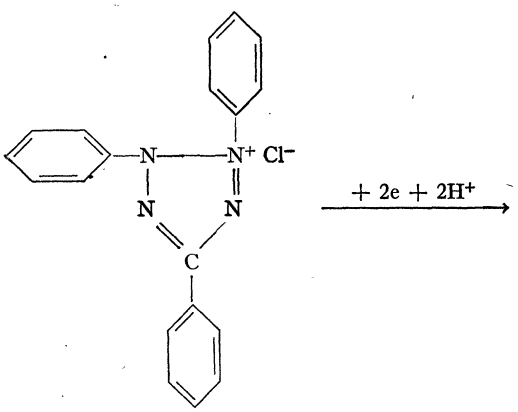
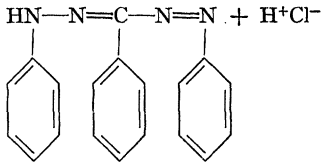
tissues. The furfuryl derivative appeared to give the same tests as the triphenyl compound. In order to prepare tetrazolium salts of suitable purity in satisfactory yields, it was necessary to make certain modifications in the method of Kuhn and Jerchel. This phase of our work will be published later.

Our work also confirms the work of Lakon with seed corn and the observations of Kuhn and Jerchel with yeast. However, we have been interested in the potentialities of the tetrazolium salt as a test reagent for living tissues in general. We have found that many other viable materials, in addition to seeds and yeast, will reduce the triphenyltetrazolium chloride at pH 6.9: the fleshy parts of apples, oranges, and grapes; the gill area of mushrooms; carrot roots, white and sweet potatoes; young leaves; the stigmas and ovaries of certain pollinated flowers; bull sperm and the blastoderm of hen's eggs. Much to our surprise, the serum of bull sperm and the chalazae of egg white give a positive reaction. The reduction of the tetrazolium salt is not due to sugars, for subsequent work has shown that reducing sugars form the red formazan only above pH 11.0, whereas the above-mentioned materials will reduce the triphenyl compound at acidities below pH 7.0.

The use of the tetrazolium reagent should have a distinct advantage over many indicators as a viability test, since it is one of the comparatively few organic compounds which is colored in the reduced state. In the presence of viable tissue the colorless solution of triphenyltetrazolium chloride forms the insoluble red triphenyl formazan by the following reaction:




2,3,5-Triphenyltetrazolium chloride




Triphenyl formazan

It is quite evident that enzyme systems are responsible when this reduction takes place in plant and animal materials, since tissues heated at 82° C. or higher lose their ability to reduce this salt. Furthermore, it is probable that this reduction is caused by dehydrogenase systems requiring coenzymes I or II, for Jerchel and Möhle (*1*) have shown that the apparent redox potential of 2,3,5-triphenyltetrazolium chloride is about −0.08 volt. Thus, it is possible for this compound to act as an electron acceptor for many pyridine nucleotide dehydrogenases. We have found that one of these holoenzymes, glucose dehydrogenase-coenzyme I, in the presence of its substrate, reduces the salt at pH 6.6.[1] Work is being continued to determine if other enzyme systems possess similar properties when treated with this reagent.

Preliminary experiments have indicated that the enzyme systems responsible for the reduction of the tetrazolium salts are present in a wide variety of living tissues. In all probability, the reduction of these compounds by enzymes of living cells cannot be considered a general test for life. Nevertheless, the unusual properties of these reagents suggest that they might be utilized in many types of biological research involving differences in tissue viability.

### References


1. Jerchel, D., and Möhle, W. *Ber. dtsch. chem. Ges.*, 1944, 77-B, 591.
2. Kuhn, R., and Jerchel, D. *Ber. dtsch. chem. Ges.*, 1941, 74-B, 941.
3. Kuhn, R., and Jerchel, D. *Ber. dtsch. chem. Ges.*, 1941, 74-B, 949.
4. Lakon, G. *Mitteilg. Intern. Ver. Samenk.*, 1940, **12**, 1; *Ber. dtsch. bot. Ges.*, 1942, **60**, 299, 434.
5. Pechman, H. V., and Runge, P. *Ber. dtsch. chem. Ges.*, 1894, **27**, 2920.
6. Porter, R. H., Durrell, M., and Romm, H. J. *Plant Physiol.*, 1947, **22**, 149.


## Oxygen and Air Pressure Effects Upon the Early Development of the Frog's Egg


Olin E. Nelsen

*Department of Zoology, University of Pennsylvania*


The following constitutes a report on a part of the author's studies of the effects upon early embryological development of (1) different pressures of normal air and (2) various proportions of the constituent substances of normal air under pressure.

Eggs of three species of frogs (*Rana pipiens*, *R. sylvatica*, and *R. palustris*) were used in these observations. Two sets were employed: normal eggs deposited under natural conditions and, in the case of *R. pipiens*, pituitary-stimulated eggs from females brought in shortly before the breeding season and kept in a state of prolonged hibernation in a cold room at approximately 1° C. In the case of eggs deposited under natural conditions, the period of development during which experiment was initiated varied between the 4- and the 16-cell condition. The pituitary-induced eggs were placed in the pressure chambers 15–20 minutes after the sperm suspension was added. Eggs from two separate females and sperm from two males were used in each experiment.

Pressures up to 3 atm. of oxygen were added to the normal air pressure in the pressure chambers. Small egg masses were placed in small glass dishes and covered with water to a depth ranging from $\frac{1}{8}$ to $\frac{3}{8}$ inch above the surface of the mass. The water remained unchanged while the eggs were in the pressure chambers. Control eggs were kept in finger bowls or crystallizing dishes in shallow water which was changed daily. The control embryos were watched until they reached the late yolk plug and early neuralation state, after which the pressure

[1] We are indebted to F. A. Baldauski, who made the glucose dehydrogenase and coenzyme I preparations.

chambers were opened. The experiments were carried out at room temperature which ranged from 14° to 20° C., with the exception of two experiments on the induced eggs, where the temperature varied between 16° and 25° C.

The following results were obtained for naturally deposited eggs:

(1) Pressures of oxygen added as above have no apparent gross morphological effect upon development through the blastular stage.

(2) Increased pressures of added oxygen have an accelerative effect upon gastrulative and neuralative processes.

(3) Embryos subjected to treatment as above, removed from the chambers, and placed in finger bowls of shallow fresh water continued to develop as follows: (a) Up to 1½–2 atm. of added oxygen no apparent deleterious effects were observed in the tadpole condition. They may show acceleration in development compared with controls; (b) embryos experiencing 3 atm. of added oxygen develop various conditions of abnormal development in the tadpole stage.

(4) Compared with the controls, oxygen added under pressure approximating 1–1½ atm. appears beneficial to early frog development.

Comparison of development of pituitary-induced eggs of *R. pipiens* with that in normally deposited and normally ovulated eggs is under investigation. Thus far the results of experiments with eggs induced during the postbreeding season indicate a greater sensitivity to oxygen administered as above. The following general statements may be made:

(1) Pressures approximating 1 atm. of oxygen added to the normal air in the chamber accelerate development.

(2) Similar pressures produce a recovery effect in some of these pituitary-induced eggs. Comparison with controls demonstrates a greater number of normal-appearing neural fold conditions, with subsequent development into vigorous tadpoles in the oxygen-treated group.

(3) Pressures of added oxygen of 3 atm. suppress development at an advanced blastular condition. A few of these embryos may make abortive gastrulation attempts.

## Red Blood Cell Sensitivity to the Blood-Group-Enzyme

PAUL M. NEUDA

*Achelis Laboratory, Lenox Hill Hospital, New York City*

The blood-group-enzyme, termed BGE, was first described by Schiff and co-workers (*4*), who considered it a physiological component of certain human secretions and excretions, being notably present in saliva and normal feces. The designation was adopted because these investigators found that the enzyme, independently of its source from an A, B, or O individual, acted on the blood group antigens of all the blood groups by destroying them. More recently, Schiff and Boyd (*3*) proposed the more inclusive designation "blood-group-property-destroying-factor."

Significant in relation to the present report is the statement by Schiff and Akune (*2*) that this enzyme was not found in the brain or the blood serum.

The method of production of the BGE by the early workers consisted in inoculating a nutrient broth either with a saline extraction of normal nondiarrheal feces or with saliva (Sievers, *5*) and then incubating the broth at 37° C. for 24 hours. By this procedure, the enzyme, which in the natural state is attached to either cellular debris or certain anaerobic bacteria, becomes enriched.

There was no evidence that BGE played any role in human pathology until 1941, when Neuda, in work done at the Pneumonia Laboratory of the Harlem Hospital, New York City, showed that the red cells of negroes suffering from sickle-cell disease developed sickle cells in from 10 to 30 minutes (at times much more rapidly) when suspended in a fluid medium prepared by the Schiff method and presumably containing the BGE. The preparation of the test fluid and the test itself were described by Neuda and Rosen (*1*) in 1945. In this paper, reasons were enumerated which suggest that Schiff's blood-group-enzyme might be identical with, or closely similar to, the sickle-accelerating substance. Whether or not this assumption is correct can be established only by future investigation. At the present stage of the work, however, this preliminary assumption seems appropriate as a working hypothesis. There are already further evidences at hand which point in the same direction. There remains the outstanding fact that a fluid produced by the Schiff method will rapidly reveal the presence or absence of the sickling quality if red blood cells obtained from a negro are suspended in this fluid.

Observations made since the previous paper appeared, which will be published shortly, have demonstrated that the sensitivity of human red cells to the BGE is not restricted to sickling negro cells. Essentially similar intermediate changes as were found to precede the final sickle-cell configuration in the red blood cells of negroes were found to occur also in red cells derived from Caucasians. The final twisted cell silhouette, however, remained a characteristic of the negro red cell.

This observation led to the conclusion that the possible damage to the red cells by this enzyme fluid might be more widespread than in sickle-cell disease alone. We have therefore coined the phrase "hemolysis of sickle-cell type" to signify this peculiar kind of blood destruction, and the expression "red cell sensitivity to the blood-group-enzyme" to indicate the liability of any red cell to react in characteristic fashion to the substance produced by the Schiff method.

The Schiff method has the disadvantage of using a material which is greatly contaminated by bacteria, so that the resultant changes in the red cells could be attributed to bacterial action. Schiff and Akune, however, have disproven this possibility regarding the BGE action by demonstrating that rigid filtration with removal of the bacteria weakened, but did not completely abolish, the action of the BGE.

A first attempt was therefore made to apply the Schiff method of enrichment by culture to plasma (or serum) of a sickle-cell case. This was done by pipetting about 2 cc. of sterile plasma (or serum) into 25 cc. of sterile nutrient broth, the mixture being corked and incubated at 37° C. for 24 hours.

This plasma(serum)-broth mixture developed the same sickle-accelerating substance as was originally produced from feces. The same procedure was successful in four more cases suffering from the following diseases: hemolytic jaundice,