TABLE III. Blood Eosinophile Response to Injection of Large (160 μg) Doses of Egg Albumin Nitrogen in 4 Normal Guinea Pigs.

Route	Time in hr 0	¼–½	1	2	5	6
I.V.	56	53		175	175	259
"	118	109		68	156	237
I.P.	188	255	350	693	394	160
S.C.	55	33	49	222	316	38

tion of this finding is not clear. The claim (8) that the polysaccharide, independent of immune reaction to it, is primarily toxic and a stimulus to histamine release is not fully supported by the evidence, though this interpretation is certainly possible. Numerous instances have been described both in man and in the experimental animal, wherein antibody to various antigens may be "naturally" acquired. In man, well-documented instances would include the "natural" isoagglutinins and "normal" antidextrans(9). It would be difficult to prove that such "natural" antibodies did not play a role in the above instances. Further, since relatively large doses of antigen have been required for the production of eosinophilia in normal animals the possibility exists that such antibodies might be antibodies to antigen impurity(4). Therefore the experiments in which eosinophilia has been observed in normal animals may need reinterpretation, and particularly is this indicated in those cases(8) in which the animals were noted to die in convulsions, or with dyspnea, after the eosinophilia-producing injections. Such symptoms were not seen in the animals reported in Table III.

Summary and conclusions. It has been shown that eosinophilia in anaphylaxis is not dependent upon active antibody formation by the shocked animal. The development in several instances of a mild eosinophilia following the injection of relatively large doses of Ea into normal guinea pigs was noted.

1. Campbell, A. C. P., Drennan, A. M., and Rettie, T., *J. Path. and Bact.*, 1935, v40, 537.

2. Samter, M., *Blood*, 1949, v4, 217.

3. Bennette, I. L., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 772.

4. Vaughan, J. H., and Kabat, E. A., *J. Exp. Med.*, 1953, v97, 821.

5. Campbell, D. H., *J. Infect. Dis.*, 1943, v72, 42.

6. Burdon, K. L., *Mechanisms of Hypersensitivity and Their Significance In Relation to Certain Aspects of the Radiation Syndrome: A Critical Review.* Project Rep. USAF School of Aviation Med., Randolph Field, Texas, 1953.

7. Vaughn, J., *Blood*, 1953, v8, 1.

8. ———, *J. Path. and Bact.*, 1952, v64, 91.

9. Kabat, E. A., and Berg, D., *J. Immunol.*, 1953, v70, 514.

Received August 5, 1955. P.S.E.B.M., 1955, v90.

Role of Hyperkalemia in Production of Ventricular Fibrillation Following Hypercapnia.* (22021)

E. B. BROWN, JR.
(With the technical assistance of Mrs. Lois Trank and Mr. Richard Clancy.)
Department of Physiology, University of Minnesota, Minneapolis.

Rapid return to air breathing following 2 to 4-hour periods of high CO_2 breathing regularly produces hypotension and cardiac arrhythmias in dogs, and may produce ventricular fibrillation and death(1). Recently Sealy, Young, and Harris(2,3) have confirmed these results and have extended them in an attempt to determine the cause of the severe cardiac effect of this rapid fall in CO_2 tension and hydrogen ion concentration. They were impressed, as we were, with the similarity between the electrocardiograms recorded in the immediate post-hypercapnic period and those characteristic of hyperkalemia. These inves-

* This study supported by funds provided under Contract with the USAF School of Aviation Medicine, Randolph Field, Texas, and by Minnesota Heart Assn.

tigators found a rise in serum potassium during hypercapnia which they believe is causally related to cardiac response.

Experiments reported in this paper suggest that the elevated plasma potassium is not sufficient of itself to produce the severe cardiac effects, but that a rapid decrease in arterial blood CO_2 tension and hydrogen ion concentration in the presence of elevated sub-lethal plasma potassium concentrations may produce cardiac arrest or ventricular fibrillation.

Methods. Five groups of experiments were carried out on dogs anesthetized with either sodium pentobarbital (Group 3) or sodium Pentothal (Groups 1, 2, 4, 5) administered intravenously. Blood pressures were recorded from a femoral artery by means of a strain gauge and one channel of a 2-channel direct writing oscillograph. Electrocardiograms were recorded on the second channel of the oscillograph using limb leads. A catheter for obtaining blood samples was passed through the femoral artery into the aorta and tied in place. Gas mixtures were administered through either an endotracheal tube or a tracheotomy tube from an open system.

In experiments in which KCl solution was administered, it was infused as a .16 M solution in an intravenous drip at approximately 100 drops per minute. Potassium determinations were carried out on a protein free filtrate of plasma with a flame photometer. Syringes for drawing 6 ml blood samples were prepared by allowing 0.1 ml of heparin solution containing 1 mg of heparin to dry overnight in each syringe in a warm oven.

Group 1. Thirteen dogs breathed 30% CO_2-70% O_2 for 2 hr followed by 40% CO_2-60% O_2 for an additional 2 hr, and were then returned to air breathing. Blood samples for assay of plasma potassium concentrations were drawn before starting the CO_2 breathing, at intervals during the 4 hours of CO_2 inhalation, and at intervals during the first hour after returning the animals to air breathing.

Group 2. Eleven dogs were administered KCl solution, while breathing air, until cardiac arrest or ventricular fibrillation appeared. Blood samples were drawn and electrocardiograms and blood pressures recorded before starting the KCl infusion and at intervals during infusion.

Group 3. Control blood samples and recordings were obtained on 8 animals who were then given 30% CO_2 in oxygen to breathe. After 10 minutes on this mixture an intravenous drip of KCl solution was started with the dogs continuing to breathe 30% CO_2. This was continued until ventricular fibrillation or cardiac arrest appeared.

Group 4. Eight dogs were given 30% CO_2 for 2 hours followed by 40% CO_2 for 2 hours and then while they continued to breathe 40% CO_2 an intravenous drip of KCl was started and continued until the heart stopped or the ventricles fibrillated.

Group 5. Thirty % CO_2 for 15 minutes followed by 40% CO_2 for 10 minutes was administered to 6 dogs. While the animals continued to breathe 40% CO_2, KCl solution was given as an intravenous drip until plasma potassium concentration was thought to be about 8 to 9 mEq/L. as judged by the amount of KCl given and by the electrocardiograms. This usually required 5 to 8 minutes. At this point the dog was changed from the high CO_2 mixture to 100% oxygen and hyperventilated at a rate of 50 breaths per minute and a positive pressure of 20 cm of water by means of a positive pressure respirator.

In all experiments during administration of KCl solution, blood pressures and electrocardiograms were recorded at intervals of one

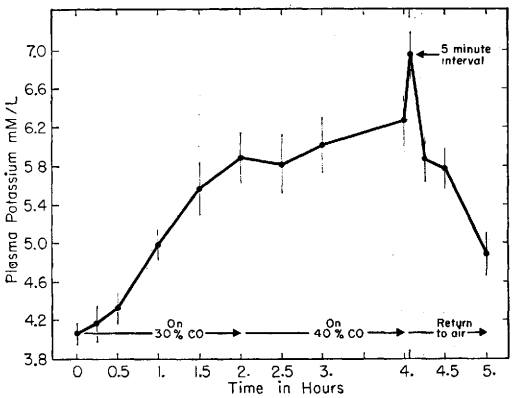

FIG. 1. Mean plasma potassium concentrations in 13 dogs before, during and following 4 hr of breathing 30% and 40% CO_2 in oxygen. Vertical bars extend one stand. error on each side of the mean.

TABLE I. Influence of Inhalation of High CO_2 Concentrations on Terminal Plasma Potassium Concentrations.

Group		No. of dogs	Plasma potassium conc., mEq/L		
			Control	Before drip	Final
2	Breathing air	11	3.62 ± .132*		14.39 ± .329
3	Breathing 30% CO_2 for 15 min.	8	3.39 ± .090	3.66 ± .131	12.98 ± .572
4	Breathing 30% CO_2 for 2 hr followed by 40% CO_2 for 2 hr	8	3.84 ± .241	7.10 ± .416	13.79 ± .890

* Stand. error of mean.

to 2 minutes until severe cardiac arrhythmias appeared, and then continuously until cardiac arrest or fibrillation occurred. Blood samples were drawn at 5 minute intervals during the first few minutes of KCl infusion and at shorter intervals during the remainder of the experiment. An attempt was made to draw the final sample just before cardiac arrest took place. By watching the electrocardiogram and blood pressure tracing this was usually accomplished successfully.

Results. Fig. 1 presents the plasma potassium concentrations of 13 dogs in Group 1 during and following 4 hours of hypercapnia. Two of these dogs died in ventricular fibrillation after 5 minutes of air breathing and are not represented in the data after that time. A gradual increase in plasma potassium concentration during the hypercapnia was a regular finding. In 12 of 13 cases a further and more rapid increase took place during the first 5 minutes of air breathing. Immediately thereafter the concentration began to decrease but was still somewhat elevated one hour after the dogs had been returned to air breathing. The highest value recorded in any of these animals was 8.2 mM/L. at 5 minutes of air breathing following the 4 hours of hypercapnia. This dog died in ventricular fibrillation within seconds after the blood sample was drawn.

To be sure the peak concentration was not being missed, blood samples were drawn at one or 2-minute intervals for 12 minutes following hypercapnia in 4 dogs. In every case the highest concentration was found between 4 and 6 minutes. Table I presents the data for Groups 2, 3, and 4. The mean plasma potassium concentration at cardiac arrest in the dogs breathing air (Group 2) was 14.39 mM/L. This value agrees well with those reported by others(4). When the dogs were made hypercapnic prior to starting the KCl drip (Group 3) cardiac arrest occurred when the plasma potassium concentration averaged 12.98 mM/L. In the 8 dogs that breathed high CO_2 mixtures 4 hours before the KCl infusion began, the average plasma potassium concentration at cardiac arrest was 13.79 mM/L. Although hypercapnia of this degree appeared to lower the plasma potassium concentration at which cardiac arrest appeared, the differences were not impressive and in no instance in any of these groups did arrest or ventricular fibrillation occur when the plasma potassium level was as low as 8.2 mM/L., the highest level found to occur spontaneously during the rapid fall in pCO_2 and hydrogen ion concentration following 4 hours of hypercapnia. In other words no overlap in the terminal plasma potassium values between Group 1 and Groups 2, 3, and 4 appeared.

The possibility still existed, however, that the hyperkalemia and a rapid rise in pH and/or fall in CO_2 tension might be the necessary conditions to produce cardiac arrest or ventricular fibrillation. In earlier work we had demonstrated many times that blood pH falls to approximately 6.7 when a dog breathes 40% CO_2 for 15 minutes to 30 minutes and that rapid reversal of this hyercapnic, acidotic state is not followed by cardiac arrest or fibrillation as it commonly is when the hypercapnia has lasted 2 or more hours. Thus it appeared that the rapid fall in CO_2 tension or rise in pH alone were not producing the untoward cardiac effects. The present data indicate that the plasma potassium level attained following 4 hours of hypercapnia was not sufficient of itself to produce this effect.

To determine whether the combination of a rapid fall in hydrogen ion concentration and pCO_2 in the presence of a sub-lethal level of plasma potassium would produce cardiac ar-

TABLE II. Effect of Rapid Reduction in pCO_2 in Presence of Elevated Plasma Potassium Concentration.

Dog No.	Plasma potassium concentrations, mM/L Control	High CO_2, 25 min.	KCl drip, 5 min.	3 to 7 min. on O_2	Result
1	4.2	4.6	10.2	9.3	Ventricular fibrillation
2	3.9	3.9	8.7	8.4	" "
3	3.7	3.8	8.4	11.5	" "
4	3.6	4.0		6.3	Cardiac arrhythmia (dog lived)
5	4.2		10.0	10.0	Ventricular fibrillation
6	3.6		11.9	11.3	Cardiac arrest

rest or fibrillation, the experiments in Group 5 were carried out. The data for this group of experiments are given in Table II. In one dog the plasma potassium concentration during the hyperventilation was only 6.3 mM/L. The electrocardiogram on this dog showed cardiac arrhythmias but the dog did not die. In 3 other cases the potassium level when fibrillation began was high enough that the hyperkalemia alone might have produced the result, although the values were well below the average for the dogs that received KCl during the hypercapnia. In 2 dogs of this series ventricular fibrillation occurred during the first 7 minutes of hyperventilation at a time when the plasma potassium concentrations were 8.4 mM/L. and 9.3 mM/L. respectively.

Comment. The pattern of changes in plasma potassium concentration during inhalation of high CO_2 mixtures appears to be different in dogs from that which has been observed in cats. Cattell and Civin(5) in attempting to separate the hypoxic effect from the hypercapnia effect of asphyxia, administered 10% CO_2 to cats and found a marked but temporary rise in serum potassium. MacKay(6) followed up this work by administering 35% CO_2 to cats and obtained similar results. An increase amounting to 3 to 5 times the normal value appeared within 10 minutes after beginning the CO_2 administration and returned to normal in 30 minutes. In addition a second rise took place when the cats were returned to air breathing. The first of these increases but not the second was blocked by tying off the blood supply of the abdominal viscera.

In dogs there appears to be a gradual increase in plasma potassium level during 4 hours of high CO_2 breathing, with an increase in slope during the first 5 minutes of air breathing following the CO_2. This latter change is similar to that reported in cats. In order to be sure that very rapid changes were not appearing during the first few minutes of CO_2 breathing in the dog, blood samples were drawn at 5-minute intervals for the first 30 minutes in several animals. All of the values obtained fell between the control and 30-minute sample. The explanation for this difference between cats and dogs in mobilization of potassium is not available at present.

Young, Sealy, and Harris(3) found a simultaneous rise in cardiac muscle potassium and plasma potassium concentration in the rat following 2 to 4-hour periods of exposure to 35 to 45% CO_2. What changes occur in heart muscle potassium during the rapid shift in blood pH are not known. The work of Fenn and Cobb(7) would suggest that heart muscle potassium should fall at the time carbon dioxide tension is falling. They found a shift of potassium from frog muscle to blood when carbon dioxide tension was reduced. Young *et al.*(3), however, found the cardiac muscle potassium still elevated after rats were removed from an atmosphere of high CO_2 to air. It may be that changes in some other electrolyte are playing a role in the cardiac effect. We have found blood pH regularly falling to approximately 6.7 when the animal breathes 40% CO_2. On changing to air the pH rises above 7.2 in 5 minutes or less. It is possible that this rapid shift in hydrogen ion concentration itself is responsible for the altered response of the heart.

A post-hypercapnic phenomenon similar to that exhibited by dogs has been demonstrated in man(8,9) and it has been suggested that

certain unexplained post-surgical deaths may be due to a rapid reduction in CO_2 tension which has become elevated during anesthesia. It would be interesting to know what changes take place in plasma potassium concentration in the human during prolonged exposure to severe hypercapnia. It seems likely that it is elevated but it would be important to know how rapidly and to what extent, as a function of time and CO_2 concentration, since this would have a bearing on the susceptibility of the heart to fibrillation or arrest when the CO_2 tension is reduced rapidly.

Summary and conclusions. 1. Dogs regularly show an increase in plasma potassium concentration during breathing of 30% and 40% CO_2 mixtures. Immediately following return to air breathing, there is a further sharp rise in this concentration. This secondary rise reaches a peak after 5 minutes of air breathing. 2. Concentration of plasma potassium following 4 hrs of high CO_2 breathing is not as high as that which is necessary to produce fibrillation or arrest when KCl solution is administered as an intravenous drip to normal or acidotic dogs. 3. Rapid return to air breathing following 30 minutes of high CO_2 breathing does not produce cardiac arrhythmias, ventricular fibrillation, or cardiac arrest in dogs. However, when this rapid elevation in blood pH and/or fall in pCO_2 takes place in the presence of sub-lethal but elevated plasma potassium concentrations, cardiac arrest or ventricular fibrillation may occur. Although this does not provide an explanation of the mechanism involved, it appears that the combination of elevated but sub-lethal plasma potassium concentration and a rapid fall in CO_2 tension and/or hydrogen ion concentration, work together to produce the damage to the heart.

1. Brown, E. B., Jr., and Miller, F., *Am. J. Physiol.*, 1952, v169, 56.

2. Sealy, W. C., Young, W. G., and Harris, J. S., *J. Thor. Surg.*, 1954, v28, 447.

3. Young, W. G., Sealy, W. C., and Harris, J. S., *Surgery*, 1954, v36, 636.

4. Huggins, R. A., Breckinridge, C. G., and Hoff, H. E., *Am. J. Physiol.*, 1950, v163, 153.

5. Cattell, M., and Civin, H., *J. Biol. Chem.*, 1933, v126, 633.

6. MacKay, J., *Am. J. Physiol.*, 1947, v151, 469.

7. Fenn, W. O., and Cobb, D., *ibid.*, 1935, v112, 41.

8. Miller, F. A., Brown, E. B., Jr., Buckley, J. J., Van Bergen, F. H., and Varco, R. L., *Surgery*, 1952, v32, 171.

9. Buckley, J. J., Van Bergen, F. H., Dobkin, A. B., Brown, E. B., Jr., Miller, F. A., and Varco, R. L., *Anesthesiology*, 1953, v14, 226.

Received September 21, 1955. P.S.E.B.M., 1955, v90.

Photometric Method for Estimation of Elastase Activity.* (22022)

LEO A. SACHAR, KATHLEEN K. WINTER, NELLE SICHER, AND SAM FRANKEL.
(Introduced by H. T. Blumenthal.)
Division of Laboratories, Jewish Hospital of St. Louis, Washington University School of Medicine, St. Louis, Mo.

The presence of an enzyme in animal pancreatic extracts which solubilizes elastin was first described by Balo and Banga(1). The activity was measured gravimetrically and by Kjeldahl nitrogen. Using the same technics, Hall(2), and later Lansing *et al.*(3), confirmed the presence of this enzyme in extracts of hog pancreas. Since both methods are somewhat cumbersome, a simpler colorimetric procedure was devised; it permits the direct measurement of the amount of dyed elastin which goes into solution when the dyed substrate reacts with pancreatic extracts.

Material and methods. Substrate. Purified elastin was prepared by the Lansing, Alex and Rosenthal(4) modification of the method of Lowry *et al.*(5). The ligamentum nuchae of horses was freed of the thin investing fascia and then ground into small pieces in a meat

* This investigation was supported by the Jacob Schultz Memorial Research Fund.