our method of assay. We believe that the basic constitution of the Rh antigen is as yet unknown. Observations of the complete inactivation of the Rh factor by trypsin suggest that the Rh hapten is very closely associated with a protein if it is not a protein itself.

Summary. 1. Elinin, a lipoprotein fraction of the red cell exhibiting Rh activity has been isolated in relatively pure form largely by physical methods.

2. Injection of elinin from Rh positive cells into animals has so far failed to yield a measurable titer of anti-Rh antibodies.

3. Injection of elinin into human subjects who have been sensitized to the Rh factor by pregnancy or transfusion failed to increase the titer of Rh antibodies in the serum. Two of the subjects injected showed subsequent response to injection of whole Rh positive cells.

4. Injection of 1 to 1.5 g of active elinin intramuscularly failed to effect a change in titer of circulating antibodies.

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Carbonic Anhydrase in Congenital Heart Disease.* (17772)

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Children with cyanotic congenital heart disease frequently tolerate relatively low degrees of oxygen saturation of the blood without any noticeable effects on the intelligence or on the state of consciousness when at rest. In connection with a study of the adaptive mechanisms involved, an investigation has been made of the possible role of carbonic anhydrase in the blood of such children. Car-

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TABLE I.
Carbonic Anhydrase Values in Patients with Different Cardiac States.

Cardiac status	Age range (years)	No. of cases	Carbonic anhydrase					Mean Hgb (g)	Mean RBC	Mean hematocrit
			Units mm³			Stand. dev.	S.E.M.			
			Max.	Min.	Mean					
Normal	13/4-30	20	2.36	1.40	1.85	.27	.06	13.0	4.78	41
Newborn	NB-2 wk	13	0.69	0.10	0.41	.19	.05	15.4	3.86	48
Cyanotic congenital heart disease	¾-24	18	4.50	2.11	3.12	.72	.17	18.3	7.84	63
Acyanotic	½-20	6	2.09	1.20	1.71	.19	.08	12.2		

bonic anhydrase was described first(1) as an enzyme which accelerates the reaction of carbonic acid to carbon dioxide and water. The same authors also showed that the enzyme is present in the hemoglobin of the red cells(2). Since the release of carbon dioxide and the uptake of oxygen are intimately related to each other, it was thought that investigation of this enzyme might throw light on such mechanisms. Using a slightly different technic than that used by ourselves, normal values for infants and children have previously been established by Stevenson(3).

Methods. The glass boat method described by Meldrum and Roughton(4) was used by us. The exact technic employed was that of Lambie(5). This consisted of using 2 ml of 0.2 molar phosphate buffer solution which was shaken vigorously with 2 ml of a 0.2 molar sodium bicarbonate in hydroxide solution, in an airtight chamber connected to a water manometer. This mixture was used as the control. With an identical setup, 0.2 ml of laked blood was added to the buffer side of the boat, and the increment in the velocity of evolution of carbon dioxide over the control was used as an index of the carbonic anhydrase activity of the blood. The enzyme unitage was calculated as described by Lambie(5). Our experiments were run at 15°C, using the same machine throughout the entire study. In each instance the blood was obtained by venipuncture and was stored until used in an oxalate tube at 4°C.

Results. The results of the studies in patients with various cardiac states can be seen summarized in Table I. Twenty patients with normal cardiac status ranging in age from 1¾ to 30 years of age had an average carbonic anhydrase value of 1.85 units. The standard deviation for this group was 0.273

1. Brinkman, R., Margaria, R., Meldrum, N. U., and Roughton, F. J. W., *J. Physiol.*, 1932, v75, 3.

2. Meldrum, N. U., and Roughton, F. J. W., *J. Physiol.*, 1932, v75, 15.

3. Stevenson, S. S., *J. Clin. Invest.*, 1943, v22, 403.

4. Meldrum, N. U., and Roughton, F. J. W., *J. Physiol.*, 1933, v80, 113.

5. Lambie, C. G., *Edinburgh M. J.*, 1938, v45, 373.

TABLE II.
Non-cardiac Infants.

Patient's No.	Age (wks)	Carbonic anhydrase (units/mm³)	Hgb (g)	RBC (millions)	Hematocrit	Diagnosis
1	1	1.2	16.5	5.50	56	Spine bifida
2	2	0.77	10.8	4.63	35	Feeding problem
3	8	1.16	7.4	2.98	28	Rectovaginal fistula
4	8	0.80	8.5	4.60	29	Pyloric stenosis
5	20	2.31	12.4	5.15	40	Convulsions
6	20	1.14	10.3	5.58	35	Hydrocephalus
7	28	1.72	10.2	5.76	35	Normal

and the standard error of the mean 0.061. Thirteen newborn infants' sera were tested, the cord blood being used in each instance except one that was two weeks of age. The average carbonic anhydrase level in these newborn infants was 0.413, the standard deviation 0.188 and the standard error of the mean 0.052. In 18 patients with cyanotic congenital heart disease, varying in age from ¾ to 24 years, the average carbonic anhydrase level was 3.12, the standard deviation 0.716, and the standard error of the mean 0.169. Six individuals with acyanotic congenital heart disease varying in age from ½ to 20 years had an individual carbonic anhydrase level of 1.71, a standard deviation of 0.190 and a standard error of the mean 0.078.

In an effort to determine the age at which the carbonic anhydrase content of the red cell changes, seven infants varying in ages from nine days to seven months were tested. These values may be seen in Table II.

Discussion. It has been suggested by Stevenson(3) that the low levels of carbonic anhydrase in premature and newborn infants is possibly a characteristic of fetal hemoglobin which is adapted to the intrauterine condition of low oxygen tension. We had thought previous to the present study that children with cyanotic congenital heart disease had possibly retained some fetal mechanism, such as fetal hemoglobin, that permitted them to utilize oxygen in the manner of the fetus. It would appear from the results of this study that carbonic anhydrase is not the factor, as the patients with cyanotic heart disease have an elevated carbonic anhydrase which seems to be proportional to the red cell mass or hematocrit. Hodgson(6) has already demonstrated this relationship in other disease conditions. In our studies, when the mean value of 3.12 for carbonic anhydrase in the cyanotic heart group is corrected for the increased hematocrit, a value of 1.83 is obtained which is almost identical with that which we obtained in the normal group, namely 1.85.

It is apparent from these studies, that heart disease of the acyanotic type places no special load on the hematopoietic mechanism, and, therefore, the carbonic anhydrase level is the same as in normal individuals. It is also apparent from our data, as from those of Stevenson(3), that the carbonic anhydrase of the normal newborn infant is low in spite of the normal or increased hematocrit. Normal adult values are not reached until the infant is between 5 and 7 months of age, as shown in Table II. Stevenson(3) has made the comment that there is not a close correlation between the carbonic anhydrase and the hematocrit, red blood cell count or hemoglobin. Our data would indicate that this is true during the first year of life, whereas thereafter the carbonic anhydrase is definitely related to the hematocrit.

6. Hodgson, T. H., *Brit. J. Exp.*, 1936, v17, 75.

Summary. In an attempt to determine the possible adaptive mechanism in children with cyanotic congenital heart disease, the carbonic anhydrase level of the blood was determined in 18 patients of different ages with proved congenital heart disease of the cyanotic type. The values found were compared with those obtained on six patients with congenital heart disease of the acyanotic type, 13 normal newborn infants and 27 patients with no cardiac disease who varied in age from one week to 20 years.

It was found that the carbonic anhydrase as determined routinely was elevated in all of the patients with cyanotic congenital heart disease as compared with that of patients with acyanotic congenital heart disease or with no heart disease. When corrected for the increased hematocrit, the blood carbonic anhydrase of the cyanotic patients did not differ significantly from that of the acyanotic subjects of similar ages.

The findings by Stevenson of excessively low values for carbonic anhydrase in the blood cells of normal newborn infants were confirmed.

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A Small Adjustable Renal Artery Clamp for Production of Chronic Hypertension in the Rat. (17773)

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In connection with studies concerning the production of arteriosclerosis in the coronary arteries, the need arose for an adequate method to induce chronic hypertension in the rat. For this purpose, a small, adjustable silver clamp, modeled after the larger Goldblatt clamp(1), has been designed and applied to the renal arteries of the rat(2). The clamp first employed measured 2 mm in length, 1.5 mm in width, and 2 mm in height. That finally used and illustrated in Fig. 1 is considerably smaller and measures 1.7 mm in height, 1.65 mm in length, 1.5 mm in width, and weighs 24 mg. It is made of sheet silver, 0.25 mm thick (0.01″), and nickel wire (0.85 mm diameter). A strip is rough cut to size for the casing (A). The edges are rounded and shaped with a fine stone to exact size and formed with a carefully machined punch die. On the top, a hole is centered, drilled, and tapped for the screw (B). Slots are cut with a saw 0.25 mm from the ends for the removable plate (C). The screw is made of nickel wire which is threaded (6 threads/mm), and one end cut with a lathe and drilled to ac-

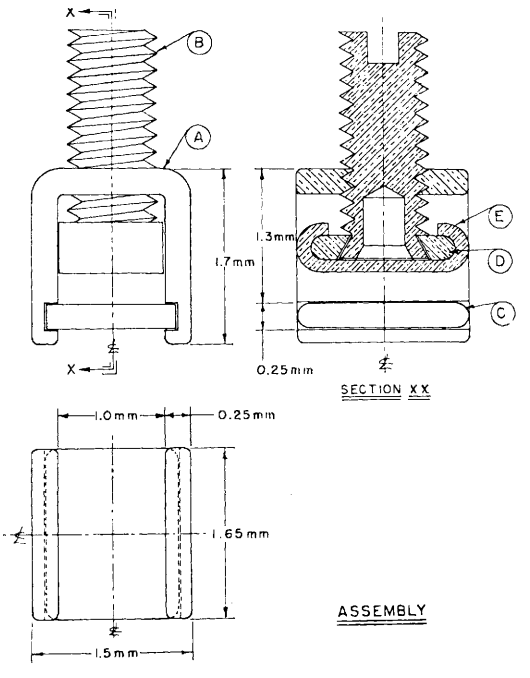

FIG. 1.
Assembly drawing of small, adjustable silver clamp.

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