Reports

Lactic Acid Accumulation as a Cause of Hypoxia-Induced Malformations in the Chick Embryo

Abstract. Most hypoxia-induced malformations are caused by a syndrome involving tremendous edema followed by formation of clear blisters and hematomas. These, in turn, mechanically interfere with development. Studies of blood *p*H and lactic acid indicate that lactate accumulation initiates this syndrome. The effects of lactic acid injections parallel those of subjection to hypoxia, confirming this conclusion.

Studies of the teratogenic and lethal effects of hypoxia on chick embryos in this laboratory (*1*) have centered around (i) a comprehensive quantitative survey of the effects of graded doses of hypoxia, and (ii) a morphogenetic and biochemical analysis of these effects. Certain conclusions are now fitting together into a total picture, which is reported here.

The earlier studies (*2, 3*) showed that the effects of oxygen deficiency could be studied quantitatively. Furthermore, within established limits, the effects were proportional to the treatment. It was also shown that, in embryos of a given age group, different levels of hypoxia (produced by a flowing mixture of air and N_2) could induce qualitatively different kinds of anomalies (*3, 4*). These differences were due to different modes of action of hypoxia. For instance, in 3-day embryos, exposure to 1 percent O_2 for 3 hours produces a variety of severe head anomalies caused by extensive cell death in this region. Mild hypoxia (6 to 12 hours at 10 to 12 percent O_2) induces little else than rumplessness of unknown cause. But malformations, especially of eyes, beak, and extremities, are induced most readily by moderate hypoxia (6 to 12 hours at 4 to 8 percent O_2). These malformations are caused not by any direct action of oxygen deficiency but by an indirect sequence of events (*5*). The primary effect of the hypoxia is to produce a tremendous edema (embryo volume increases up to tenfold) which persists for several hours. Numerous subcutaneous blisters appear over the head and trunk. Most of them disappear, but some persist either as clear blisters or as hematomas after neighboring blood vessels rupture into them. Additional hematomas may appear, especially in the eye cup and in head mesenchyme. Persistent blisters and hematomas are evidently responsible for the maldevelopment of adjacent structures, since (i) incidence of malformations of a given region agreed with incidence of hematomas found 24 hours after treatment, and (ii) abnormal development of these structures does not begin until 2 or 3 days after treatment.

Further study was concentrated on this circulatory syndrome, since (i) it was responsible for most of the observed anomalies and (ii) it seemed amenable to experimental analysis. It was postulated that the primary effect —edema—may be due to the accumulation of metabolites from prolonged anaerobiosis, such as lactic acid, and resulting disturbances in *p*H and salt balance. Consequently, the *p*H of venous blood samples from over 50 embryos, 3 and 4 days of age, were checked before and after treatment. A Beckman *p*H meter with capillary glass electrode was used. Normal *p*H was 8.0 to 8.2. This level was maintained in most embryos checked immediately after mild and moderate subjection to hypoxia except for an occasional individual in which *p*H dropped to 7.6 or even to 6.8. This drop was always associated with a very feeble heartbeat and other signs of imminent death. If the circulatory disturbance was caused by the accumulation of acidic metabolites, it was apparent that the buffering capacity of the embryonic blood stream could, up to a certain point, keep the *p*H constant.

Direct assay of the lactic acid content of blood of 3- and 4-day embryos was then attempted, with 0.01-ml samples and a method sensitive to ± 0.4 μg of lactate (*6*). The lactic acid level in the blood of 10 controls ranged from 50 to 150 μg/ml. The lactate levels of embryos subjected to hypoxia ranged from 400 to 1300 μg/ml, and this in embryos swollen to 10 times normal volume. The average for embryos subjected to moderate hypoxia was around 800 μg/ml. This is equivalent to a concentration of 0.08 percent —certainly enough to affect the salt balance of an embryo containing 0.85 percent of total salts. Clearly, lactic acid accumulates in quantity in the blood stream of embryos subjected to hypoxia.

Was this increase in blood lactate a significant effect of hypoxia? Direct applications of lactic acid solutions (0.01 to 0.03 ml of 0.01 percent lactic acid in saline) were made to the vitelline membrane, amniotic cavity, or subgerminal cavity of 3-day embryos and into the allantoic cavity of 4-day embryos. Regardless of method of injection or age of embryo, all the early effects of lactic acid injection precisely paralleled those of subjection to moderate hypoxia—namely, extensive edema followed by the appearance of subcutaneous blisters and hematomas. The death rate was generally high, around 90 percent, but only 70 percent of embryos injected with lactic acid by way of the allantoic route died. Of the 79 survivors in this group, 10 (12.7 percent) developed anomalies. Only one of the 85 surviving saline-injected controls was abnormal. Furthermore, the malformations obtained with lactic acid injections were the same ones induced by hypoxia at this age—namely, defective eyelids, exocephaly, and short upper beaks. Many details remain to be studied, but the conclusion is clear. The simple accumulation of lactic acid —the physiological consequence of prolonged anaerobiosis—is responsible for

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

most of the abnormal development which follows exposure to oxygen-deficient atmospheres.

The significance of these data may extend beyond that of interest in chick embryos subjected to hypoxia. Vascular anomalies have been described in mouse fetuses subjected to anoxia (*7*). A comparable syndrome of edema, subcutaneous blisters, hematomas, and maldevelopment of adjacent tissues has been described in mammalian and avian embryos in deficiencies of pantothenic and linoleic acids, after administration of the redox dye trypan blue, as well as in Little and Bagg's famous mutant strain of mice (*8*). Is it not possible that some of these agents (and others too) and hypoxia have a common mode of action—that is, an interference with oxidative metabolism and the accumulation of metabolites?

CASIMER T. GRABOWSKI
Department of Zoology, University of Miami, Coral Gables, Florida

References and Notes

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Conformal Transformations and Space Travel

Abstract. Conformal transformations are applied to the motion of a space ship experiencing a constant acceleration. The role of proper time is interpreted in terms of atomic periods, and the relationship between the conformal transformations and the general theory of relativity is clarified.

Conformal transformations, connecting an inertial observer to a non-inertial observer experiencing a constant acceleration, were developed by Bateman (*1*), Cunningham (*2*), and Page (*3*). Further interesting work on these transformations has been carried out by Engstrom and Zorn (*4*), Robertson (*5*), Hill (*6*), and others.

This report applies the conformal transformations to the problem of space travel and establishes the equivalence of the resulting formulas with those obtained by standard relativistic methods. It also discusses the physical significance of proper time in space travel and clarifies the relationship between the conformal transformations and the general theory of relativity.

An observer in an arbitrary frame of reference in general experiences two types of gravitational fields, which I shall call the *real* and the *apparent* gravitational fields. A real gravitational field is produced by bodies consisting of all types of elementary particles, while an apparent gravitational field is produced by the acceleration of the observer's frame of reference. According to Einstein's ideas, there is a certain equivalence between the two types of gravitational fields, but this does not mean that they are indistinguishable. In fact, in nature it is always possible to distinguish between the real and the apparent gravitational fields. For instance, the gravitational field around the earth can possibly be produced either by the matter contained in the earth or by an outward acceleration of the surface of the earth. Since we know from observation that the surface of the earth is not exploding, we must conclude that the gravitational field around the earth is a real gravitational field. It is also evident that a real gravitational field must vanish at an infinite distance from all gravitating bodies, while an apparent gravitational field can exist even at infinity. A frame of reference in which the apparent gravitational field vanishes everywhere is an inertial frame of reference.

We can easily pass over from one inertial frame of reference to another by means of the Lorentz transformations, but a passage from an inertial frame of reference to a non-inertial frame of reference is not so simple. A particularly simple example of a non-inertial frame of reference is one in which the observer experiences a constant acceleration (*7*). The relationship between such a non-inertial frame of reference and an inertial frame of reference can be stated as follows:

Let there be an inertial frame of reference S and a non-inertial frame of reference S' which is moving with respect to S along the x-axis in such a way that the acceleration experienced by an observer at the origin of S' has the constant value α. Further, let the coordinates of any event be denoted by (x, y, z, t) in S and by $(x', y', z' t')$ in S', and let the origins of the two frames of reference meet at rest at $t = t' = 0$. Then, the conformal transformations connecting the two frames of reference are (*3*) as follows (Eq. 1):

$$X' = \frac{X}{X^2 + Y^2 + Z^2 - c^2T^2},$$
$$X = \frac{X'}{X'^2 + Y'^2 + Z'^2 - c^2T'^2},$$
$$Y' = \frac{Y}{X^2 + Y^2 + Z^2 - c^2T^2},$$
$$Y = \frac{Y'}{X'^2 + Y'^2 + Z'^2 - c^2T'^2},$$
$$Z' = \frac{Z}{X^2 + Y^2 + Z^2 - c^2T^2},$$
$$Z = \frac{Z'}{X'^2 + Y'^2 + Z'^2 - c^2T'^2},$$
$$T' = \frac{T}{X^2 + Y^2 + Z^2 - c^2T^2},$$
$$T = \frac{T'}{X'^2 + Y'^2 + Z'^2 - c^2T'^2},$$

where (Eq. 2)

$$X = 1 + \frac{\alpha x}{2c^2}, \qquad Y = \frac{\alpha y}{2c^2},$$
$$Z = \frac{\alpha z}{2c^2}, \qquad T = \frac{\alpha t}{2c^2},$$
$$X' = 1 - \frac{\alpha x'}{2c^2}, \qquad Y' = \frac{\alpha y'}{2c^2},$$
$$Z' = \frac{\alpha z'}{2c^2}, \qquad T' = \frac{\alpha t'}{2c^2}.$$

I shall apply the conformal transformations to the motion of a space ship whose occupants experience a constant acceleration α. Let the space ship be located at the origin of the frame of reference S'. Then, putting $x' = y' = z' = 0$ in Eq. 1, we have

$$1 + \frac{\alpha x}{2c^2} = \left(1 - \frac{\alpha^2 t'^2}{4c^2}\right)^{-1}, \quad (3)$$
$$t = t'\left(1 - \frac{\alpha^2 t'^2}{4c^2}\right)^{-1}, \quad (4)$$

which give us

$$x = \frac{\alpha t'^2}{2}\left(1 - \frac{\alpha^2 t'^2}{4c^2}\right)^{-1}, \quad (5)$$

or

$$x = \frac{c^2}{\alpha}\left[\left(1 + \frac{\alpha^2 t^2}{c^2}\right)^{\frac{1}{2}} - 1\right], \quad (6)$$

where x is the distance traveled by the space ship with respect to S, and t and t' are the times of travel according to the observers in S and S' respectively.

Differentiating Eq. 6 with respect to t, we obtain for the velocity of the space ship relative to the frame of reference S

$$v = \alpha t\left(1 + \frac{\alpha^2 t^2}{c^2}\right)^{-\frac{1}{2}}, \quad (7)$$