Anoxic endurance of cardiac and respiratory function in the adult and infant rat

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METHODS

The adult rats used were, in all cases, Sprague-Dawley males ranging in weight from 250 to 340 gm. Most of the infants used were also Sprague-Dawley, of both sexes, obtained usually 12-48 hours after birth. A few measurements are also included on infants up to 60 hours of age, and a few on Wistar infants. In neither case are these determinations out of line with the rest.

A plastic chamber (volume, 1 l) large enough for one adult rat was connected with rubber tubing to a tank of 100% commercial nitrogen. A rat was placed in the chamber, and the gas flushed through at a rate of 18-20 l/min., a rate previously found sufficient to bring the oxygen content of the empty chamber to less than 1% alter 10 seconds of flushing. The nitrogen flow was continued during the total period of anoxia, whereupon the rat was rapidly removed from the chamber into room air, and observed for respiratory activity. For the infants, a smaller chamber was used (about 250 ml volume) and 6-10 infants were exposed to nitrogen at one time. The infant chamber was submerged in a 37°C constant temperature bath, the infants were introduced, and 20-30 minutes were then allowed for temperature equilibrium to occur before exposure to nitrogen was begun. Rectal temperatures of several infants were determined under these circumstances using a 0.5 mm diameter thermometer tip probe and found to be 36°-37°C.

In those animals in which cardiac activity was observed, only one rat at a time was placed in the chamber. The ECG was recorded on a Sanborn ‘Viso-cardiette,’ using subcutaneous needle electrodes, inserted after nitrogen narcosis had occurred.

RESULTS

Adult anoxic time. Four adult rats, placed successively in the gas chamber for 1 minute each, all showed struggles, muscular spasms, and running and gasping motions, beginning 3-5 seconds after the nitrogen was turned on. After 45-50 seconds, all collapsed completely, their
muscles became flaccid, and they appeared to be in deep narcosis. At about 50-55 seconds detectable breathing movements stopped and when the animals were removed to room air at 60 seconds, none of them began respiring. None recovered. A second group of four animals was treated similarly, but was exposed to nitrogen for only 30 seconds. Upon removal from the gas chamber, all of these rats were gasping violently. These gasps and muscle spasms continued for 10-20 seconds, after which breathing quieted down, muscles relaxed, and in less than a minute breathing was regular and apparently normal. All four of these rats were alive and apparently unaffected 3 hours after the period of anoxia. These animals showed no sign of any deleterious effects during an observation period of several weeks, at which time they were sacrificed for other purposes. On the basis of even this small sample, it seems safe to conclude that the median lethal exposure time to 100% nitrogen for adult rats of this strain lies between 30 and 60 seconds.

Infant anoxic group. A total of 141 infants, 10-60 hours old, were divided into eight groups and each group exposed to nitrogen for a period of from 8 to 20 minutes. An 8-minute period of anoxia killed none of the infants, whereas none of them survived a 20-minute period. This type of data is generally plotted as percentage mortality against 'dose' (in this case, time in nitrogen). This has been done in figure 1. The two points of 0 and 100% mortality at 30 and 60 seconds, for the adults, are also plotted on this figure. Since the range is so small, they have been connected by an arbitrary straight line. The asymmetrical sigmoid curve connecting the infant points was obtained by probit analysis of the data (4), from which resulted an estimate, at the 95% confidence limits, of the median lethal dose of 13 minutes (S.D. = 0.4 min.) in nitrogen for the infant rat (cf. 5, for the details of these statistical analyses).

Cardiac versus respiratory resistance. Although none of the adult rats mentioned above recovered from a period of 1 minute of total anoxia, in all cases, upon removal of the rat into room air violent pulsation of the heart could be seen through the chest wall for a matter of minutes. During this time, no breathing or gasping movements occurred. It was therefore assumed that the immediate cause of death in these animals was the functional failure of the central nervous respiratory mechanism.

To test this assumption, a series of 12 adult and 13 infant rats were each exposed to anoxia, electrocardiograms being recorded after the animals had succumbed to nitrogen narcosis. The results are summarized in table 1. Complete respiratory collapse occurred in the adult, as seen previously, after about 1 minute of anoxia. In the infant the situation was much less clear-cut. After the first 10 minutes in the nitrogen chamber during which all of the animals continued gasping, respiratory movements became highly variable. In some infants a period of complete apnea occurred after 10-12 minutes, lasting sometimes as long as 2-3 minutes, after which intermittent periods of gasping often began again. In other animals respiratory movements continued at a fairly regular rate, but gradually decreased in force until they were barely discernible, and remained at such a level for 2-3 minutes. During most of these last minutes it is impossible to say with certainty whether respirations were occurring or not. For these reasons, time of respiratory cessation in the infant is not listed in table 1. It would appear, however, that this value is not dissimilar to the infant LD₅₀ of 13 minutes, noted above. The mean time of cessation of ventricular electrical activity for adult and infant (at comparable temperatures) is seen to be about 5 minutes and 31 minutes, respectively. Thus, we may list in order of decreasing resistance to the effects of anoxia (with approximate time of cessation of activity): infant rat ventricle (31 min.); infant rat respiratory center (13 min.); adult rat ventricle (5 min.); adult rat respiratory center (1 min.).

The range of one standard deviation from the mean is also shown for each column in table 1. These were calculated from the log values since the dose-response relation derived from probit analysis of the data appeared to be of the log-normal type (5). Several statistical tests were applied to confirm this log-normal distribution. For example, when the increment in percentage mortality per unit increment in dose was calculated and plotted against dose, the Gaussian curve generated was found to be positively skewed, as predicted in the case of an exponential effect. Furthermore, probit analysis on the assumption of log-normalcy, yields a set of theoretical results which can be compared with the experimentally observed values. Application of the (Chί)² test resulted in a probability of 0.98 that differences between them were fortuitous.

**DISCUSSION**

Our findings are in agreement with the classical observations of Legallois (6) of the decreasing tolerance of animals to anoxia with increasing age, which have been repeatedly confirmed (7-9). Kabat (10) has reported that the respiratory center in the newborn dog continues to function 17 times as long as that in the
ANoxic ENDURANCE IN ADULT AND INFANT RATS

TABLE 1. Respiratory and Cardiac Activity in the Infant and Adult Rat in Nitrogen

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Respiratory activity</th>
<th>Ventricular electrical activity</th>
<th>Ventricular electrical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sec.</td>
<td>mm.</td>
<td>mm.</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>6.5</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>5.8</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>5.0</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
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<td>28</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>2.7</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>3.0</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>6.0</td>
<td>22</td>
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<tr>
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<td>61</td>
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<tr>
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<td>70</td>
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</tr>
<tr>
<td>11</td>
<td>66</td>
<td>5.5</td>
<td>33</td>
</tr>
<tr>
<td>12</td>
<td>52</td>
<td>6.5</td>
<td>42</td>
</tr>
<tr>
<td>13</td>
<td>52</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Mean (log)</td>
<td>1.769</td>
<td>0.697</td>
<td>1.489</td>
</tr>
<tr>
<td>S.D. (log)</td>
<td>0.152</td>
<td>0.155</td>
<td>0.197</td>
</tr>
<tr>
<td>Mean</td>
<td>58.7</td>
<td>4.98</td>
<td>30.84</td>
</tr>
<tr>
<td>Range of 1 S.D.</td>
<td>52.1-66.2</td>
<td>3.48-7.11</td>
<td>23.02-41.30</td>
</tr>
</tbody>
</table>

adult, following complete arrest of blood flow to the brain.

Our finding that breathing fails before ventricular electrical activity in both infant and adult rats is in line with the results of Fazekas et al. (7) on newborn dogs. It also agrees well with the demonstration by Kabat et al. (11) of irreversible anoxic damage to the dog brain in 6-8 minutes, as compared with the 25 minutes or longer required to produce areas of myocardial necrosis and persistent electrical irregularities, as shown by Blumgart et al. (12).

While not revealing any new relations, our observations do provide a more precise determination of the differences between infant and adult survival in anoxia than has previously been available. Since hypothermia was prevented in the infant animals, their longer survival was not attributed to lower body temperatures. Moreover, we believe that the method of statistical treatment which has been applied to our data is some improvement over previous methods.

There have been several mechanisms postulated to account for this resistance of the newborn to anoxia. The only sources of energy for the anaerobic animal must involve the glycolytic breakdown of sugars. Thus the supply of carbohydrates available should be critical (13, 14). Britton and Kline (15) have demonstrated the protective effect of glucose injections on hypoxic animals, and Winbury (16) has shown that cat papillary muscle survives anoxia longer in the presence of glucose than in its absence. The infant may then owe its survival, in part, to the relatively high glycogen content of its tissues (15, 17).

Fitzgerald (18) emphasizes the extremely small energy requirement of the nonhomeostatic newborn mammal as a factor in its resistance to anoxia, while Brodie, Cross and Lomer (19) suggest that even when body temperature is maintained constant, hypoxic infants are capable of decreasing their heat production and reducing their oxygen demand.

The accumulation of lactic and pyruvic acids as a consequence of anaerobiosis may be a major source of tissue damage (14, 20, 21). Whereas the anoxic adult is largely incapable of metabolizing these products, there is now evidence that the infant is able to convert pyruvate and lactate to neutral lipids (22, 23).

Yet another factor contributing to anoxic death is the formation of blood clots in the cerebral and coronary capillaries, resulting from the depressed plasma pH. Crowell and Smith (24) have found that Varidase, a fibrinolytic activator, extends the survival time of adult dogs to 15 minutes after circulatory arrest. The prothrombin level of the newborn is normally very low (25), a situation tending to prevent multiple clot formation. Thus again, the infant is protected from anoxic damage.

Undoubtedly the resistance of the infant mammal to anoxia represents the cumulative effect of most or all of the factors mentioned. In addition, we shall show in the accompanying paper (20), that isolated hearts from newborn animals react to anoxia with less radical biochemical changes than does cardiac muscle from adults. Thus one more protective agent is added to the armamentarium of the infant in its defense against anoxic damage.

REFERENCES
