The Enzymatic Characteristics and the Control of Glycogen Phosphorylase during Early Amphibian Development*

DAVID J. GUSSECK† AND JERRY L. HEDRICK

From the Department of Biochemistry and Biophysics, University of California, Davis, California 95616

SUMMARY

Glycogen phosphorylase has been studied in Rana pipiens eggs and embryos with regard to the nature of the enzyme, its control possibilities, and its involvement in preneurula development. On the basis of enzyme activity, immunological, and electrophoretic comparisons, R. pipiens egg phosphorylase is not wholly identical with the phosphorylases characteristic of skeletal muscle, liver, or kidney tissues. The possibility exists that the egg activity consists of a mixture of types or is a single type peculiar to the embryo.

Amphibian egg phosphorylase possesses the same control possibilities as adult tissue forms. It can be activated by AMP. It appears to exist in active and inactive forms, the interconversion of which is catalyzed by endogenous factors having properties of a phosphorylase kinase and phosphorylase phosphatase. The egg phosphorylase kinase is capable of activating purified frog and rabbit muscle phosphorylase, and the purified rabbit muscle phosphorylase b kinase can activate the egg and frog muscle enzymes. However, the egg and rabbit muscle phosphatases are species specific.

If the activity of the activated form of egg phosphorylase in the presence of AMP is assigned a value of 100%, its activity in the absence of AMP is 96%. The activity of the inactivated form in the presence of AMP is 35%. In the absence of AMP, the inactivated form has no activity.

Phosphorylase in unfertilized R. pipiens eggs is mostly in the inactive form. Shortly after fertilization and at gastrulation, increases in activity and in the --AMP: +AMP activity ratio occur. A cyclic adenosine 3',5'-monophosphate-mediated control mechanism is possible as a cyclic AMP-activated protein kinase has been detected in the fractionated egg supernatant. This enzyme mimics the behavior of rabbit muscle phosphorylase kinase.

For instance in the case of Rana pipiens, some 58% of the glycogen originally present at the time of fertilization is utilized by the time of hatching to meet the metabolic needs of the developing embryo (3–5). Although much information has accumulated on the potential for glycolysis during early embryogenesis, very little is known about the enzymes involved in glycogenolysis and their control during early amphibian development.

On the other hand, the regulation of glycogenolysis in adult tissues is known in considerable detail to proceed via hormone-induced alterations in an enzyme chain resulting in the eventual modification of glycogen phosphorylase activity (6, 7). However, little is understood about the control processes which couple the activity of phosphorylase (and, consequently the rate of glycogenolysis) to the energy requirements of the developing embryo. Given the clarity of understanding as to how glycogenolysis is controlled in adult tissues and the importance of glycogen metabolism during early amphibian development, we therefore undertook the study of the nature and control of glycogen phosphorylase in developing R. pipiens embryos.

Two aspects of this problem have been studied here. First, it was necessary to determine the molecular type or types of phosphorylase present in the embryo. In adult tissues, different tissue-specific molecular forms of phosphorylase have been demonstrated as well as isoenzyme forms within a given tissue (8, 9). Since these different tissue types ultimately arise from the single cell of the fertilized egg, any one or a combination of these enzyme types could exist in the early embryo. Furthermore, a type of enzyme peculiar to embryonic life might be present (e.g. by analogy with adult and fetal forms of hemoglobin). Second, the hormonal control of phosphorylase which occurs in differentiated tissues (10) may not be applicable to preneurula embryos. It is thought that the appearance of various hormones coincides with the differentiation of their respective endocrine glands (see for example Reference 11); since organ differentiation has not yet occurred in preneurula embryos, the involvement of hormones in the control of phosphorylase must be questioned. Thus a different control mechanism may be present at this stage of development. Therefore, we have investigated the type of glycogen phosphorylase present during early developmental stages and the potential for control via a hormone-triggered chain of enzymatic events resulting in the activation of glycogen phosphorylase.

MATERIALS AND METHODS

R. pipiens obtained from Hazen and Co., Alburt, Vermont, in the fall or spring were kept at 4° in bowls (9 1/4 inches in diameter by 5 inches) filled with water which was changed twice a week.
Eight to 10 frogs per bowl were satisfactory. Ovulation was induced by injecting a pituitary gland intraperitoneally (12) and 10 mg of progesterone (suspended in corn oil) into the dorsal lymph sac. Eggs were fertilized by stripping a primed female onto a gently washed testes suspension. Eggs and early embryos were dejellied by exposing them to a cysteine solution (0.5 to 1.0 M) at pH 10 for 2 to 4 min (13). The eggs were then gently rinsed and allowed to develop in tap water. Dejellied embryos so treated developed normally to the feeding stage (when observations were terminated). Unless otherwise stated, all embryos used in an experiment were dejellied simultaneously shortly after fertilization. Only embryos which appeared to be normal were used.

Eggs and pregastrula embryos were homogenized with three passes of a Potter-Elvehjem homogenizer at about 200 rpm. After gastrulation, six passes were required to completely break the cells.

For enzyme activity determinations after fertilization, embry concentrations of 20 per ml were used (a total of 60 to 70 embryos in 3.0 to 3.5 ml were selected and homogenized for each assay in order to minimize random variation), and the homogenates were assayed within 20 s after homogenization. For all other types of studies, more concentrated egg supernatant solutions were used. Eggs were homogenized in 1.5 times their volume of buffer and the supernatant solution (less the floating lipid layer) was removed following centrifugation at 45,000 x g for 10 min.

For experiments with unfertilized eggs, both mature ovarian and dejellied eggs have been used. (Critical experiments have been repeated on both and, to date, no significant differences have been found regarding phosphorylase activity.)

Eggs and embryos were homogenized in a buffer (Buffer A) containing 8-glycerophosphate (20 mM), sodium fluoride (20 mM), bovine serum albumin (0.1%), and mercaptoethanol (40 mM) at pH 6.8. In those experiments where supernatant solutions were in a buffer other than Buffer A, or at a different pH, or when Mg++ATP was added for activation, aliquots of the supernatant were diluted 1:10 in Buffer A immediately prior to being assayed.

The addition of Mg++ATP means one part of a solution containing ATP (18 mM) and magnesium acetate (90 mM), pH 7.0, was added to five parts of a phosphorylase-containing solution. Homogenates and supernatants were assayed for phosphorylase activity by the coupled, fluorescence procedure of Lowry (14) which was modified and used as follows. To 0.2 ml of substrate (sodium phosphate, 100 mM; glycogen 2%; \pm AMP; 4 mM; at pH 6.7) was added 0.2 ml of diluted enzyme (or egg homogenate, supernatant, etc.). The assay mixture was incubated at 30°C for exactly 8 min, then stopped by the addition of 0.1 ml of HCl of sufficient concentration (about 0.38 M) to lower the pH of the mixture to 2.0 to 2.5. After 2 min at the low pH, the mixture was neutralized by adding 0.4 ml of an unneutralized imidazole (200 mM) magnesium acetate (4 mM) solution. If crude homogenates were being assayed, the tubes were centrifuged at this point (45,000 x g, ten minutes) and 0.8-ml aliquots were taken.

The glucose-1-P formed by the phosphorylase reaction was measured by the addition of 0.1 ml of a solution containing phosphoglucosemutase (0.01 mg per ml), glucose 6-phosphate dehydrogenase (0.005 mg per ml), NADP+ (2.1 mg per ml), and mercaptoethanol (40 mM) in an imidazole (100 mM) magnesium acetate (2 mM) buffer at pH 7.0. The NADPH formed was measured fluorometrically following the addition of 2.0 ml (2.1 ml if 0.8-ml aliquots had been taken) of a sodium carbonate (40 mm) EDTA (1 mM) buffer at pH 10 (which stabilizes the NADPH fluorescence). Blanks were prepared by adding the 0.1 ml of HCl stop solution before addition of the enzyme.

Assay of 32P incorporation into casein was by the method of Walsh et al. (15).

Crystalline rabbit muscle phosphorylase b was prepared according to Fischer et al. (16) with the modification of Krebs et al. (17). Phosphorylase a was prepared from phosphorylase b, using purified phosphorylase b kinase. Phosphorylase b kinase was purified from rabbit muscle and assayed by the method of Krebs (18). Phosphorylase a phosphatase was prepared from rabbit muscle and assayed according to Hurst et al. (19). Frog skeletal muscle phosphorylase was prepared by the procedure of Metzger et al. (20).

Rabbit antiserum to frog muscle phosphorylase was obtained by injecting New Zealand white female rabbits with the purified enzyme. The first injections were subcutaneous (10 mg of antigen in complete Freund’s adjuvant) and intraperitoneal (2.5 mg in aqueous solution). Three additional injections at weekly intervals (5.0, 7.5, and 10.0 mg, respectively) were given only intraperitoneally. Antiserum was prepared from the whole blood by the method of Brown (21). Antibody content was estimated by assaying a diluted antigen solution before and after incubation for 10 min at 30°C with antisem diluted in 0.9% NaCl. The reduction in activity was compared with samples incubated similarly with a control serum taken before inoculation of the animals with antigen. Mercaptoethanol was omitted from the phosphorylase assay mixture for these tests.

Disc gel electrophoresis was on 6% acrylamide gels after the method of Hedrick and Smith (22). Phosphorylase activity stains followed the method of Hedrick et al. (23).

[y-32P]ATP was graciously provided by Dr. Donal Walsh. All other chemicals were obtained from commercial sources and were of the highest grade obtainable.

RESULTS

General Characteristics of Egg Phosphorylase—Eggs and embryos of the amphibian R. pipiens possess glycogen phosphorylase activity sufficient to produce 10⁻⁴ to 10⁻⁵ pmoles of glucose-1-P from glycogen per min per egg. The activity resides in a soluble fraction; following centrifugation of egg homogenates for 10 min at 45,000 x g, all of the phosphorylase activity remains in solution. The activity is labile to cold storage and is destroyed upon freezing. In R. pipiens eggs, the activity in the crude homogenate is optimum between pH 6.5 and 7.0.

Disc gel electrophoresis of a R. pipiens ovarian egg supernatant solution stained for phosphorylase activity in the presence of AMP showed a band with a relative mobility of 0.350; gels stained in the absence of AMP showed no band (therefore, the band had activity characteristics of an inactive phosphorylase). By comparison, purified frog muscle phosphorylase subjected to electrophoresis before and after activation with phosphorylase b kinase and Mg++ATP showed relative mobilities of 0.325 for the activated enzyme (+AMP) and 0.268 for the inactivated enzyme (+AMP). Therefore, the relative mobility of inactive frog muscle phosphorylase differs from that of the egg enzyme, indicating that the two enzymes may differ in charge or size, or both. The frog muscle phosphorylase can be compared with rabbit skeletal muscle phosphorylase which exhibits relative mobilities on disc gel electrophoresis of 0.230 and 0.204 for the active and inactive forms, respectively (23).

Further structural differences between the frog egg and skeletal muscle enzymes are shown by the absence of a cross-reaction.
between antibody elicited against the purified muscle enzyme, and the egg phosphorylase. Fig. 1 shows that, while the antiserum was effective in inhibiting frog muscle phosphorylase activity, it had no effect on the egg enzyme.

Since the egg phosphorylase was not identical with the skeletal muscle enzyme, it was of interest to compare it with the liver enzyme which can be distinguished from muscle phosphorylase on the basis of assays carried out in the presence of 0.7 M sodium sulfate (24). Under these conditions, the activity of muscle phosphorylase b is inhibited while that of the inactive liver enzyme is enhanced.

When an inactivated (see following section) ovarian egg solution was assayed in the presence of 0.7 M sodium sulfate, phosphorylase activity was depressed 22% in the presence of AMP but was increased 3-fold in the absence of AMP (over the corresponding activity in the absence of the salt). These data rule against the possibility that the egg phosphorylase is identical with liver phosphorylase. The increased activity in the absence of AMP, however, suggests that the egg activity may consist of a liver-type activity in part.

**Activation and Inactivation of Egg Phosphorylase**—When egg supernatants are treated with Mg\(^{++}\)ATP (see "Materials and Methods"), the endogenous phosphorylase activity increases markedly, with activity measured in the absence of AMP rising faster than that measured in its presence (Fig. 2). Thus, the -AMP: +AMP activity ratio increases to a maximum of 0.96. Addition of Mg\(^{++}\)ATP about 3 hours after homogenization showed that the previously untreated homogenate was still capable of responding. (It does not respond fully, however, for reasons that are mentioned later.)

That this phenomenon is indeed phosphorylase activation mediated by an endogenous egg phosphorylase kinase is substantiated by the ability of the egg supernatant to activate crystalline rabbit muscle phosphorylase b. The egg supernatant can also activate the purified frog muscle enzyme (25).

When egg homogenates are incubated in the absence of fluoride, a rapid decline in phosphorylase activity to a plateau is seen with the activity in the absence of AMP falling faster than that in its presence (Fig. 3). Therefore, a decline in the -AMP: +AMP activity ratio is observed which proceeds to a value of zero (i.e., no activity in the absence of AMP). This decrease in the ratio is consistent with a process of phosphorylase inactivation. That the decline is the result of an in vitro conversion of active to inactive phosphorylase mediated by an endogenous phosphorylase phosphatase is further indicated by the fact that the decline is inhibited by the fluoride anion which is a general inhibitor of phosphatase activity.

When a supernatant solution (lacking fluoride) was divided into two portions, Mg\(^{++}\)ATP added to one, and the phosphorylase activity of both followed for several hours, the results shown in Fig. 4 were obtained. The two plateaus reached

---

**Fig. 1.** Frog muscle and egg phosphorylase activities in the presence of rabbit antiserum (against frog muscle phosphorylase). Four-tenths milliliter of an ovarian egg supernatant (standard dilution buffer less mercaptoethanol) or diluted frog muscle phosphorylase was combined with 0.4 ml of antiserum (diluted in the standard buffer; see "Materials and Methods") or control serum. After incubating mixtures at 25° for 10 min, 0.2-ml aliquots were assayed for phosphorylase activity (stated in terms of fluorescence units).

**Fig. 2.** The effect of Mg\(^{++}\)ATP on phosphorylase activity in a *Rana pipiens* ovarian egg supernatant. To one sample of an ovarian egg supernatant adjusted to pH 8.5 with unneutralized 2 M Tris was added Mg\(^{++}\)ATP. To another sample an equal volume of deionized water was added. Fluorescence units are proportional to phosphorylase activity.

**Fig. 3.** Egg phosphorylase inactivation during incubation of homogenates. A dejellied, uterine egg supernatant containing 80 eggs per ml (in 0.05 M Tris, 0.03 M mercaptoethanol, pH 7.4) was incubated at 25° and assayed after various time intervals.
correspond to the activity levels (±AMP) of the fully activated and inactivated states. If the activity of the activated form +AMP is assigned a value of 100%, then its activity −AMP is 96%. The activity of the inactivated form +AMP is 35% and −AMP is zero. Fig. 4 also shows that Mg++ATP addition to the previously untreated supernatant fraction 2¾ hours after homogenization did not result in complete activation. We now know that under these conditions, the capacity of the endogenous kinase to fully activate phosphorylase is lost within an hour of homogenization. However, the inclusion of purified rabbit muscle phosphorylase kinase in the mixture results in rapid and complete activation several hours after homogenization (Fig. 5). Since the fully inactivated egg phosphorylase can be activated to the originally attainable level, the activity decline seen in the absence of fluoride must indeed be due to reversible phosphatase-mediated conversion to an inactive form and not to a loss of activity due to enzyme destruction. Furthermore, this shows that the rabbit muscle phosphorylase kinase is capable of activating frog egg phosphorylase.

When purified rabbit muscle phosphorylase phosphatase was applied to partially and fully activated solutions, no enhanced rate of inactivation was seen. This indicates that the active frog egg phosphorylase is not a substrate for the rabbit muscle phosphatase. Hurd et al. (19) have noted the high specificity of this enzyme.

Using the relative activity levels of the active and inactive forms of egg phosphorylase, it is possible to mathematically estimate the proportions of active and inactive forms present at any time in the supernatant. For example, for the data of Fig. 2, it can be shown that activity lost by the active form (while the eggs were being incubated in the absence of Mg++ ATP) is fully compensated by activity gained by the inactive form; there is essentially no loss of total enzyme.

**Phosphorylase Activation in Intact Eggs**—In these studies, the question arose as to whether the phosphorylase activation phenomenon seen when Mg++ATP was added to egg supernatant solutions had significance in vivo. Insight pertinent to this question was gained when it was observed that unfertilized eggs devoid of jelly show phosphorylase activation when allowed to remain in tap water for several hours.

Phosphorylase in unfertilized *R. pipiens* eggs initially appears to be mostly in the inactive form. That is, if eggs were rapidly obtained, manipulated, and homogenized in thoroughly chilled equipment, in buffer containing high levels of fluoride (up to

---

**Fig. 4.** Phosphorylase activation and inactivation carried out on two fractions of an ovarian egg supernatant solution (0.05 M Tris, 0.03 M mercaptoethanol, pH 7.4). The numbers appearing above each vertical pair of points represent the −AMP: +AMP activity ratios.

**Fig. 5.** Activation of inactive frog egg phosphorylase catalyzed by rabbit muscle phosphorylase kinase. To a 2.0-ml sample of ovarian egg supernatant solution (0.05 M Tris, 0.03 M mercaptoethanol, pH 7.5) was added Mg++ATP and 0.01 ml of a purified phosphorylase kinase suspension. To other aliquots, Mg++ATP alone was added at the times indicated. For clarity, the levels of the fully inactivated and activated egg phosphorylase are shown by the dotted lines.
0.075 M sodium fluoride was used) so as to minimize phosphatase activity. 

-AMP : +AMP activity ratios were low—around 0.2. On the other hand, if eggs were placed in water, homogenized and assayed over a period of several hours, large changes in activity were seen. These phenomena seem related to the fact that unfertilized, dejellied (or ovarian) eggs are unable to regulate their internal osmotic concentration and swell in hypotonic environments. This swelling coincides at first with activation of the egg phosphorylase. After a time, however, as it continues to swell, the egg appears to undergo "metabolic death," and phosphorylase activity is completely lost. Fig. 6 shows the phenomenon in ovarian eggs placed in tap water contrasted with eggs left in the animal's peritoneal cavity. After being placed in water, the egg phosphorylase is rapidly and completely activated.

In the body cavity, a more gradual activation is seen which may be related to the accumulation of fluids in the cavity after death. A similar activity pattern occurs when the phosphorylase activity of dejellied eggs placed in tap water is compared to that of eggs dejellied just prior to assay. If eggs are harshly treated during dejellying (i.e. if high concentrations of cysteine are used and the eggs are exposed for several minutes after jelly dissolution), they become more permeable. The rapidity with which the eggs swell in water appears to be related to the rate of phosphorylase activation (and the subsequent loss of activity).

Dejellied, fertilized eggs do not swell like unfertilized eggs. Likewise, the activity behavior of phosphorylase in dejellied embryos does not resemble that of unfertilized eggs (to be discussed in the following section).

**Phosphorylase Activity during Early Amphibian Development**—Phosphorylase activity as a function of time after fertilization is shown in Fig. 7. Shortly after fertilization phosphorylase activity increases to a plateau which persists until the late blastula. Concomitant with gastrulation an increase in activity to another plateau is observed, and during these periods increases in the -AMP: +AMP activity ratio are seen. Estimates of glucose-1-P phosphatase activity during this time (which could change with development and thus lead to erroneous phosphorylase estimations) showed that a small and approximately constant amount of glucose-1-P was lost from assay mixtures. This amounted to about 8% of the glucose-1-P produced by the phosphorylase reaction during assays.

**Possibility of Control by Cyclic AMP**—Although cyclic AMP has been shown to initiate phosphorylase activation in adult tissues (26), attempts to find an increased rate of phosphorylase activation in egg supernatants in the presence of cyclic AMP (0.04 M) have been unsuccessful. Similar experiments on rat kidney phosphorylase by Villar-Palasi and Gazquez-Martinez (27) were also negative. However, such experiments involve the attempted measurement of an effect three steps removed from the presumed site of action. Thus, for an effect to be noted, the activation of phosphorylase kinase kinase (15) by cyclic AMP must cause activation of phosphorylase kinase which must activate phosphorylase (17) which is the final activity measured.

Since Walsh et al. showed that the cyclic AMP-activated phosphorylase kinase kinase from rabbit muscle is capable of phosphorylating casein, a cyclic AMP-enhanced phosphorylation of casein catalyzed by egg supernatants was sought. Using complete egg supernatants, however, such an effect could not be shown. The background incorporation of 32P into endogenous

---

**Fig. 6.** Phosphorylase activation in intact ovarian eggs. One-half of an ovarian mass was placed in water, and the other half was allowed to remain in the animal's body cavity. Eggs were alternately sampled from the two halves and assayed.

**Fig. 7.** Phosphorylase activity in *Rana pipiens* embryos as a function of time after fertilization.
proteins was so substantial that a small cyclic AMP enhancement could not be resolved.

Accordingly, egg supernatant solutions were fractionated by isoelectric precipitation in an attempt to magnify the possible contribution of cyclic AMP-activated protein kinase activity. When the supernatant was fractionated at pH 6.0 and pH 5.5, the pH 5.5 pellet (which was dissolved at pH 6.3—the pH at which these assays were carried out) produced a 3-fold cyclic AMP stimulation of 32P incorporation into casein but had no effect on the incorporation into endogenous protein (Table I). These results are compatible with presence of a phosphorylase kinase in the eggs. Thus, the possibility of a cyclic AMP-mediated phosphorylase activation during development exists.

**DISCUSSION**

Glycogen phosphorylase present in the *R. pipiens* egg and early embryo possesses the same potential for modulation of its activity as does the adult enzyme (10, 28). From the effects of ATP and AMP on enzyme activity, we conclude that two interconvertible forms of the enzyme are present. This interconversion is undoubtedly a phosphorylation-dephosphorylation reaction as both a phosphorylase kinase and phosphatase are present. The inactive form of the enzyme can be activated either by phosphorylation or by AMP.

The molecular form of phosphorylase in the adult organism varies from tissue to tissue (8, 9). In rabbit, at least four tissue-specific forms are present. Fig. 8 compares the enzyme activity characteristics of various tissue-specific forms. Clearly, the inactive form of frog egg phosphorylase has activity characteristics distinct from the muscle type (activities of purified *R. pipiens* skeletal muscle phosphorylase were identical with those of the rabbit muscle enzyme). Furthermore, electrophoretic and immunological evidence indicates physicochemical differences between the frog muscle and egg enzymes. Rabbit and pig liver phosphorylase enzymes also have activity characteristics different from the frog egg enzyme (21). However, the egg enzyme did exhibit some activity increase when 0.7 M sodium sulfate was added, a response peculiar to the liver type of enzyme. The frog egg enzyme most closely resembles the rat kidney type (27).

**TABLE I**

**Effect of cyclic AMP on capacity of a pH 5.5 pellet from Rana pipiens ovaarium eggs to incorporate radioactive phosphate into casein**

Counts per min values are averages of three assays except for the blanks which show the averages of seven assays and the casein samples which show the average of six assays. All tubes were counted to within 1.5%.

<table>
<thead>
<tr>
<th></th>
<th>Average counts per min</th>
<th>Per cent standard deviation</th>
<th>Total 32P incorporation into protein</th>
<th>32P incorporation into casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanks</td>
<td>123</td>
<td>±20%</td>
<td>590</td>
<td>188</td>
</tr>
<tr>
<td>+Cyclic AMP</td>
<td>713</td>
<td>±30%</td>
<td>455</td>
<td>65</td>
</tr>
<tr>
<td>+Cyclic AMP</td>
<td>578</td>
<td>±5%</td>
<td>392</td>
<td></td>
</tr>
<tr>
<td>+Casein</td>
<td>815</td>
<td>+4.1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Since there were no significant differences among assays in the absence of casein, these values were combined.

The frog kidney enzyme, however, has activity characteristics similar to the frog muscle phosphorylase rather than egg phosphorylase (25). *R. pipiens* kidney phosphorylase does not cross-react with *R. pipiens* muscle phosphorylase antigen, thus, although they are similar in terms of their enzymatic activity, they are immunologically distinct. The frog egg enzyme appears, therefore, to be different from the adult type of phosphorylase. There may be a type of the enzyme peculiar to the egg or embryo, or alternatively, it is possible that a mixture of types is present, e.g. liver and kidney types but not muscle. To decide between these possibilities it will be necessary to purify the egg form of the enzyme.

Phosphorylase regulation has been recently studied in other embryonic tissues and developing systems (29-31); phosphorylase activity during early amphibian development was first reported by Kurata et al. (32). In the toad *Bufo vulgaris* formosus phosphorylase activity increased 15-fold from fertilization to the blastula stage then rapidly decreased at the onset of gastrulation. However, the assay procedures employed would have detected only the activated (phosphorylated) form of the enzyme. Furthermore, the activity changes detected appear to conflict with the rates of glycogenolysis reported by Jaeger (33) and Boell (34) who showed that glycogen degradation greatly increased after gastrulation. We have not observed the dramatic changes in phosphorylase activity during development reported by Kurata et al., although species differences may explain the discrepancy; indeed, differences in phosphorylase activity do exist between *Xenopus laevis* and *R. pipiens* (25).

The increase in total phosphorylase activity which coincides with gastrulation in *R. pipiens* may be due to *de novo* synthesis of the enzyme. Gastrulation is known to be a period of extensive gene activity. The increase at gastrulation coincides with, and therefore may be the immediate cause of, the increased rate of glycogen disappearance at this time (33, 34). To test for *de novo* synthesis, it will be necessary to measure the amount of enzyme protein present at various developmental stages, rather than the amount of enzyme activity.

Although the evidence suggests control of glycogenolysis by phosphorylase during early embryogenesis, glycogen degradation could also be accomplished in part through amylase-hexokinase coupling, although it would be metabolically less efficient than...
phosphorolysis (5). Whereas the extent to which these activities may be important in early development has yet to be determined, the existence of hexokinase and glucokinase activities in R. pipiens eggs have been observed.3

The potential for control by a cyclic AMP-mediated system in the frog egg has been clearly demonstrated by the presence of a phosphorylase kinase, a phosphorylase kinase kinase (protein kinase), and a cyclic AMP effect on the protein kinase. It is perhaps surprising to find such a control possibility present at such early developmental stages (i.e. the egg). Castenada and Tyler (35) have observed that a cyclase activity in the sea urchin stages, the nature of the initiating events in utilizing such a mediated system is present in the egg and early developmental pre-organ differentiation stages of development. Thus, although the potential for phosphorylase control by a cyclic AMP-anaerobically or aerobically. No differences in the ratios active forms of the enzyme by anaerobic conditions have been determined, the existence of hesokinase and glucokinase activities in R. pipiens eggs have been observed.2

Determination of the phosphorylase control system and its potential physiological significance during early development has yet to be established. Phosphorolysis (5). Whereas the extent to which these activities may be important in early development has yet to be determined, the existence of hexokinase and glucokinase activities in R. pipiens eggs have been observed.2

Attempts to trigger the interconversion of the inactive and active forms of the enzyme by anaerobic conditions have been unsuccessful. Activity measurements in the presence and absence of AMP were made on pregastrular embryos developing anaerobically or aerobically. No differences in the ratios of active to inactive enzyme as a function of oxygen tension were found.3 This finding is indeed puzzling as this ratio would have been affected in most adult tissues by anaerobic-aerobic conditions. No differences in the ratios of active to inactive enzyme as a function of oxygen tension were found.3 This finding is indeed puzzling as this ratio would have been affected in most adult tissues by anaerobic-aerobic conditions. No differences in the ratios of active to inactive enzyme as a function of oxygen tension were found.3 This finding is indeed puzzling as this ratio would have been affected in most adult tissues by anaerobic-aerobic conditions. No differences in the ratios of active to inactive enzyme as a function of oxygen tension were found.3 This finding is indeed puzzling as this ratio would have been affected in most adult tissues by anaerobic-aerobic conditions. No differences in the ratios of active to inactive enzyme as a function of oxygen tension were found.3 This finding is indeed puzzling as this ratio would have been affected in most adult tissues by anaerobic-aerobic conditions. No differences in the ratios of active to inactive enzyme as a function of oxygen tension were found.3 This finding is indeed puzzling as this ratio would have been affected in most adult tissues by anaerobic-aerobic conditions.

Acknowledgments—The authors wish to thank Mr. Ed Carroll and Mr. Curtis Roster for their technical assistance in certain aspects of this study.

REFERENCES

4. COHEN, A. I. (1964) Physiol. Zool. 27, 128
22. HEDRICK, J. L., AND SMITH, A. J. (1968) Arch. Biochem. Biophys. 120, 152-164
32. KURATA, Y., MAEDA, S., IWATA, T., AND MIKUAMA, I. (1958) Igaku to Seibutsugaku 36, 154