Reports and Letters

Action of Parapyruvate on Early Development of Strongylocentrotus Purpuratus

The metabolic sequence followed by the sea urchin egg during its early development is not known with certainty, although the change in oxygen uptake on fertilization, first described in 1908 (*1*), has been studied by numerous workers (*2*). The presence of the conventional glycolytic pathway and of the enzymes of the Krebs tricarboxylic acid cycle has been demonstrated in unfertilized eggs (*3*), and oxidative phosphorylation has been demonstrated in cell-free particles (*4*). However, the importance of the tricarboxylic acid cycle as a metabolic pathway in early development of sea urchin embryos has not been determined. If the cycle functions as a major source of high energy phosphate during this period, it is possible that specific inhibitors might have some effect on sea urchin morphogenesis. Accordingly, the effects of parapyruvate, a specific inhibitor of the α-ketoglutaric oxidase (*5*), on the early development of *Strongylocentrotus purpuratus* were investigated (*6*).

Fertilizable eggs from several ovaries were pooled, strained through cheese cloth, washed, and allowed to stand in sea water (10°C) for 6 hours before they were divided into the various cultures and fertilized. Freshly fertilized eggs were washed essentially free of excess sperm. Embryos were cultured in shallow dishes on a rocking device where they were kept in constant gentle agitation throughout the experiment. At appropriate intervals the culture fluid was replaced with fresh medium. Test cultures were fertilized and grown in the inhibitor solutions made up in sea water and adjusted to the normal *p*H of the sea water.

The effects of 25-millimolar parapyruvate were as follows. (i) *Fertilization.* Fertilization was normal in both the controls and in the cultures grown in parapyruvate. (ii) *Cleavage through the 32-cell stage.* There was no obvious difference in the rate and appearance of cleavage in the cultures grown in parapyruvate and in the controls. However, at the 32-cell stage almost all of the fertilization membranes of the parapyruvate cultures were badly wrinkled and many had disappeared, although the membranes in the controls were intact. (iii) *Early blastulae.* The blastulae of the control and the parapyruvate cultures looked identical except for fertilization membranes that were intact in the controls but missing from more than 50 percent of the parapyruvate cultures. (iv) *Late blastulae.* The blastulae of all cultures had hatched and were actively moving. Those of the control cultures were becoming oval with a marked thinning of the blastula wall and were developing a conspicuous aggregation of primary mesoderm. Those of the parapyruvate cultures still resembled early blastulae in that the embryos were quite spherical, had a relatively thick wall, and had developed no mesoderm. (v) *Early gastrulae.* The control cultures had commenced to gastrulate. The archenteron was indented about one-eighth the diameter of the blastocoel and the primary mesoderm was well developed. The parapyruvate cultures were essentially still blastulae, except that primary mesoderm was now visible. (vi) *Mid to late gastrulae.* When the control cultures had completed gastrulation, the parapyruvate cultures were only very early gastrulae, with the archenteron merely a slight depression. Twenty-four hours later there had been no further development in these parapyruvate cultures.

Eggs that had been placed in 25-millimolar parapyruvate for 1 hour before fertilization, and that were cultured in parapyruvate, developed in an identical manner to that just described. Eggs kept in 25-millimolar parapyruvate for 6 hours prior to fertilization, and cultured in parapyruvate, failed to form an archenteron although some primary mesoderm was developed. Cultures in 5-millimolar parapyruvate were almost identical with the control cultures. Cultures grown in 25-millimolar fluoroacetate, which blocks the aconitase reaction in the tricarboxylic acid cycle (*7*), developed as did those that were incubated for 6 hours prior to fertilization and grown in 25-millimolar parapyruvate.

Thus it appears that these inhibitors prevent differentiation and arrest the development of sea urchin embryos in the late-blastula to early-gastrula stage. A similar suppression of development in the blastula stage of sea urchins owing to the addition of thiocyanate was described as early as 1896 (*8*). This arrested development was preceded by a depression of respiration before the morphological differences between the control cultures and those grown in thiocyanate became apparent (*9*). Iodosobenzoate inhibits archenteron formation (*10*), and it has been suggested (*11*) that this is caused by inhibition of the selective distribution of mitochondria involved in gastrulation. The late blastula stage seems to be characterized by increased metabolic activity antecedent to differentiation. There is a sudden increase in mitochondrial number (*12*) and a major increase in protein synthesis (*13*) coinciding with a period of increased respiration (*14*).

Perhaps cleavage in the sea urchin is independent of the tricarboxylic acid cycle since the cycle inhibitors used in this study had no detectable effect until the blastula stage. The fact that parapyruvate had no apparent effect prior to differentiation involving gastrulation might indicate a change in the metabolism of the blastula whereby the tricarboxylic acid cycle becomes a necessary pathway; thus, further development would be depressed by cycle inhibitors. The increased protein and enzyme synthesis necessary for differentiation may possibly require the cycle. Before the blastula stage the cycle may function at a comparatively low level, but during the blastula stage it may enter a phase of activity geared to the increased metabolic requirements. An alternative to this hypothesis is that the tricarboxylic acid cycle does operate during the early stages and actually is blocked by parapyruvate, but its function is to produce a store of high energy phosphate only utilized later during periods of synthesis, when the effects of the inhibitor become apparent.

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Evidence That Serotonin Has a Role in Brain Function

The finding of serotonin (5-hydroxytryptamine) in brain (*1, 2*) has aroused speculation that it may have a role in brain function (*3*), perhaps as a neurohumoral agent (*4*). Studies from our laboratory that support this view have shown that lysergic acid diethylamide (LSD), a hallucinogenic agent, suppresses an action of serotonin on the central nervous system (*5*), while reserpine, a drug used in treatment of mental disorders, liberates serotonin from body depots, including intestines (*6*) and platelets (*7*).

The development of a specific fluorometric assay for serotonin in brain (*2*) has now made it possible to test our hypothesis that reserpine action may be mediated through the liberation of serotonin in brain. The serotonin content of brain, normally about 0.55 μg/g, declined rapidly after the intravenous injection of 5 mg/kg of reserpine. Within 30 minutes the total brain serotonin declined by about 75 percent and within 4 hours it declined by about 90 percent. The low level persisted for about 24 hours and then increased slowly, attaining the normal value after about 7 days. The brain is particularly sensitive to the serotonin-releasing properties of reserpine—doses as low as 0.1 mg/kg appreciably lowered the serotonin content.

Reserpine measured fluorometrically was no longer detectable in brain 12 hours after administration (*8*), whereas sedative effects and changes in brain serotonin persisted longer than 48 hours. The sedative effects thus seem to be related to the change in brain serotonin rather than to the concentration of reserpine. This may be interpreted as further evidence that reserpine acts through liberation of serotonin. Throughout the period of low serotonin content in brain, 5-hydroxyindoleacetic acid, the metabolic product of serotonin, appeared in urine in appreciable amount. Presumably, therefore, serotonin was still being formed in the body but was not accumulating in brain tissue. Thus it seems that reserpine causes an alteration in the serotonin-binding capacity of brain cells that persists long after reserpine can no longer be detected.

Our present concept, on the basis of the available facts, is as follows: serotonin in brain is normally present mainly in a bound form, thus being protected from the highly active enzyme, monoamine oxidase (*9*). After reserpine administration, brain cells lose, in part, their capacity to retain serotonin. As a result, serotonin is liberated and metabolized by the action of monoamine oxidase. Although reserpine rapidly disappears from the brain, the effect on the capacity of cells to retain serotonin persists. Since serotonin is still being formed during this period, much of it is presumably present in a free or physiologically active form. This free form of serotonin is considered as the mediator of the prolonged reserpine action. Since free serotonin is rapidly metabolized, the total serotonin remains at a low level.

The data described in this communication are in accord with the view that serotonin has an important role in brain function, possibly as a neurohumoral agent.

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Systematic Effect of C^{14}-Labeling on Ion-Exchange Chromatography of Amino Acids

In the course of studies on amino acid metabolism in mammalian cells in tissue culture (*1*), protein hydrolysates from cells fed C^{14}-labeled glucose or glutamine were chromatographed on an ion exchange column. It was observed that the peaks of radioactivity in the effluent did not precisely coincide with amino acid peaks located by the ninhydrin color reaction, but in every case followed them closely. The two curves were identically shaped, and activity did not occur elsewhere in the effluent. It seemed likely that the heterogeneity was the result of the partial resolution of labeled from unlabeled amino acid. This was shown to be the case by chromatography of known C^{14}-labeled amino acids. Some preliminary observations concerning the nature of the effect are reported in this paper.

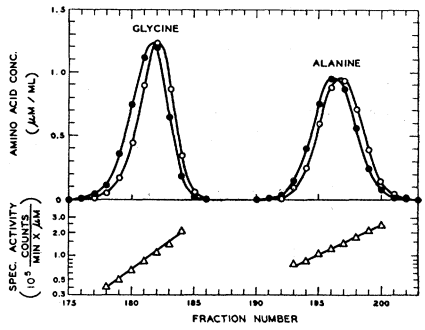

Fig. 1. Portion of the effluent curve from the chromatography of C^{14}-labeled amino acids on a 100- by 0.9-cm column of Dowex 50: ●, amino acid concentration as determined by ninhydrin; ○, amino acid concentration as determined by C^{14} count assuming 100 percent recovery; and △, calculated specific activities.

The ion-exchange separation used was a modification of the methods of Moore and Stein (*2, 3*). A 100- by 0.9 cm column of Dowex 50 (*4*), operated at 50°C and 6 ml/hr throughout the run, was employed. Elution was started from a reservoir containing 250 ml of a 0.37*M* citrate buffer (0.25*N* in sodium citrate) of *p*H 3.10. A continuous *p*H gradient was produced by the addition of 0.25*N* sodium hydroxide to the buffer to give a ratio of flow rates into and out of the reservoir of 1 to 5.7. After about 170 ml of effluent had been collected, the ratio of flow rates was changed to 1 to 1. The degree of resolution provided was significantly greater than it was in the original Moore and Stein procedure (*2*) with a 100 cm column and in some respects was equal to that of their newer method (*3*) employing a 150 cm column.

Approximately 4 μmoles each of L-aspartic, L-threonine, L-serine, L-proline, glycine, L-alanine, and L-valine (*5*), all uniformly labeled with C^{14} at a level of about 0.1 μc/μmole, were chromatographed. One-milliliter fractions were collected and 3 ml of water were added to each. One milliliter was taken for determination of amino acid concentration with ninhydrin (*6*). Carbon-14 was determined on a 0.5-ml portion of the remainder after diluting 1 to 50, except at the leading and trailing edges of the peaks, where 0.5 ml of undiluted sample