

treatment of patients suffering from congestive heart failure or the anginal syndrome. These authors have obtained low basal metabolic rates in their patients. In fact, their object seems to be to keep the basal metabolic rate of their patients between —20 to —30%, results that we obtain on an 800 calorie diet. It is therefore suggested that this diet may produce over a period of one to 3 months or more, results comparable to those obtained by complete thyroidectomy over a long period of time.

It appears that in a patient with coronary artery thrombosis kept on an 800 calorie diet the basal metabolic rate may be lowered just as in a normal person.

A lowered basal metabolic rate is associated with a diminished velocity of the blood flow and a decreased amount of work of the heart. Hence low caloric diets should help patients with myocardial impairment. Lusk² and DuBois⁵ have already discussed this theoretical phase. One of us (A.M.M.) has been treating his coronary thrombosis patients by bed rest and an 800 calorie diet for 7 years with excellent results.

7860 C

Peroxidases and Cell Activity in Developing Egg (Orthoptera).*

JOSEPH HALL BODINE AND EDGAR JOHN BOELL.

From the Zoological Laboratory, State University of Iowa.

Cellular activity during normal embryonic development of the common grasshopper, *Melanoplus differentialis*, at constant temperature (25°C.) is characterized by 3 distinct periods: (a) a period of rapid cell proliferation (pre-diapause), (b) a period of developmental block or cellular inactivity in which mitosis, growth, etc., are absent (diapause), (c) a period of marked differentiation and growth terminating in the hatching of the embryo (post-diapause).¹ It becomes of some interest, therefore, to determine physiological changes which accompany the various phases of cellular activity. The present discussion has to do with results of studies on the peroxidase reaction during the entire course of embryonic development.

⁵ DuBois, E. F., *Bull. N. Y. Academy of Medicine*, 1933, **8**, 680.

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¹ Bodine, J. H., *Physiol. Zool.*, 1932, **5**, 549.

TABLE I.
Guaiac Reaction of Embryos in Different Stages of Development.

Developmental age at 25°C. days	Pre-diapause		Diapause		Post-diapause	
	Guaiac Reaction Eggs at constant temp. 25°C.	Developmental age at 25°C. days	Guaiac Reaction Eggs at constant temp. 25°C.	Developmental age at 25°C. days	Guaiac Reaction Eggs at constant temp. 25°C.	Developmental age at 25°C. days
1	—	21	+	50	+	+
4	—	22	+	51	+	+
6	—	23	—	52	+	+
8	+	24	+	53	+	+
10	+	25	+	54	+	+
11	+	26	+	55	+	+
12	+	27	+	56	+	+
13	+	28	+	60	+	+
14	+	30	+	hatching nymph	+	+
15	+	32	+		+	+
16	+	35	+		+	+
17	+	38	+		+	+
18	+	40	+		+	+
19	+		+		+	+
20	+		+		+	+

— = negative reaction.

+ = faint reaction.

++ = positive reaction.

+++ = strongly positive reaction.

++++ = very strongly positive reaction.

* After developing at 25°C. for days shown.

In each test the eggs (usually 10 in number) were crushed with glass rods in narrow test tubes. Small amounts of distilled water (1 cc.) were next added. After the addition of a few drops of hydrogen peroxide, tincture of guaiac was added, the contents of the tubes shaken and the tubes allowed to stand at room temperature until the characteristic blue color appeared. Check experiments were run in which pH values were controlled by phosphate buffers (pH 7.3) but since the eggs are themselves buffered no significant differences were noted between samples buffered with and without phosphates. Several hundreds of eggs were used and each was carefully examined so that both chronological and morphological histories could be accurately determined. This procedure, as will be noted below, is of much importance in attempting a correlation between cellular and physiological behavior of the embryos.

A summary of results is given in Table I. It will be noted that for pre-diapause eggs kept at constant temperature (25°C.) no positive peroxidase reactions are obtained until the 19th or 20th day when diapause or cellular block normally occurs.² During diapause the reaction gradually increases, reaching a maximum as diapause disappears. In the post-diapause period, with rapid differentiation and growth, the reaction reaches a maximum intensity just before hatching. Curiously, after the embryo hatches no positive reactions are obtained.³ If pre-diapause eggs are subjected to low temperatures (0-5°C.) for appropriate periods of time (1 month) and then tested, a positive reaction is always obtained. Obviously, exposure to low temperature in some way or other destroys an inhibitory mechanism which at normal temperature (25°C.) prevents the reaction.

Thus it appears that the peroxidases are, at constant temperature (25°C.), normally present in the embryonic cell from the time of fertilization but are inhibited (as judged by negative guaiac reaction) by some mechanism already present in the cell. The potency of this inhibitor at constant temperature (25°C.) gradually diminishes until at diapause or developmental block the peroxidase reaction normally becomes positive. Exposure to low temperature (0-5°C.) during this period (pre-diapause) in some way or other destroys the inhibitor and a positive peroxidase reaction is then obtained.

Additional evidence supporting such a concept is furnished by the results of mixing extracts of pre-diapause eggs kept only at constant

² Slifer, E. H., *J. Morph.*, 1932, **53**, 1.

³ Bodine, J. H., *Biol. Bull.*, 1925, **48**, 79.

TABLE II.
Inhibition of the peroxidase activity of post-diapause developing embryos by substances contained in pre-diapause developing embryos.

No. of eggs crushed		Guaiac reaction
Pre-diapause (16 days at 25° C.)	Post-diapause (17 days from hatching)	
15	0	—
0	5	+++
15	5	±
20	5	—

TABLE III.
Inhibition of the peroxidase activity of embryos exposed to 5° C. (14 day morphological stage) by substances contained in embryos of similar morphological stages but kept only at 25° C.

No. of eggs crushed		Guaiac reaction
14 day embryos at 25° C.	14 day embryos exposed to 5° C.	
10	0	—
0	10	+++
40	10	±

(This inhibition is not a dilution effect. Peroxidases may be demonstrated in extracts of cold treated embryos or of post-diapause embryos diluted 4 to 6 times.)

temperature (25°C.) with those of post-diapause eggs showing marked peroxidase reactions (Table II). Extracts of pre-diapause eggs exposed to 5°C., when added to extracts of similar embryos kept only at constant temperature (25°C.) give the same general result (Table III).

Other characteristics of these enzymes such as cyanide sensitivity, thermolability, etc., point to the fact that they undoubtedly are peroxidases. Positive reactions also indicate the presence of indophenol oxidases and tryosinases in addition to peroxidases. Enzymes are always present in the embryo and not in the yolk.

It has previously been shown¹ that exposure of pre-diapause eggs to low temperatures will also prevent or destroy the factor producing cellular block or diapause. Curiously cellular activity is absent or at a minimum during diapause even though positive peroxidase reactions are found. During pre-diapause development at constant temperature (25°C.) marked proliferation of cells occurs even though negative peroxidase reactions are given. It seems reasonable, therefore, to infer that peroxidases are *not primarily* concerned with cellular activity as shown in this particular type of embryonic cell.

Summary. 1. A study has been made of the peroxidase activity (guaiac test) during the embryonic development of the grasshopper, *Melanoplus differentialis*. 2. At constant temperature (25°C.)

negative peroxidase reactions are found for pre-diapause eggs. Positive reactions are given for diapause and post-diapause eggs, Embryos after hatching give negative reactions. 3. Exposure of pre-diapause eggs to low temperatures (0-5°C.) apparently destroys a naturally occurring inhibitor so that eggs thus treated always give positive peroxidase reactions. 4. Ideas are expressed as to possible manner in which peroxidase inhibitors function. 5. No correlation between cellular and peroxidase activity seems apparent.

7861 P

Action Potentials of the "Respiratory Center."

ROBERT GESELL, JOHN BRICKER, AND CONWAY MAGEE.

From the Department of Physiology, University of Michigan, Ann Arbor.

A systematic study of localized potentials lends itself well to the localization of the respiratory center and to an analysis of its still unknown mode of function. After removing the skull cap and the cerebral and cerebellar hemispheres in the dog, we explored the depths of the brain stem from the thalamus through the upper portion of the cervical cord with needle electrodes. A variety of potentials were encountered but in the medulla and upper cervical cord discrete potentials, of orderly sequence, associated with the respiratory act, have been definitely established. These potentials are readily counted and appear to arise from individual nerve cells or neuraxones.

Inspiratory potentials commonly accelerate and deaccelerate with the waxing and waning of inspiration. These potentials cease during the phase of expiration, or continue at a lowered rate during this period. As a rule, the amplitude of discrete potentials remains moderately constant, but instances of gross change of intensity associated with little change in rate have been encountered.

Expiratory potentials during active expiration may accelerate and deaccelerate with waxing and waning of the expiratory act. Usually discrete potentials progress at a uniform rate throughout expiration regardless of its duration indicating a tonic nature of discharge. They are inhibited in rate or number, only during the phase of inspiration. These results fit in with classification of types of breathing previously recorded by the potential method in respiratory muscles of the dog (Gesell).