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THE CONCENTRATIONS OF SOME ENZYMES OF THE CITRIC ACID CYCLE AND ELECTRON TRANSPORT SYSTEM IN THE LARGE GRANULE FRACTION OF EGGS AND TROCHOPHORES OF THE OYSTER, CRASSOSTREA VIRGINICA[1]

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In an accompanying report Black (1962) has shown that considerable changes in the relative activities of several enzymes of the tricarboxylic acid cycle occur during early development of the oyster. Thus, between the blastula and trochophore stages two enzymes, TPN-specific isocitric dehydrogenase and alpha-ketoglutaric dehydrogenase, increase 2- to 3-fold, roughly paralleling the increase in respiration during this same period. Five other enzymes were found not to change appreciably during development to the trochophore. These were: aconitase, succinic dehydrogenase, malic dehydrogenase, DPNH oxidase, and cytochrome oxidase. Following the trochophore stage cytochrome oxidase decreased somewhat in activity, while the other enzymes remained constant.

With the exception of isocitric dehydrogenase, most of the enzymes studied are known to be present in considerable concentration (though not usually localized) in the mitochondrial fraction of vertebrate tissues. The relationship of enzymic changes to the possible biochemical differentiation of these respiratory granules must therefore be considered. Previous investigations pertaining to this phenomenon include the finding that the content of cytochrome oxidase increases in mitochondria of differentiating rat muscle (Shen, 1955), the work reported by Weber and Boell (1955) and Boell and Weber (1955) in which an increase in the content of cytochrome oxidase and succinoxidase in mitochondria of *Xenopus laevis* was found during development, and the investigation of Mahler, Wittenberger and Brand (1958) in which changes in the relative activities of several respiratory enzymes were found to occur in the large granule fraction of homogenates of the chick between 2 and 6½ days of development.

In eggs of marine invertebrates few studies have been made of the distribution of respiratory enzymes in the various cell fractions which can be obtained from homogenates. In the sea urchin mitochondria isolated in sucrose from unfertilized eggs have been shown to contain succinoxidase and cytochrome oxidase (Maggio and Ghiretti-Magaldi, 1958). The latter enzyme undergoes a 30% increase in the mitochondria at fertilization, but shows no further change until the blastula stage is reached (Maggio, 1959). Cytochemical studies of eggs of various invertebrates, in which respiratory enzymes (usually indophenol oxidase and succinic dehy-

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drogenase) have been reported to be associated with granules, are reviewed by Brachet (1960). In eggs of the oyster Cleland (1951) has studied oxygen uptake by homogenates in the presence of substrates of the citric acid cycle, and has found that removal of the large granules by centrifugation results in a decreased ability of the homogenates to respire in the presence of such intermediates. In addition, he has shown that succinoxidase and cytochrome oxidase are localized in the large granules.

In the present study an attempt has been made to determine the distribution of several enzymes involved in aerobic respiration between the "large-granule" fraction (yolk, mitochondria and other granules) and the "supernatant" fraction (submicroscopic and soluble elements) derived from sucrose homogenates of fertilized eggs and trochophores of the oyster, *Crassostrea virginica*. Several changes in enzyme distribution and in enzyme content of the large granules will be shown to occur during this developmental period.

Materials and Methods

The oysters used in this study were collected by dredging from the Rappahannock River and stored in trays at Gloucester Point, Virginia. The eggs were removed from gonads, inseminated, and cultured by methods described previously (Black, 1962). The stages used for homogenization were cleaving eggs (1½ hours after fertilization) and late trochophores (20 hours after fertilization when cultured at 22° C.). The eggs and larvae were collected by centrifugation and homogenized in 10–15 volumes of 0.55 *M* sucrose buffered with 0.05 *M* Tris (hydroxymethyl) aminomethane, pH 7.35. This medium is approximately isotonic to the dilute sea water in which eggs were grown. The addition of as much as 5% polyvinyl pyrrolidone to the medium caused considerable clumping of the granules in homogenates; this component was therefore omitted from the homogenization mixtures. Homogenization of cleaving eggs was performed by forcing the suspension through a 22-gauge needle; usually 3–5 minutes were required for complete disruption of the eggs. Partial homogenization of trochophores required 15–20 minutes of this treatment, or two minutes of blending in a Lourdes multimixer at 16,000 rpm. No attempt to achieve complete homogenization of the trochophores was made. The homogenization and all subsequent operations were carried out at 0° C.

The homogenates were divided into two aliquots of 2 ml. each, and each aliquot was centrifuged for 10 minutes at 1,000 × gravity in a Lourdes Model LR refrigerated centrifuge containing a swinging bucket rotor. The sediment from the first centrifugation was washed once with 2 ml. of buffered sucrose, and the two supernatant fractions were combined in each of the two aliquots. The washed, low-speed sediment contained some nuclei but consisted chiefly of whole cells and embryonic coats, and it was discarded from both aliquots.

Both aliquots of the homogenate, minus nuclei, whole cells, and embryonic coats, were centrifuged at 18,000 × gravity for one hour in order to sediment the granules. This centrifugal force was near the maximum which could safely be obtained with the rotor available. In preliminary experiments a centrifugation time of one hour was found to be barely sufficient to sediment nearly all of the visible granules as well as all of the succinic dehydrogenase and DPNH oxidase from homogenates of fertilized eggs. In the preliminary experiments, the supernatant fluid recovered

from this high-speed centrifugation of egg homogenates was centrifuged for an additional hour at the same force. This second treatment failed to cause the sedimentation of a usable quantity of granules.

The high-speed sediment in one of the aliquots of homogenate was resuspended in 4 ml. of buffered sucrose and washed by centrifugation for an additional hour. The other aliquot served as a control, in which the sediment was merely resuspended in the original supernatant fluid. This aliquot, labelled "whole homogenate" (minus nuclei), was centrifuged again during the washing of the granules. Supernatant fractions from the first and second centrifugal treatments of the "experimental" aliquot were combined, and the volume was noted. The washed granules were diluted to 4.0 ml. in buffered sucrose. Enzyme determinations were then made on the washed granules, the combined supernatant fractions, and the "whole homogenate" (combined granule and supernatant fractions). In addition, the fatty fraction found at the top of the first supernatant portion of the fertilized eggs was removed with a spatula, suspended in 1 ml. of buffered sucrose, and assayed for all enzymes. Since no enzyme was found to be concentrated in this fraction, the assays were not repeated on the fat from homogenates of trochophores.

The enzymes measured in the separate fractions were: aconitase, isocitric dehydrogenase, alpha-ketoglutaric dehydrogenase, succinic dehydrogenase, fumarase, malic dehydrogenase, DPNH oxidase (with and without added cytochrome *c*), and DPNH-cytochrome *c* reductase. Simultaneous assays of each enzyme were usually conducted on corresponding fractions from three separate batches of eggs or embryos. Each experiment was repeated once, so that all of the reported values represent the averages of data which were obtained from at least six batches of eggs or embryos. Usually not more than one or two enzymes could be measured in any one experiment since it was desirable to complete the determinations within 1 or $1\frac{1}{2}$ hours after preparation of the fractions. Succinic dehydrogenase was measured in all of the granule preparations for use as a reference enzyme.

Spectrophotometric methods used in assaying all of the above enzymes except DPNH-cytochrome *c* reductase have been listed previously (Black, 1962). The latter enzyme was determined by the method of Strittmatter and Velick (1956). The cuvettes contained 10^{-4} *M* cytochrome *c*, 10^{-3} *M* sodium cyanide, 1.7×10^{-4} *M* DPNH, 0.05 *M* phosphate, pH 7.4, and 0.1 or 0.2 ml. of homogenate or homogenate-fraction in a total volume of 3.0 ml. The changes in A_{550} were followed at 15-second intervals for one or two minutes after the addition of enzyme. Assays of all of the enzymes were performed at 25° ± 1° C.

Results

Microscopic examination of homogenate fractions. No serious effort was made to characterize any component of the large-granule fraction. Under oil phase the granules were seen to consist of both spherical and rod-shaped bodies of a wide range of sizes. Attempts to stain with Janus green were partially successful, as determined on masses of granules which were collected by centrifugation after staining; however, it was difficult to observe staining of individual granules by use of the microscope. The supernatant fraction was not found to possess visible granules under ordinary lighting or phase; however, under dark-field illumination small light-scattering particles could be seen.

Enzyme distributions in granule and supernatant fractions. The percentages of total enzyme activities recovered in the large granules of eggs are listed in Table I, column 2. These percentages are based on the sums of the activities recovered in both fractions. In the egg, fumarase, malic dehydrogenase, and DPNH-cytochrome *c* reductase are found almost entirely in the supernatant fluid, whereas appreciable percentages of all of the other enzymes are found in the granules. The electron-transport enzymes, succinic dehydrogenase and DPNH oxidase, are recovered almost exclusively in the granules, as might be expected if this fraction contains

TABLE I

Distribution and total recovery of respiratory enzymes in homogenate-fractions of fertilized eggs and trochophores. Homogenates in 0.55 M sucrose containing 0.05 M Tris, pH 7.35, were freed of nuclei and centrifuged at 18,000 × gravity for one hour. The sediment was washed once by the same treatment and the two supernatant fractions were combined. Whole homogenates were also freed of nuclei and whole cells for comparison with above fractions. Standard deviations are based on six determinations, in separate batches of embryos, of each value

Enzyme	Percentage of total recovered enzyme present in granules based on 100% for the sum of the activities in granules and supernatant fraction		Percentage recovery of enzyme in granules plus supernatant fluid based on 100% for the whole homogenate	
	Fertilized egg	Trochophore	Fertilized egg	Trochophore
Aconitase	37.5 ± 9.1	25.7 ± 5.0	88.7 ± 8.1	108.6 ± 27.3
Isocitric dehydrogenase	65.1 ± 4.9	63.3 ± 1.6	80.5 ± 12.8	91.3 ± 18.0
Alpha-ketoglutaric dehydrogenase	63.9 ± 6.7	100.0 ± 0.0		
Succinic dehydrogenase	100.0 ± 0.0	67.1 ± 2.0	77.6 ± 13.4	98.9 ± 8.9
Fumarase	3.0 ± 1.3	16.1 ± 3.5	302.8 ± 128.6	135.9 ± 15.0
Malic dehydrogenase	8.5 ± 2.4	11.2 ± 2.2	89.5 ± 9.1	112.6 ± 11.7
DPNH oxidase (without cytochrome *c*)	92.0 ± 1.6	46.9 ± 5.8	84.0 ± 13.2	87.3 ± 15.6
DPNH oxidase (with added cytochrome *c*)	92.1 ± 2.9	60.1 ± 5.3	84.7 ± 6.1	81.9 ± 10.6
DPNH-cytochrome *c* reductase	6.3 ± 1.8	23.9 ± 4.8	123.7 ± 14.5	94.5 ± 15.7

nearly all of the mitochondria. The finding that an active DPNH-cytochrome *c* reductase is almost entirely localized in the supernatant fraction from egg homogenates is of interest, since this enzyme has been reported to be present in high concentration in the microsomal fraction of mammalian liver (see Strittmatter and Velick, 1956). None of the enzymes listed were found to be present in quantity in the fatty fraction of the egg.

The average per cent recovery of each enzyme in the granule fraction of trochophores is given in Table I, column 3. Major increases in the percentages of alpha-ketoglutaric dehydrogenase, fumarase, and DPNH-cytochrome *c* reductase recovered in the granules are found when these data are compared with those which were obtained for egg homogenates. Alpha-ketoglutaric dehydrogenase appears to be localized in the granules of trochophores, and the percentage of fumarase and

DPNH-cytochrome *c* reductase in the trochophore granules are 4 to 5 times higher than in the granules obtained from eggs. In contrast to these enzymes, succinic dehydrogenase and DPNH oxidase in the trochophore homogenates were found to be distributed between the granule and the supernatant fractions, so that about $\frac{1}{3}$ of the total recovered enzyme was found in the supernatant fluid in each case. Centrifugation of the trochophore homogenates for 2 hours at 18,000 × gravity did not result in increased sedimentation of either enzyme.

In Table 1, columns 4 and 5, the sums of the recovered enzyme activities in the separate fractions are expressed as percentages of the activities found in the "whole homogenates" (minus nuclei). With the exceptions of fumarase and DPNH-cytochrome *c* reductase, the recoveries of most enzymes are somewhat higher in the trochophore fractions than in those of fertilized eggs. This difference may be an indication that there are substances in whole homogenates of trochophores which inhibit enzyme activities, or that the enzymes in the separated fractions from trochophores are somewhat more stable than in those from eggs. Because of the high endogenous activity of whole homogenates with the dye, 2, 6-dichlorophenolindophenol, the activities of alpha-ketoglutaric dehydrogenase were not measured in the whole homogenates. Endogenous reduction of this dye was almost negligible in the separated fractions.

The extremely high recovery of fumarase in separated fractions of eggs and trochophore homogenates is of particular interest. As noted previously (Black, 1962), fumarase activity is extremely variable in whole homogenates of all stages. In the separated fractions of eggs, nearly all of the activity was present in the supernatant fluid; the inhibition in whole homogenates therefore appears to be caused by the presence of the granules. Since the inhibition is obtained when either fumarate or malate is used as the substrate, it does not appear to be a result of any competing reaction which might be catalyzed by the granules. A marked reduction in the total recovery from separate fractions is observed in the trochophore; this may indicate that trochophore granules inhibit the enzyme to a lesser extent than granules from eggs. A calculation of the total fumarase activity in eggs has been made from the data obtained on the separate fractions. This calculation shows that one million eggs have sufficient enzyme to convert 0.442 ± 0.098 micromoles of malate to fumarate per minute. The ratio of total fumarase to total succinic dehydrogenase in the trochophore is not appreciably different from that in the egg; fumarase activity therefore probably does not change during this period of development.

An excessive recovery of DPNH-cytochrome *c* reductase is also found in separated fractions of the egg, but not in those of the trochophore (Table I). A slight inhibition of this enzyme by the granules of the egg again appears to be responsible for the high recovery. The total activity of this enzyme in all fractions of the egg is calculated to be sufficient to reduce 0.448 ± 0.056 micromoles of cytochrome *c* per minute per million eggs, and the ratio of total reductase to total DPNH oxidase is the same in eggs and trochophores.

Ratios of enzyme activities in granules. The findings summarized in Table I, together with the data available from assays of enzymes in whole homogenates (Black, 1962), indicate that changes in the relative activities of respiratory enzymes in the large granules must occur during development. In order to determine more

precisely the extent of the changes in the granules, measurements of the ratios of the activities of these enzymes to that of succinic dehydrogenase have been made on the granule fractions prepared from eggs and from trochophores. The average ratios obtained are presented in Table II. The averages have been calculated from data obtained in 6 to 10 separate determinations of each ratio. The only enzyme which was found to be constant in the two stages in comparison to the reference enzyme was DPNH oxidase in the presence of added cytochrome *c*. This enzyme system had an activity which was almost exactly equal to that of succinic dehydrogenase in the granules of both eggs and trochophores (ferricyanide was used as the electron acceptor in all assays of the reference enzyme). In the absence of

TABLE II

Ratios of activities of respiratory enzymes to that of succinic dehydrogenase in granules of fertilized eggs and trochophores. Ratios are expressed as micromoles of substrate utilized per minute divided by micromoles of ferricyanide reduced per minute by succinic dehydrogenase. All ratios were determined at 25° C. Standard deviations are based on 10 determinations of each ratio for isocitric dehydrogenase and 6 determinations of all other ratios

Enzyme	Fertilized egg	Trochophore	Per cent change in ratio	't'
Aconitase	0.431 ± 0.160	0.606 ± 0.155	+41	2.33*
Isocitric dehydrogenase	1.350 ± 0.370	2.530 ± 0.460	+88	7.00**
Alpha-ketoglutaric dehydrogenase	0.083 ± 0.013	0.170 ± 0.050	+105	3.78*
Succinic dehydrogenase	1.0	1.0		
Fumarase	0.196 ± 0.138	0.930 ± 0.430	+382	3.61*
Malic dehydrogenase	8.16 ± 3.30	23.50 ± 5.90	+188	5.01**
DPNH oxidase (without added cytochrome *c*)	0.524 ± 0.088	0.370 ± 0.077	−29	9.06**
DPNH oxidase (with added cytochrome *c*)	0.990 ± 0.080	1.020 ± 0.240	+3	0.08
DPNH-cytochrome *c* reductase	0.343 ± 0.130	1.560 ± 0.350	+355	14.31**

*P < 0.005
** P < 0.001

added cytochrome the activity of DPNH oxidase in the egg granules was about ½ of the maximum activity. A decrease of 29% in the activity of the unsupplemented DPNH oxidase system, relative to that of the reference enzyme, was found in the trochophore granules; presumably a loss of endogenous cytochrome *c* from the granules was responsible for this change.

With the exception of the above enzyme system, all of the enzymes investigated were found to have considerably higher activities, relative to that of succinic dehydrogenase, in the trochophore granules than in those of fertilized eggs. The percentage increases in ratio range from 41 for aconitase to 382 for fumarase (Table II). An analysis of the data given in Table II shows that the probability is less than 0.005 that the differences found between the egg and trochophore granules for all enzymes except cytochrome-supplemented DPNH oxidase are due to random variations in ratios.

Discussion

The differences in enzyme ratios in the granules between the two stages investigated are undoubtedly related to changes in distribution and in total amounts of the enzymes. These may be summarized as follows: (1) about ⅓ of the succinic dehydrogenase and DPNH oxidase are present in the supernatant fraction of the trochophores, whereas these enzymes are almost entirely localized in the granules of eggs (Table I); (2) in contrast to these enzymes, higher proportions of alpha-ketoglutaric dehydrogenase, fumarase, malic dehydrogenase, and DPNH-cytochrome *c* reductase are associated with the granules of trochophores than with the granules of eggs (Table I); and (3) a 170% increase in the total activity of isocitric dehydrogenase and a 140% increase in that of alpha-ketoglutaric dehydrogenase are found in whole homogenates during development (Black, 1962). The distribution of isocitric dehydrogenase between the two fractions is the same in eggs and trochophores, about 63–65% of this enzyme being present in the granules. The increase in relative activity of this enzyme in the granules should therefore be at least 170%. In the case of alpha-ketoglutaric dehydrogenase a change in distribution apparently occurs during development, so that 100% of the activity is recovered in the trochophore granules, whereas only 64% is present in the egg granules. The percentage increase in the granules should therefore amount to (140 × 100/64) or at least 210%. The changes actually found in these two enzymes are only about half as great as the predicted changes (Table II). One possible explanation for these discrepancies is that selective destruction of some enzymes might occur in one of the homogenate-fractions of either developmental stage, giving erroneous values for distribution of the enzymes or for the ratios of activities in the granules. This possibility seems especially applicable to alpha-ketoglutaric dehydrogenase, since the activity of this enzyme was always found to decline very rapidly during the assays.

In evaluating the data given in Tables I and II it is necessary to consider possible artifacts other than the one given above. The presence of succinic dehydrogenase and DPNH oxidase in the supernatant fraction of the trochophores could have been caused by disruption of mitochondria during homogenization; however, such disruption of mammalian mitochondria usually results in the solubilization of many of the enzymes of the citric acid cycle (see Hogeboom, 1954). In the trochophore aconitase is slightly less concentrated in the granules than in the egg, and some loss of cytochrome *c* from the trochophore granules also appears to be probable. Except for these two enzymes and the terminal enzymes mentioned above, however, no enzyme investigated is less concentrated in the trochophore granules than in the egg granules. Since the total amounts of succinic dehydrogenase and DPNH oxidase do not change during development, there appear to be only two possible ways to account for their presence in the supernatant fraction of the trochophore. These are: (1) a specific loss (either natural or artificial) of the electron-transport enzymes from the granules of the trochophore without corresponding losses of other enzymes; and (2) natural or mechanical splitting of the granules of the trochophore in such a way that the submicroscopic fragments retain a full complement of enzymes. In the latter case enzymes of the citric acid cycle might be associated with succinic dehydrogenase and DPNH oxidase in submicroscopic particles which were not recovered in the granule-fraction of the trochophore. The first possibility seems unlikely in view of the results other workers have ob-

tained with mammalian mitochondria—if ⅓ of the terminal enzymes are lost from the granules then one would expect that even larger proportions of the other enzymes would be lost. In any case the relative increases in the granule-fraction of all of the enzymes except aconitase are too large to be accounted for by the selective loss of the reference enzyme from the granules.

The simplest interpretation of the data given in Table II is that isocitric dehydrogenase, alpha-ketoglutaric dehydrogenase, fumarase, malic dehydrogenase, and DPNH-cytochrome *c* reductase increase in the large granules during development to the trochophore. The increases in some of the enzymes are a result of the incorporation of existing enzyme molecules into the granules. This appears to be true for fumarase and DPNH-cytochrome *c* reductase and possibly also for alpha-ketoglutaric dehydrogenase and malic dehydrogenase. The increases in other enzymes (isocitric and alpha-ketoglutaric dehydrogenases) result from the incorporation of newly-synthesized enzyme molecules into the granules or from their actual synthesis by the granules. One possible result of such changes in the granules is an increase in their capacity for catalyzing oxidations via the citric acid cycle. This may be related to the large increase in respiration which occurs during development.

It is interesting to speculate on the possibility that the populations of granules in the egg and trochophore are heterogeneous with respect to the enzyme content of individual granules. Thus the relative changes noted in Table II might represent increases in the number of large granules containing high concentrations of some enzymes (fumarase and DPNH-cytochrome *c* reductase, for example) but low concentrations of others. If such heterogeneity exists it may be expected that a diversity of aerobic metabolic pathways also exists in the embryo. In marine animals several workers have obtained cytochemical evidence for the existence of more than one kind of enzyme-containing granule in the egg; however, few of the enzymes investigated are directly involved in respiration. These findings are reviewed by Pasteels (1958).

Summary

1. The enzymes aconitase, TPN-specific isocitric dehydrogenase, alpha-ketoglutaric dehydrogenase, succinic dehydrogenase, fumarase, malic dehydrogenase, DPNH oxidase, and DPNH-cytochrome *c* reductase have been assayed in two fractions, large granules and supernatant, prepared from sucrose homogenates of fertilized eggs and trochophores of the oyster, *Crassostrea virginica*. Succinic dehydrogenase and DPNH oxidase are almost completely localized in the granules of the fertilized egg, but in the trochophore about ⅓ of each enzyme is found in the supernatant fraction. High percentages of aconitase, isocitric dehydrogenase, and alpha-ketoglutaric dehydrogenase are found in the granules of both stages; the latter enzyme appears to be localized in the trochophore granules. Fumarase, malic dehydrogenase and DPNH-cytochrome *c* reductase are almost absent from granules of the egg, but considerable proportions of these enzymes are found in trochophore-granules.

2. Ratios of enzyme activities in the granules relative to that of succinic dehydrogenase have been determined at the two developmental stages. All of the enzymes except DPNH oxidase increase in activity, relative to the reference enzyme, in the granules during development to the trochophore stage. The activity of

DPNH oxidase in the presence of cytochrome *c* is about the same as that of the succinic dehydrogenase in both stages. These changes appear to indicate that differentiation of the population of respiratory granules occurs during development of the oyster.

LITERATURE CITED

BLACK, R. E., 1962. Respiration, electron-transport enzymes, and Krebs-cycle enzymes in early developmental stages of the oyster, *Crassostrea virginica*. *Biol. Bull.*, **123**: 58–70.

BOELL, E. J., AND R. WEBER, 1955. Cytochrome oxidase in mitochondria during amphibian development. *Exp. Cell Res.*, **9**: 559–567.

BRACHET, J., 1960. The Biochemistry of Development. Pergamon Press, New York.

CLELAND, K. W., 1951. The enzymatic architecture of the unfertilized oyster-egg. *Australian J. Exp. Biol. and Med. Sci.*, **29**: 34–45.

HOGEBOOM, G. H., 1954. The isolation and biochemical properties of liver mitochondria. *In:* Fine Structure of Cells, VIII Congress of Cell Biology, Leiden. Interscience Publishers, New York.

MAGGIO, R., 1959. Cytochrome oxidase activity in the mitochondria of unfertilized and fertilized eggs. *Exp. Cell Res.*, **16**: 272–278.

MAGGIO, R., AND A. GHIRETTI-MAGALDI, 1958. The cytochrome system in mitochondria of unfertilized sea urchin eggs. *Exp. Cell Res.*, **15**: 95–102.

MAHLER, H. R., M. H. WITTENBERGER AND L. BRAND, 1958. Biochemical studies of the developing avian embryo. II. Enzymes of the citric acid cycle. *J. Biol. Chem.*, 233: 770–782.

PASTEELS, J. J., 1958. Comparative cytochemistry of the fertilized egg. *In:* A Symposium on the Chemical Basis of Development, ed. by W. D. McElroy and B. Glass. Johns Hopkins Press, Baltimore.

SHEN, S. C., 1955. Enzyme development as ontogeny of specific proteins. *In:* Biological Specificity and Growth, ed. by E. G. Butler. Princeton Univ. Press, Princeton, N. J.

STRITTMATTER, P., AND S. F. VELICK, 1956. A microsomal cytochrome reductase specific for diphosphopyridine nucleotide. *J. Biol. Chem.*, **221**: 277–286.

WEBER, R., AND E. J. BOELL, 1955. Über die Cytochromoxydase-aktivität der Mitochondrien von frühen Entwicklungsstadien des Krallenfrosches (*Xenopus laevis* Daud.). *Rev. Suisse Zool.*, **62**: 260–268.