The heart of the chick embryo has been used as a bioassay tool in the evaluation of cardiotonic agents such as digitalis because of the ease of observation, ready availability and reproducible response. In a study of metabolic intermediates which might augment the activity of the heart it became apparent that the chick heart had to be stressed to an embarrassing degree in order to make an accurate appraisal of the compound tested. The stress induced would have to be less than lethal, constant in character, easily reproduced and promptly reversible. The most apparent approach to the induction of such a state of stress was the alteration of the concentrations of oxygen and carbon dioxide in the gaseous environment of the heart preparation. The results of these studies form the substance of this report.

METHODS
Hearts isolated from 72 hour chick embryos were microscopically observed in 32 ml. vials stoppered with paraffined corks which contained sealed inlet and outlet tubes. One heart was implanted in each of 6 separate drops of chicken plasma which were placed en the inner wall of the vial with a platinum loop 4 mm. in diameter. After the plasma had clotted, 1 ml. of Tyrode solution containing phenol red as an indicator was pipetted into the vial, and the hearts were bathed with the solution for 10 min. in atmospheric air. Three minutes later (a total of 13 min. after the introduction of the Tyrode solution), the vials were rotated through 180° to permit microscopic examination of the hearts, and the first observation was made (figs. 1 and 2, control). One liter of the gas mixture to be studied was then run through the vial, which was being rotated, and after a 13 min. equilibration period, the second observation was made (13 min.). The 13 min. period was selected because preliminary experiments indicated that this period of time was necessary to establish equilibrium. After another 13 min. period in the gaseous atmosphere being tested, the third observation was taken (26 min.). Finally, the test gas mixture was flushed from the tube with 1 L. of air, and after 13 min. of equilibration, the final observation was made (39 min. or recovery observation). All observations were made at a temperature of 38 °C and consisted of notations of the heart rate and of the appearance of arrhythmias or asystole.

The gases tested were: 20 (air), 16, 12, 8, 4 and 0 per cent O₂ in the absence of CO₂; the same concentrations of O₂ in the presence of 4 per cent CO₂; and 1, 2, 4, 8, 12, 25, 50 and 100 per cent CO₂. Nitrogen was used to bring the gases to volume. For each gaseous mixture, 5 vials containing 6 hearts each were used. Because one or more hearts were sometimes washed from the clots, the number of hearts in an experiment varied from 24 to 30.

The data from a series of studies were incorporated into a single illustration for clarity by plotting the mean and the standard deviation of heart rate or the appearance of arrhythmias at the four 13-min. observation periods (figs. 1 and 2).

0.02; MgCl₂·6H₂O, 0.01; NaH₂PO₄, 0.005; NaHCO₃, 0.04; pH, 7.4; no glucose.
RESULTS

In figure 1, the effects of a graded reduction in oxygen concentration in the absence of carbon dioxide upon the activity of the chick embryo heart are plotted. Exposure of the hearts to air throughout the four separate equilibration periods caused no alteration in heart rate. Except for an occasional instance of arrhythmia, the mixtures containing 16 and 12 per cent O₂ were without deleterious effects. Oxygen concentrations of 8 and 4 per cent produced a slowing of the heart with the appearance of arrhythmias and asystole. The deleterious effects of these low oxygen concentrations were not completely reversed, in a large number of hearts, by the flushing of the vials with air. Exposure of the hearts to 100 per cent N₂ induced asystole and apparent irreversible damage as observed by the failure to recover. In this series of studies the pH of the Tyrode solution as determined colorimetrically by phenol red was 7.8 to 8.0.

Decreasing oxygen concentration from 20 to 0 per cent in the presence of 4 per cent CO₂ (fig. 2) produced a distinctly different effect from that observed in the absence of carbon dioxide. The mean heart rate was reduced upon the addition of 4 per cent CO₂ to the 20, 16 or 8 per cent O₂ and nitrogen mixtures. A marked bradycardia occurred at 4 per cent O₂ concentration and asystole developed at 0 per cent O₂. Arrhythmias and asystole which were observed at 8 per cent O₂ concentration in the absence of carbon dioxide were less frequent or absent in the experiments in which 4 per cent CO₂ was added to the 8 per cent O₂ but were noted in the mixtures containing 4 per cent CO₂ and 4 or 0 per cent O₂. In contradistinction to the irreversible effects of progressive hypoxia in the absence of carbon dioxide, reduction or complete withdrawal of oxygen in the presence of carbon dioxide produced myocardial effects which were promptly reversed by the introduction of 20 per cent O₂ into the vials. The addition of 4 per cent CO₂ to the gaseous environment lowered the pH of the Tyrode solution to a range of 6.8 to 7.0 (bromthymol blue).

Since the presence of 4 per cent CO₂ in the gaseous environment permitted the hearts to withstand total anoxia for two successive 13 min. periods without irreversible damage, experiments were designed to determine the period of total anoxia in the presence of 4 per cent CO₂ that the chick heart could tolerate before permanent damage occurred. The maximum exposure time after which consistently rapid and complete recovery occurred was found to be 30 min.
To test the effects of different concentrations of carbon dioxide in the absence of oxygen upon the activity of the chick heart, mixtures of 1, 2, 4, 8, 12, 25, 50 and 100 per cent CO₂ in nitrogen were used. Hearts exposed to carbon dioxide concentrations of 1 and 2 per cent did poorly in that a large number of the hearts failed to return to normal upon flushing the vial with atmospheric air. Carbon dioxide concentrations of 4, 8, 12 and 25 per cent produced no irreversible damage. Exposure of the hearts to 50 and 100 per cent CO₂ resulted in a sluggish return to the control level upon flushing the vials with atmospheric air. Thus, in this series, 4 per cent CO₂ in the presence of 96 per cent N₂ was the lowest concentration of carbon dioxide which permitted the hearts to return promptly and consistently to control values of rate and rhythm upon flushing with atmospheric air.

**DISCUSSION**

The chick embryo hearts reacted in a reproducible manner to alterations in the concentrations of oxygen and carbon dioxide with the appearance of a bradycardia, arrhythmias or asystole. The ability of the chick heart to tolerate absolute anoxia for a period of 0.5 hour without detectable permanent damage is somewhat surprising because cardiac muscle is known to function poorly during hypoxia. Survival of the heart after anoxia or prolonged hypoxia is dependent upon the activity of the heart during anaerobiosis and upon the capacity for buffering incompletely metabolized end products. The chick heart reacted to anoxia by becoming completely quiescent, i.e., asystolic, thereby operating at the lowest possible metabolic level. The 0.5 hour period during which the chick heart tolerated total anoxia is in surprisingly good agreement with the findings for the isolated dog heart perfused with blood which had been exposed to 5 per cent CO₂/N₂; the mammalian heart began to manifest failure by elevation of venous pressure or by reduction in cardiac output after 32 to 106 min. of perfusion.

Recovery of the chick heart after exposure to total anoxia for a period of 30 min. occurs only if carbon dioxide is present in the gaseous environment during the exposure period. The concentration of carbon dioxide in the oxygen-free mixture significantly affects the degree of recovery. Carbon dioxide appears to protect the heart from the irreversible effects of anoxia at concentrations of 4, 8, 12 or 25 per cent. This interesting effect is probably related to the formation of bicarbonate and the resultant augmentation of buffering capacity of the plasma clot for the acid metabolic intermediates of the chick heart. Lower concentrations of carbon dioxide, i.e., 1 and 2 per cent, apparently did not sufficiently increase the buffering capacity of the plasma clot, and the anoxia produced in the 1 or 2 per cent CO₂/N₂ mixture was more lethal to the chick heart than the 4 per cent CO₂/N₂ mixture. Exposure of the chick heart to a gaseous environment devoid of oxygen even in the presence of 4 per cent CO₂ for a period longer than 30 to 60 min. resulted in irreparable damage. This irreversible effect may be the result of cellular damage caused by prolonged anoxia or by the accumulation of acid intermediates.

When large concentrations of carbon dioxide (50 and 100 per cent) in nitrogen were used as the gaseous perfusate, a marked lag in recovery was observed. With these concentrations of carbon dioxide the pH of the plasma clot was dropped below 6.8. Previous work indicates that the isolated heart of the frog withstands acidosis poorly. The addition of 4 per cent CO₂ to 20 per cent O₂ (air) caused a reduction in the pH of the plasma clot to 6.8 to 7.0 and a reduction of heart rate. Carbon dioxide concentrations of 4, 8, 12 and 25 per cent in nitrogen alone appeared to have similar cardiogenic effects although the pH change in the plasma clot was not critically followed. However, carbon dioxide concentrations greater than 4 per cent in nitrogen alone produced some deleterious effects which were presumably the result of an excessive depression of pH in that some of the hearts did not promptly recover from the total anoxia.

It should also be noted that the addition of 4 per cent CO₂ to the gaseous environment
during progressive hypoxia resulted in a steady and reversible state of stress, i.e., bradycardia, arrhythmia or asystole (fig. 2), whereas in the absence of CO\textsubscript{2}, deterioration of the heart preparation occurred during the hypoxic state (fig. 1).

It would appear from the results of these studies that the gaseous environment which more easily induces a reversible and steady state of stress in the chick embryo heart is the 4 per cent O\textsubscript{2}/4 per cent CO\textsubscript{2}/N\textsubscript{2} mixture. The state of cardiac irregularities which resulted in this environment remained constant during the approximately 0.5 hour of observation and was promptly reversed to the control level of rate and rhythm upon flushing the vial with air.

**Summary**

Progressive reduction of oxygen in the gaseous environment of the 72 hour chick embryo heart resulted in the following changes: Hypoxia in the absence of carbon dioxide resulted in alterations in rhythm and rate of the heart at 12 and 8 per cent O\textsubscript{2} concentrations. Four per cent and 0 per cent O\textsubscript{2} produced irreversible arrhythmia or asystole. Similar degrees of hypoxia in the presence of 4 per cent CO\textsubscript{2} did not irreversibly damage the chick heart. The embryonic chick heart can withstand total anoxia in the presence of 4 per cent CO\textsubscript{2} for a period of 30 min. without sustaining damage. Concentrations of CO\textsubscript{2} below 4 per cent failed to prevent the damage to the chick heart resulting from anoxia. A gaseous environment of 4 per cent O\textsubscript{2}/4 per cent CO\textsubscript{2}/92 per cent N\textsubscript{2} induced a reproducible, steady and an entirely reversible state of cardiac stress.

**Summary in Interlingua**

Le reduction progressive del oxygeno in le ambiente gassoso del corde de embryones de gallina post 72 horas resultava in le sequente alterationes: Hypoxia in le absentia de bioksidio de carbon resultava in alterationes del rhythmio e del velocitate del corde a concentraciones de 12 e de 8 pro cento de O\textsubscript{2}. Quatro e zero pro cento de O\textsubscript{2} produciva irreversibile arrhythmia o asystole. Simile grados de hypoxia in le presentia de 4 pro cento de CO\textsubscript{2} non produciva irreversible dannos in le corde embryonie de gallina. Ilio pote endurar anoxia total in le presentia de 4 pro cento de CO\textsubscript{2} durante 30 minutas sin suffrer ulle dannio. Concentrationes de CO\textsubscript{2} de infra 4 pro cento non preveniva le damno resultante in le corde embryonie de gallina in consequentia de anoxia. Un ambiente gassoso de 4 pro cento de O\textsubscript{2}, 4 pro cento de CO\textsubscript{2}, e 92 pro cento de N\textsubscript{2} induceva un reproducibile, constante, e completamente reversibile stato de stress cardiac.

**References**

Effects of Different Oxygen and Carbon Dioxide Concentrations on the Activity of the Embryonic Chick Heart
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