effect of diet on survival time of chicks was not significant. However, the survival times were 72.8 and 68.0 minutes for the control and fat-fed birds, respectively. The significant run × diet interaction apparently caused the main effect of diet to be obscured.

The chicks used in Experiment 2 were a commercial strain not previously selected for heat tolerance. Therefore, the variation in this population was considerably greater than that of the selected population used in Experiment 1.

Differences between runs and run × diet interaction were both significant. This was attributed to the greater individual variation in the chicks used.

Results from these two experiments indicated that it was of utmost importance to know the previous breeding program of the experimental birds. Since the background of the chicks used in Experiment 1 was known, it was possible to explain why they were less variable than the chicks in Experiment 2.

Summary. Two experiments were conducted to test the effect of diet composition on heat tolerance. The addition of 12% animal fat to the diet significantly reduced the survival time of chicks when they were exposed to 40.8 ± 0.3°C and 75 ± 5% relative humidity. The level of protein in the diets had no effect. The birds used had been previously selected for either a high or low tolerance to heat. In Experiment 2, the differences between runs and the run × diet interaction were both highly significant. The interaction obscured the main effect of diet which was not significant. This population which was unselected with regard to heat tolerance had such great variation that large numbers of birds would be required to test effects of diet on heat tolerance.

1. KheirEldin, M. A., Shaffner, C. S., Poultry Sci., 1954, v33, 1064.
2. Wilson, H. R., Armas, A. E., Ross, I. J., Dorminey, R. W., Wilcox, C. J., ibid., 1966, v45, 784.
3. Clark, C. E., Amin, M., ibid., 1965, v44, 1003.
4. Dorminey, R. W., Ross, I. J., Wilson, H. R., Proc. So. Agri. Workers, 1966, 315.
5. Mickelberry, W. C., Rogler, J. C., Stadelman, W. J., Poultry Sci., 1966, v45, 313.
6. Heywang, B. W., ibid., 1947, v26, 38.
7. Baldini, J. T., Rosenberg, H. R., ibid., 1957, v36, 432.
8. Mitchell, P. H., A Textbook of Biochemistry, 1950, 408.
9. Forbes, E. B., Voris, L., Ann. Rev. Physiol., 1943, v5, 105.
10. Combs, G. F., Feedstuffs, 1964, v36, 30.
11. Adams, R. L., Andrews, F. N., Gardener, E. E., Fontaine, W. E., Canick, C. W., Poultry Sci., 1962, v41, 588.
12. Snedecor, G. W., Iowa College Press, 1956, 5th Ed.
13. Robinson, K. W., Lee, D. H., J. Animal Sci., 1947, v6, 182.
14. Fox, T. W., Poultry Sci., 1951, v30, 477.

Received July 5, 1967. P.S.E.B.M., 1967, v126.

Effect of Hypocapnia and Respiratory Alkalosis on Cardiac Contractility.* (32519)

WILLIAM B. WEAD AND ROBERT C. LITTLE
Department of Physiology, College of Medicine, Ohio State University, Columbus

The effect of hypocapnia on cardiac performance remains open to question. Studies on isolated muscle by Vaughan Williams(1) and others(2,3) suggested that a reduction in P_{CO_2} is accompanied by an increase in myocardial contractility. This finding has been supported by data from unanesthetized human studies(4) and the dog heart-lung preparation(5). Recently, Little and Smith(6) and Theye and coworkers(7) have presented evidence that the drop in arterial P_{CO_2} that accompanies hyperventilation in anesthetized dogs or man is associated with a reduction in cardiac performance. Other investigators

* Condensed report of thesis presented by W. B. W. in partial fulfillment of the requirements for M. S. degree in Physiology. Supported in part by a grant from the Central Ohio Heart Association.

(8,9,10,11) have shown that a low arterial Pco_2 may be present without significant change in myocardial function. Some of these studies were complicated by respiratory and other effects of hypocapnia. We have, therefore, elected to restudy this relationship in the isolated cardiac muscle preparation where Pco_2, pH, and bicarbonate concentration could be closely regulated.

Methods. Atrial strips, approximately 2 $\times$ 10 mm, were prepared from the right atrial appendage of rabbits lightly anesthetized with sodium pentobarbital. These strips were placed in an oxygenated modified Ringer-Locke's solution containing the following components in mM per liter of solution: NaCl, 124.8 $NaHCO_3$, 20.0; Dextrose, 10.0; KCl, 5.63; $CaCl_2$, 2.16 and $MgCl_2$, 2.10. Each strip was secured by stainless steel hook electrodes placed at each end. Resting tension was adjusted to 2 g and essentially isometric contractions were produced by external stimulation at a rate of 60 per minute. Contraction force was measured with a Grass Model Ft .03 Force Displacement Transducer.

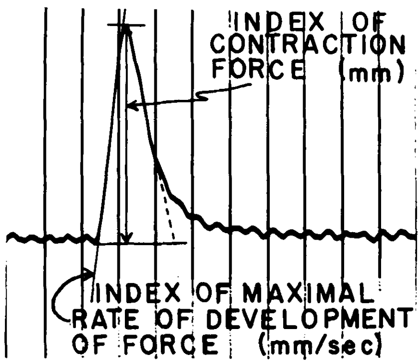
The control bathing solution was adjusted to a pH of 7.38, Pco_2 of 34 mm Hg, and bicarbonate concentration of 20 mM per liter. Thirty-minute periods of exposure to this bathing solution were alternated in most experiments with an equal exposure to an experimental solution. In this way, each experimental solution was preceded and followed by a control exposure. Solution A had a Pco_2 less than 10 mm Hg and a bicarbonate concentration of less than 10 mM per liter. The pH of this solution was the same as the control. Solution B had the same Pco_2 as solution A but a pH of 7.7 to 7.9 with a bicarbonate concentration the same as control. Osmotic changes caused by reducing the $NaHCO_3$ concentration in solution A were adjusted by reciprocally increasing the sodium chloride concentration.

The isometric contraction force was recorded and evaluated by measuring (1) the maximum rate of force development (force-time ratio) and (2) the maximum force produced. The height of the contraction record was measured in mm and used as an index of contraction force. A typical record labeled to show these measurements is shown in Fig. 1. Each observation reported here is the average of 5 consecutive contractions.

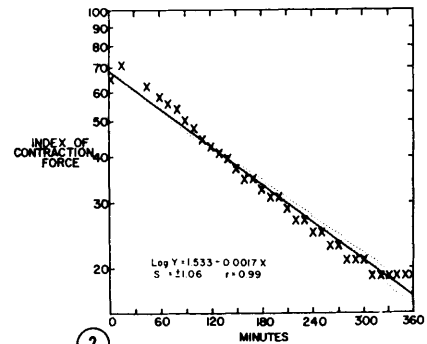
Results. A series of control observations was made over a 6-hour period. A semilog plot of force index data from such an experiment is shown in Fig. 2. A similar linear regression with time was observed in all our experiments for both the index of contraction force and the force-time ratio.

Experimental data suitable for analysis were obtained from 8 muscle preparations. In these experiments the control and the experimental bathing solutions were alternated. The index of contraction force data from a typical experiment is shown in Fig. 3 as a semilog plot against time. Only data from the control periods are shown in this figure, and the calculated control regression line is extended through the time period when experimental bathing solutions were used. It is interesting that this regression line calculated for interrupted exposure to the control solution is very similar to the regression line shown in Fig. 2 where the muscle was continuously exposed to the control solution. This plot serves to establish the control regression line which by extrapolation can be utilized to predict the expected performance during the experimental periods.

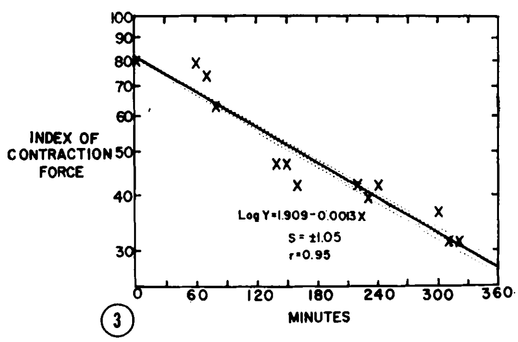
Data from this same experiment but obtained during the exposure to experimental solutions A and B are plotted in Fig. 4. The solid line shows the same control regression line plotted in Fig. 3. The data obtained during exposure to solution A are shown by triangles and coincides very closely with the extrapolated control regression line. The data obtained with solution B, which had the same low Pco_2 as solution A but an alkaline pH, are shown by the open circles. They are clearly displaced above the control regression line. The dashed line in the Figure shows the calculated regression line for the data obtained with solution B. Force-time data from the same experiment are plotted in Fig. 5. The control regression line for this parameter was plotted in the same manner as was done for the index of contraction force. This is shown as the solid line in



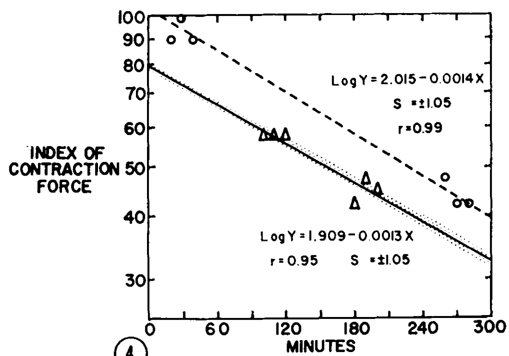
①



②



③



④

FIG. 1. A typical record of a muscle contraction labeled to show the measurement used for evaluation of myocardial performance.

FIG. 2. Semilog plot of index of contraction force during exposure to the control bathing solution. The calculated regression line and its formula are shown. The stippled area comprises one sample standard deviation (S) on each side of regression line.

FIG. 3. Semilog graph of index of contraction force showing data obtained during exposure to the control bathing solution. The calculated regression line and sample standard deviation are plotted as in Fig. 2. See text for discussion.

FIG. 4. Semilog plot of index of contraction force from same experiment shown in Fig. 3 but showing data obtained during exposure of a low P_{CO_2}, control pH (triangles), and low P_{CO_2} high pH (circles). The calculated regression line for the open circles is shown by the dashed line. The solid line and stippled area are the same as shown in Fig. 3. See text for further discussion.

the Figure. The maximal rate of tension development ($\Delta F/\Delta t$) during exposure to the low P_{CO_2}, control pH bathing solution (triangles) also agrees closely with the extrapolated control regression line while the data for this parameter recorded during exposure to the same P_{CO_2} but a higher than control pH (open circles) are again displaced above the regression line.

Data from this study were subjected to an analysis of covariance. The results indicate that the data obtained with the low P_{CO_2} control pH bathing solution were not significantly different ($P>0.05$) from results predicted from the control observations. However, the index of contraction force and force-time ratio were significantly different when exposed to the low P_{CO_2} solution if the pH was permitted to rise above control ($P<0.01$).

Discussion. The data presented above suggest that cardiac contractility is increased above control when the isolated myocardium is exposed to an environment with a low P_{CO_2} and a pH above the usual level of 7.38. Our findings do not permit a final explanation for this positive inotropic effect. Various mechanisms have been postulated to explain the action of CO_2 on cardiac contraction. These include changes in intracellular pH

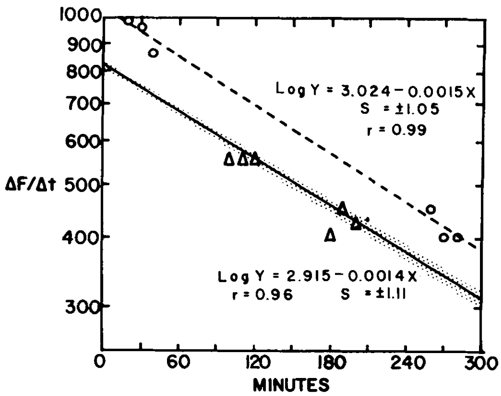


FIG. 5. Semilog graph of force-time data plotted in same manner as Fig. 4. The solid regression line and its formula shows the control data. Data points for this measurement are not included in Figure. The dashed liine shows the calculated regression line for the low Pco_2, high pH data (circles). Data for exposure to a low Pco_2, control pH is shown by triangles. See text for further discussion.

and movement of ions(1,12), release of intracardiac potentiating substances(13), and changes in enzyme kinetics(14). The data from this study suggest that the change in pH that accompanies the reduction in Pco_2 is the important factor and not the level of CO_2 itself.

It would have been of value in our study to have used an alkaline bathing solution with a Pco_2 in the control range. However, this required a bicarbonate concentration above 20 mM per liter. At these levels we observed the same wide fluctuation in results that were first reported by Vaughan Williams (1) and thus were not able to secure useful data when this solution was used.

Summary. Isolated myocardial strips of rabbit atrium were exposed to an oxygenated, modified Ringer-Locke's solution having various levels of Pco_2, pH, and bicarbonate. Peak contraction force and maximum rate of tension development were used to evaluate myocardial contractility. Exposure to a low Pco_2 and control pH environment did not cause a significant change in contractility. Myocardial performance was significantly increased by exposure to a bathing solution containing a low Pco_2 and high pH. The results are interpreted to indicate that the change in pH is the significant factor in the positive inotropic response to low Pco_2.

1. Vaughan Williams, E. M., J. Physiol., 1955, v129, 90.
2. McElroy, W. T., Gerdes, A., Brown, E. B., ibid., 1955, v105, 412.
3. Wang, H., Katz, R. L., Circ. Res., 1965, v17, 114.
4. Burnum, J. F., Hickman, J. B., McIntosh, H. D., Circulation, 1954, v9, 89.
5. Areskog, N., Acta Physiol. Scand., 1962, v55, 139.
6. Little, R. C., Smith, C. W., Am. J. Physiol., 1964, v206, 1025.
7. Theye, R. A., Milde, J. H., Michenfelder, J. D., Anesthesiology, 1966, v17, 778.
8. Lentini, E. A., Billings, S. D., Proc. Soc. Exp. Biol. & Med., 1964, v116, 484.
9. Monroe, R. G., French, G., Whittenberger, J. L., ibid., 1960, v199, 1121.
10. Opie, L. H., ibid., 1965, v209, 1075.
11. Row, G. G., Castillo, C. A., Crumpton, C. W., Am. Heart J., 1962, v63, 67.
12. Adler, S. R., Arlene, R., Relman, A. S., J. Clin. Invest., 1965, v44, 8.
13. Whalen, Wm. J., Fishmen, N., Erickson, R., Am. J. Physiol., 1958, v194, 573.
14. Kaye, L., Mommarts, W. F. H. M., J. Gen. Physiol., 1960, v44, 405.

Received July 5, 1967. P.S.E.B.M., 1967, v126.