corticosterone and ascorbic acid changes in the same gland indicated that no response occurred until the animals were approximately 8 days old, at which time stress caused changes in both these substances. The early postnatal period in the rats' life when stress does not result in pituitary-adrenal activation we propose to call the stress-non-responsive period (S-N-R period). Both synthetic and commercial vasopressin administered to rats during the S-N-R period caused an increase in corticosterone content of the adrenal gland, and less consistently a decrease in ascorbic acid. It is suggested that the S-N-R period may be related to immaturity of the hypothalamic osmoreceptor mechanism which underlies adjustments in vasopressin secretion.

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Effect of Oxygen at High Pressure (OHP) on Asphyxial Survival Time of Rats.* (27385)

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Hypothermia is well known to be effective in increasing the tolerance of animals to the deleterious effects of anoxia and asphyxia(1). Theoretically, another means whereby the oxygen demands of tissue metabolism can be met, even in absence of effective O_2 transport by hemoglobin, is to increase the amount of physically dissolved O_2 in blood and tissue fluids by exposure to O_2 at high pressure(OHP). The possibility that combining OHP and hypothermia might significantly increase the tolerance of animals to the effects of anoxia and asphyxia above that seen with either treatment alone thus appeared worthy of investigation.

This report presents the results of experiments designed to test whether or not moderate OHP (*i.e.,* 3 atmospheres absolute) has any effect on asphyxial survival time of normothermic and hypothermic rats. Experiments are also presented in which the effects

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of varying the amounts of O_2 and CO_2 under pressure have been studied.

Materials and methods. Male Long-Evans rats weighing between 300-400 g were used. Experiments were divided into 8 groups. *Group I.* Normothermic anesthetized (20 mg/kg pentobarbital, IP) rats breathing room air at ambient temperature and pressure (ATP). *Group II.* Normothermic, anesthetized rats breathing 100% O_2 for 10 minutes at ATP. *Group III.* Hypothermic (20°C rectal temperature) anesthetized rats breathing room air (ATP). *Group IV.* Hypothermic, anesthetized rats breathing 100% O_2 for 10 minutes (ATP). *Group V.* Normothermic anesthetized rats breathing 100% O_2 at 2 atmospheres (in a pressure chamber) for 10 minutes. *Group VI.* Hypothermic, anesthetized rats breathing 100% O_2 at 2 atmospheres for 10 minutes. *Group VII.* Normothermic anesthetized rats breathing 99% O_2-1% CO_2 at 2 atmospheres for 10 minutes. *Group VIII.* Normothermic, anesthetized rats breathing 90% O_2-10% CO_2 at 2 atmospheres for 10 minutes. Animals in Groups I, II, V, VII, and VIII were first anesthetized, immobilized on a board, and a polyethelene cannula inserted into the trachea. Animals in Groups III, IV, and VI were anesthetized, cooled to 20°C by immersion in an ice bath, immobilized on a board, and tracheal cannulas inserted.

In Groups I-IV inclusive, the animals were allowed to breath the appropriate gas mixtures for 10 minutes following tracheal cannulation. At the end of 10 minutes, the trachea was occluded at the peak of an inspiratory movement. In Groups V-VIII inclusive, the procedure was to place the immobilized animals in a 36″ × 12″ × 20″ pressure chamber (which had been previously flushed with the test gas), seal the chamber, and bring the chamber pressure to 2 atmospheres in 2 minutes. The animals were allowed to breath the test gases for 10 minutes at 2 atmospheres, then the tracheal cannula was occluded by a remotely actuated device which permitted clamping the cannula without having to open the chamber. Thus, the chamber pressure was held at a constant 2 atmospheres throughout the experiment, avoiding the effects of decompression. In some experiments Lead II ECG's were recorded. Chamber pO_2 values were occasionally monitored by a Beckman oxygen analyzer attached to a vent circuit from the chamber interior. Seven animals were studied in each group.

Results. In all animals studied, except those of Group VIII, occluding the trachea produced 2 distinct changes in respiratory patterns which could be considered as indices of "asphyxial survival time." First, there was the characteristic disappearance of regular diaphragmatic and thoracic respiratory movements. Following this last regular respiratory effort, an interval was noted where no visible respiratory excursion occurred (apnea). After this period of total respiratory arrest, there was a sudden onset of progressively deepening "gasping" or "convulsive" movements of the diaphragm and body. Once these movements ceased, no further respiratory or other gross muscular activity was seen. This was noted as the time of "last gasp or convulsion." Recording of ECG's indicated, however, that electrical activity from the heart persisted for many minutes. Considerable variation in ECG persistence time was found, and was not considered as valid an end point for "physiological death" under the conditions of these experiments.

Fig. 1 shows the effects of the various treatments on time of disappearance of last regular respiration and time of last gasp or convulsion following tracheal occlusion. Note the consistent relationship between the disappearance of regular respiration and last

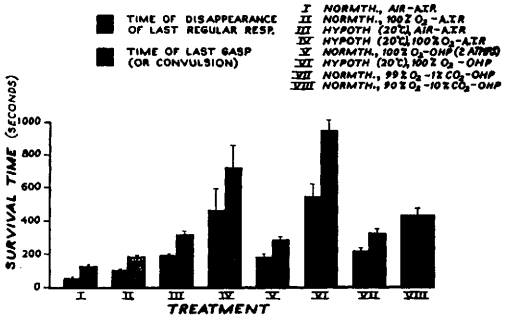

FIG. 1. Effects of O_2 and O_2-CO_2 mixtures at ATP and OHP on asphyxial survival time of normothermic and hypothermic rats. See text for complete description of treatments and criteria for measurement of survival. Vertical bar represents one S.E. of mean.

gasp in all groups except those animals exposed to the 90% O_2-10% CO_2 mixture at 2 atmospheres. In this group, no clear distinction was noted between these 2 respiratory events.

Although mean survival time for animals in Group VI was the highest of any of the treatments studied, there was no significant difference from the survival times of Group IV ($p>0.05$). Treatment of normothermic animals with 99% O_2-1% CO_2 at 2 atmospheres (Group VII) afforded approximately the same protection as observed with hypothermia-air at ATP (Group III) and normothermia-100% O_2-OHP (Group V).

Discussion. Tracheal occlusion in rats produces 2 distinct periods of respiratory activity separated by a period of apnea (Tschirgi and Gerard)(2). On the basis of differences in gasping time noted in rats with intact and denervated carotid chemoreceptor mechanisms, these investigators felt that the time for appearance of last gasp may be considered as a measure of "survival" of the primitive respiratory center concerned with the gasping reflex. Selle(3) and Hiestand *et al.*(4) had previously shown, using substances blocking or stimulating oxidative or anerobic reactions, that the first series of gasps (induced by cessation of cerebral blood flow) depends on aerobic mechanisms, the second period on anerobic ones.

Although gasping probably has little or no physiological function in normal respiratory patterns in the mammal, our work and that of others suggests that the two patterns of respiratory activity following tracheal occlusion provide an index of respiratory neurone activity. The fact that the respiratory-gasping reflex can be differentially affected by glycolytic and aerobic oxidations(3,4) as well as by O_2 and CO_2 and temperature (as seen in the present study), suggests that gasping induced by tracheal occlusion serves as a valid and simple procedure for estimating functional CNS survival under anoxic and asphyxial conditions.

Of primary interest is the effect of the combined use of OHP and hypothermia in increasing survival time of the animals. The results suggest that the apparent protective effects of hypothermia and OHP treatments alone are additive rather than synergistic since Group VI survival times are approximately equal to the combined survival time of Groups IV and V.

Boerema(5), studying the combined use of moderate OHP (3 atmospheres) and hypothermia in animals and man, has concluded that the effectiveness of OHP and hypothermia, in preventing anoxic damage resulting from total circulatory arrest could be based on 2 factors: An increase in physically dissolved O_2 in body fluids, and a reduced oxygen requirement. Hence, in the absence of circulatory perfusion, tissue function is adequately met by the physically dissolved O_2 in plasma and body fluids.

The toxic effects of OHP are now generally agreed to be potentiated by the presence of increased CO_2 concentrations(6). Moreover, survival time of rats made "anoxic" with 90% N_2 and 10% CO_2 is longer than survival time of rats asphyxiated by clamping the trachea(2). Of interest, then, is the observation that with treatment VIII (90% O_2-10% CO_2-OHP, normothermic) survival time was approximately twice that seen with comparable normothermic animals breathing 100% O_2 at OHP (Group V). However, survival with 99% O_2-1% CO_2 OHP treatment in normothermic animals (Group VII) was approximately the same as those of Group V. The apparent beneficial effect of high CO_2 (*i.e.*, 10%) may be due to (1) CO_2 potentiation of gasping by possibly stimulating carotid chemoreceptors(2); (2) hyperpnea produced by increased CO_2 in hypothermic rats (7); and (3) cerebral vasodilation resulting in increased availability of O_2 to the brain (8). This does not explain, however, the lack of the usual period of apnea preceding onset of gasping.

It should be emphasized that our investigations were based on the rationals of increasing survival time by the combined effects of reduced O_2 requirements and increased physically dissolved O_2. No direct measure of either parameter has been made. Other variables cannot be excluded in determining survival under the conditions of the experiments. Thus, barbiturate anesthesia, changes in

blood pH, age of the animals, and duration of OHP treatment may influence respiration and gasping. These factors are known to be important in other aspects of hypothermic and OHP stress(1,9).

Summary. The effects of combining pressure oxygenation at 3 atmospheres with hypothermia (20°C) on asphyxial survival of rats following tracheal occlusion have been studied. Exposure to 100% oxygen at high pressure (OHP) without hypothermia increased survival times about 55-80% over those seen in animals breathing 100% oxygen at ATP. Oxygenation at 3 atmospheres combined with hypothermia at 20°C gave maximal prolongation of survival, although this was not significantly greater than seen with hypothermia alone. Addition of CO_2 to the inspired O_2 under pressure also afforded additional protection, even in absence of hypothermia. The action of OHP and hypothermia in increasing survival is additive rather than synergistic.

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Further Evidence of a Relationship between Live Fowl Pox Virus and Parthenogenesis in Turkey Eggs. (27386)

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Data presented in 1956 indicated a possible relationship between live fowl pox and pigeon pox viruses with parthenogenesis in turkeys and chickens(1). Later, live Roux sarcoma virus was shown to be effective in enhancing an unorganized type of parthenogenetic development in turkeys(2). A relationship was found by Gill and Stone at the Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Md., between live Newcastle disease virus and the level of parthenogenesis in turkey eggs (personal communication). Each of these 3 live viruses, however, upon being inactivated with beta-propriolactone and subsequently used to vaccinate virgin turkey hens proved to be ineffective in increasing the level of parthenogenetic development(3). Spontaneous parthenogenesis in unfertilized White Leghorn chicken eggs also has been noted following a natural outbreak of fowl lymphomatosis of the visceral type(4).

Data presented earlier on the effectiveness of live fowl pox and pigeon pox viruses involved use of unfertilized eggs of Dark Cornish chickens and Beltsville Small White turkeys. The effects of live virus vaccines were measured by comparing the average level of parthenogenesis in eggs of the same birds before and after vaccination. This method, while keeping genetic influences constant, failed to take into consideration minor seasonal fluctuations in the level of parthenogenesis. Therefore, it was desirable to conduct additional tests with live fowl pox virus so that daily collections of eggs from 2 similar groups of birds could be incubated and examined simultaneously for parethenogenetic development.

Material and methods. These tests involved a total of 50 virgin Beltsville Small White (BSW) females from a flock in which no selection for increased incidence of parthenogenesis had been practiced. Each of these