TECHNICAL PAPERS

Factors in Neonatal Resistance to Anoxia. I. Temperature and Survival of Newborn Guinea Pigs Under Anoxia[1]

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It is standard hospital procedure to place anoxic babies in a warmed bassinet or incubator and give them oxygen. Since the speed of chemical reactions of importance to life is, according to Van't Hoff's rule, doubled or trebled with each 10° C rise in temperature, some doubts appeared in the mind of the writer as to the advisability of warming the anoxic child. Accordingly, an investigation of the effects of temperature upon survival of anoxic newborn animals was undertaken.

This short preliminary account of results of this investigation is being presented at this time in the hope that it may stimulate clinical studies on the treatment of asphyxiated newborn infants.

The experiments were performed as follows: Littermate guinea pigs 24 hr old or less were used. Temperatures were taken by using a U. M. A. skin thermocouple with the sensitive element modified so that it could be used as a small colonic thermometer. Each reading was taken with the element inserted into the anus for a distance of 20 mm. Temperatures were taken immediately before and after the experiment.

The temperature differentials were produced as follows: In litters of three or more, one animal was left untreated, one was incubated for ½ hr, and one or more was cooled. In litters of two, one was cooled or warmed, the other left untreated.

The colonic temperatures of the untreated animals usually varied between 29° and 33° C. This is an example of the well-known fact that the temperature-regulating mechanism is either very inefficient or nonfunctional in neonatal mammals.

Cooling was produced by wetting all but the head and circumanal region with 95% alcohol and exposing the animal to air currents from a 12-in. electric fan. When its temperature had fallen 2° to 5° C it was placed with a littermate under a glass bell jar with a current of 95% N_2 and 5% CO_2 flowing through. Records were kept of the time of appearance of all visible reactions, timed by means of a stop watch.

In a preliminary series of experiments the animals were exposed to anoxia for 4¼ min (4 experiments, 10 animals) or 4½ min (4 experiments, 14 animals) and then removed. This length of exposure was sufficient to kill the warmed and the room temperature animals and, with but one exception, their cooled littermates recovered from the treatment. The exceptional animal withstood 4½ min of exposure but succumbed 52 sec after removal of the bell jar.

Next, experiments were performed in which the animals were exposed until dead. The time of appearance of the last slight movement of the abdominal wall in an attempt to gasp was recorded as the time of death (T.O.D.). Preliminary experiments had established that, even though spontaneous recovery may occur if the bell jar is removed before the last few weak and ineffective gasps, it does not happen when the animals are exposed to the gas mixture until gasping has stopped completely.

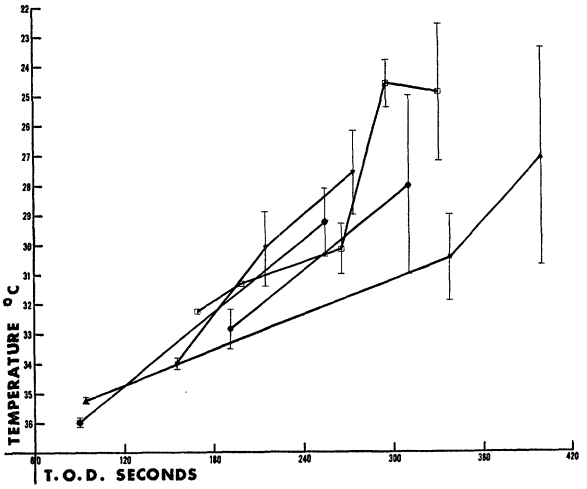

FIG. 1. Five typical experiments showing the effects of temperature on time of death (T.O.D.) of day-old guinea pigs exposed to 95% N_2 and 5% CO_2 until dead.

Fig. 1 is a graph of 5 experiments with a total of 15 animals, giving a typical picture of the effect of temperature on the survival of day-old guinea pigs. On the abscissas are shown seconds survival, on the ordinates the temperature. Each point on the graph represents one animal, with its temperature range during the experiment indicated by the thin vertical line. The midpoint of this range is used as the temperature of the animal for purposes of calculations. Littermates are indicated by the same symbol and are connected together by a heavy line.

A glance at the graph shows that there was less variation in temperature and greater consistency in results in the case of animals kept at higher temperatures than in those kept at lower temperatures. Because the method of cooling stimulated motor activity, which might be expected to reduce the animals' potential for anaerobic survival, the cooled animals were placed in the bell jar while still wet. This reduced the total motor activity of the

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animal, since part of the cooling period was passed while the animal was unconscious. This procedure was instituted because in exploratory experiments the animals which were cooled for a long time exhibited both struggling and shivering. The performance of these animals in the bell jar was poorer than their uncooled littermates, and much poorer than those in which cooling took place for the most part while the animals were unconscious.

From these five typical experiments it appears that the effects of reduction of colonic temperatures on one-day-old guinea pigs subjected to anoxic anoxia is to prolong life at about the same rate as might be expected from Van't Hoff's rule—i.e., about three times for the first 10° C differential.

Additional experiments, confirming the findings reported here and extending them to include also nembutalized animals, will be reported later when the statistical analyses of these data are complete.

It is concluded that elevation of body temperature is deleterious to day-old guinea pigs subjected to anoxic anoxia. Reduction of temperature, at least within the limits reported here, is beneficial when cooling is accomplished with minimal motor activity and shivering.

The Genera of Amoebae

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In recent years there has been considerable discussion about the correct scientific names of certain fresh-water amoebae. The recent articles by King and Jahn (*3*) and Wilber (*5*) include references to other work on the subject.

The three species concerned are: (I) *Amoeba proteus* Leidy (*Chaos diffluens*), (II) *Chaos chaos* Schaeffer (*Pelomyxa carolinensis, Amoeba carolinensis, Chaos carolinensis*), and (III) *Pelomyxa palustris* Greeff.

The controversy embraces two separate questions: one about the relative historical validity of the generic names *Amoeba, Chaos* and *Pelomyxa.* To this question we have no contribution to make. The other question is whether (I) and (II), or (II) and (III) should be placed in the same genus, or whether they belong to three separate genera.

Since the morphological criteria seem to be insufficient to allow a clear decision of the issue, we have tried to supplement the morphological evidence with a biochemical comparison of the three organisms.

Organisms (I) and (II) are readily available and their morphology and physiology is comparatively well known. Organism (III) is, at least in Denmark, comparatively rare, and in spite of persistent search we were able to obtain only four specimens. This limited material forced us to restrict our investigation to a few characteristic properties easily determined with the micromethods at our disposal. We decided therefore to determine the contents of two proteolytic enzymes, catheptic proteinase (substrate caseinogen, pH 4.0) and peptidase (substrate alanyl-glycine, pH 7.4). In addition we measured the approximate total protein content colorimetrically by means of the Folin-Ciocalteau reagent against a tyrosine standard. Details of these methods are described in a recent publication from this laboratory (*2*). As a basis of comparison we chose the cell volume rather than weight as suggested by Zeuthen (*6*), since the cytoplasm of *Pelomyxa palustris* (III) contains a great deal of foreign inclusions, e. g., grains of sand, which could be expected to falsify the density much more than the volume.

Organism (III) was identified as *Pelomyxa* in the following way: all four specimens were examined while alive with a water immersion objective giving 40 times magnification. The sand grains coating the surface and enclosed in the cytoplasm; the broad, slowly forming pseudopodia; the vacuolar appearance; and the bacteria in the cytoplasm, were easily seen. One of the specimens was crushed between cover slips and showed numerous nuclei about 10 μ in diameter, and "glycogen bodies" to 5–30 μ in size, which varied in shape from spherical to ellipsoid, some being quite irregular. Another specimen was fixed, sectioned, and stained. The details of bacteria, nuclei, and "glycogen bodies" which are described by Leiner (*4*), were confirmed. These are the characteristic features used for determining the species *Pelomyxa palustris,* and all of them are quite different from the corresponding features in *Amoeba proteus* (I) and *Choas chaos* (II). The third specimen was used for preliminary enzyme measurements, and the last and largest animal (total volume 6.5 μl) was used for final analyses in triplicate. The calculations in Table 1 for Species (III), therefore, all refer to this one specimen but they were roughly checked by preliminary experiments which indicated the order of magnitude of enzyme activity.

The values for species (I) and (II) are averages for a great number of individuals. The values for species (III) are the means of triplicate analyses. All values refer to a standard volume of 1 μl of cytoplasm. For

TABLE 1

	Protein	Peptidase	Proteinase	Peptidase / proteinase
	μg tyrosine	0.06 N HCl/hr	arbitrary units	
Species (I) *A. proteus*	4.0	53	43	1.2*
Species (II) *Ch. chaos*	2.6	62	28	2.2*
Species (III) *Pelom. pal.*	1.0	0.31	0.25	1.2*
Ch. chaos / *A. proteus*	0.65*	1.2*	0.65*	
Ch. chaos / *Pelom. pal.*	2.6*	200*	112*	

* These figures give ratios.