Table 1. Properties of triosephosphate dehydrogenase from *Chromatium*. Purified enzyme was oxidized or reduced as described in text. The K_m is an average based on five independent experiments. DiPGA is used as an abbreviation for 1,3-diphosphoglyceric acid.

Source of enzyme	K_m				Moles —SH group per mole enzyme
	DiPGA	NAD	G-3-P	NADH	
Untreated CO_2*	3×10^{-3}	5×10^{-5}	1.7×10^{-4}	3.0×10^{-6}	4.2
Oxidized CO_2	9×10^{-3}	6.5×10^{-5}	5.0×10^{-4}	4.0×10^{-6}	2.4
Untreated mal†	1×10^{-2}				2.4
Reduced mal	4.0×10^{-3}				3.3

* CO_2 refers to cells that were grown photolithotrophically. † Mal refers to cells that were grown photoorganotrophically.

per protein molecule was measured by the nitroprusside reaction (*7*) in both treated and untreated TPD's from both sources of cells (Table 1). The untreated enzyme from cells grown on CO_2 contained 4.2 reactive —SH groups per molecule, and the untreated enzyme from cells grown on malate contained 2.4 —SH groups per protein molecule—with the assumption of 100 percent purity of the preparations. Oxidation of the former, which increased the K_m threefold, also reduced the number of reactive —SH groups to 2.4. Conversely, reduction of the latter enzyme, which reduced the K_m, raised the number of reactive —SH groups to 3.3. All changes in K_m and in reactive —SH content were reversible.

These results indicate that *Chromatium* many contain a single NAD-dependent TPD, the physical and chemical properties of which are controlled by the chemical environment of the cells. Whereas, in higher plants, the NAD-dependent TPD probably functions primarily in the direction of glycolysis and the NADP-dependent TPD functions primarily in the photosynthetic Calvin cycle, the NAD-dependent TPD of *Chromatium* functions in the synthetic direction during both lithotrophic and organotrophic growth conditions. During photolithotrophic growth, TPD must support synthesis of C_6 compounds and support the function of a Calvin cycle (*5*). Organotrophic growth results in a tenfold reduction of Calvin-cycle activity (*5*) and, under such conditions, TPD activity would be necessary only for the production of compounds for carbon storage and for glycolysis. In an organism with small intracellular pools of both 1,3-diphosphoglyceric acid and G-3-P (*5*), and a high content of TPD with a relatively low affinity for these substrates, a small change in K_m could easily control the rate of the TPD reaction. Such a control mechanism might be based on direct, environmentally caused, changes in the tertiary structure of the protein molecule. These results are interesting in view of the results of Horecker and Cremona (*8*) who found that two —SH groups might play a role in the regulation of activity of fructose 1,6-diphosphate aldolase and in view of the work of several authors demonstrating the reversible loss of antibody activity produced by reduction of S—S linkages (*9*).

Regulation of TPD activity in *Chromatium* by such a mechanism would provide control of a key reductive reaction in photosynthesis and would be rapid in both directions since the *de novo* synthesis of a protein is not required.

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References and Notes

1. M. Gibbs, *Nature* **170**, 164 (1952); R. H. Hageman and D. T. Arnon, *Arch. Biochem. Biophys.* **57**, 421 (1955); G. A. Hudock and R. C. Fuller, *Plant Physiol.*, in press.
2. R. C. Fuller and M. Gibbs, *Plant Physiol.* **34**, 324 (1959).
3. U. Heber, N. G. Pon, M. Heber, *ibid.* **38**, 355 (1963).
4. R. M. Smillie and R. C. Fuller, *Biochem. Biophys. Res. Commun.* **3**, 368 (1960).
5. R. C. Fuller, R. M. Smillie, E. C. Sisler, H. L. Kornberg, *J. Biol. Chem.* **236**, 2140 (1961).
6. P. Andrews, *Biochem. J.* **91**, 222 (1964).
7. R. R. Grunert and P. H. Phillips, *Arch. Biochem.* **30**, 217 (1955).
8. B. L. Horecker and T. Cremona, *Science* **148**, 665 (1965).
9. P. L. Whitney and C. Tanford, *Proc. Nat. Acad. Sci. U.S.* **53**, 524 (1965); E. Haber, *ibid.* **52**, 1099 (1964).
10. Supported in part by GB 2631 from the National Science Foundation, and in part by a traineeship 5TL GM 961-03 (to G.A.H.) from the National Institute of General Medical Sciences.
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1 July 1965

Protein Synthesis in Enucleated Eggs of Rana pipiens

Abstract. *Tritiated leucine of high specific activity was injected into enucleated* Rana pipiens *eggs. After 6 hours, there was significant incorporation of the label into protein. The amount of incorporation in these eggs was as great as that in fertilized, normally developing eggs.*

Many kinds of experiments have shown that early development in the amphibian egg can occur in the absence of obvious nuclear control. For example, partially cleaved blastulae have been obtained by fertilizing frogs' eggs with heavily irradiated sperm and then mechanically removing the egg nucleus (*1*). Likewise, development to the blastula or gastrula stage occurs in the presence of lethal hybrid nuclei (*2*), and in the presence of nuclei with very abnormal chromosomal complements (*3*). However, neither the nature of the synthetic processes occurring during early development nor the importance of nuclear participation in the control of these processes is clearly understood.

Early investigations had suggested that very little, if any, net RNA, DNA, or protein synthesis occurs before gastrulation (*4*), but recent reports have indicated that RNA synthesis (*5, 6*), and possibly protein synthesis (*5, 7*), does take place during cleavage stages.

Tiedemann and Tiedemann (*8*) have presented evidence for protein synthesis in anucleate halves of ligated *Triton* eggs, indicating that protein synthesis can occur in the absence of a nucleus. Further evidence for the nonparticipation of DNA in the control of early synthesis was reported by Brachet *et al.* (*7*) who showed that inhibition by actinomycin D of DNA-directed RNA synthesis had little effect on cleavage even when the antibiotic was injected directly into fertilized eggs. On the other hand, injection of puromycin, an inhibitor of protein synthesis, effectively stopped cleavage shortly after treatment. These results, although somewhat indirect, suggest that protein synthesis which does occur during early development is not necessarily subject to direct nuclear control. The following experiments provide direct evidence for the synthesis of protein in enucleated eggs of the leopard frog, *Rana pipiens*.

Ovulation was induced in mature

Rana pipiens females by the injection of anterior pituitary suspensions. Eggs from an ovulated female were stripped into clean watch glasses and were either fertilized, activated, or enucleated. Eggs were artificially activated by pricking them with a clean glass needle. Activated eggs were enucleated by flicking the chromatin out of the egg (*9*). Fertilized eggs from each frog were allowed to develop to stage 25 of Shumway (*10*) in order to ascertain the percentage that exhibited normal development. This percentage did not differ significantly among eggs from the various frogs, and, on an average, normal development was observed in 96 percent of the eggs. The enucleation procedure was controlled by enucleating a number of eggs shortly after fertilization. When the operation is successful, the enucleated eggs develop as androgenetic haploids. A total of 147 control eggs were fertilized and then enucleated. All developed as haploids.

After fertilization, activation, or enucleation, the jelly was removed from the eggs manually; the eggs were rinsed several times in sterile 10 percent Steinberg's solution (*11*) and transferred to the operating dish which contained sterile full-strength Steinberg's solution. Tritiated leucine [1.0 mc/ml; 4 c/mmole (*12*)], was injected with a Leitz micromanipulator directly into each test egg with a micropipette calibrated to deliver 7.1 ± 0.4 mμl of the solution. The eggs were injected at 18°C within a period of 35 to 100 minutes after activation or fertilization.

After injection, the eggs were transferred in groups of five or ten to small Stender dishes that contained sterile Steinberg's solution (10 percent) where they remained at 18°C for 6 hours. The fertilized eggs developed normally to the stage of 8 to 16 cells during this time. In the case of activated eggs no development occurred, although abortive cleavage divisions were occasionally observed.

Controls were prepared by injection into unactivated eggs of the same amounts of tritiated leucine used in the experimental groups, and then the eggs were immediately homogenized.

Groups of eggs were homogenized in 5 ml of 0.15*M* NaCl by repeated passage through a Pasteur pipette. Homogenates were extracted in the saline solution overnight at 4°C and centrifuged in the cold at 12,000 g for 10 minutes. The saline extract was separated, made 0.5*M* in perchloric acid, and heated in a water bath at 60°C for 30 minutes. The material precipitated by perchloric acid was removed by low-speed centrifugation, washed in 0.5*M* perchloric acid and dissolved in hydroxide of hyamine (*13*) at 60°C (3 to 5 minutes). The hyamine solutions were quantitatively transferred to scintillation vials with the xylene-dioxane-cellosolve scintillation fluid of Bruno and Christian (*14*). The volumes of hyamine and scintillation fluid were varied. Tritium was counted in a Tri-Carb liquid scintillation spectrometer (*13*). Activity in the control groups of eggs, which had been homogenized immediately after injection, was slightly higher than instrument background. Counts in these controls were subtracted, as background, from the counts obtained in the experimental groups.

Table 1. Incorporation of tritiated leucine in *Rana pipiens* eggs.

No. eggs per group	Eggs injected		Radioactivity per egg (count/min)
	Type	Total (No.)	
	*Experiment 1**		
5	Fertilized	25	58
5	Enucleated	35	54
5	Activated	35	54
	Experiment 2†		
5	Fertilized	30	43
5	Enucleated	30	64
5	Activated	20	85
	Experiment 3‡		
10	Fertilized	40	151
10	Enucleated	70	141
10	Activated	30	178

* 1.0 ml of hyamine, 10 ml of scintillation fluid.
† 0.5 ml of hyamine, 10 ml of scintillation fluid.
‡ 0.5 ml of hyamine, 20 ml of scintillation fluid.

Protein in the extracts from the groups of eggs was determined (*15*), with crystalline bovine serum albumin as the protein standard. The unactivated egg contains about 90 μg of saline-soluble protein (approximately 13 percent of the total protein). Six hours after activation, enucleation, or fertilization there was no measurable change in the amount of protein extractable in saline.

The results (Table 1) from 315 treated eggs showed that incorporation of isotope occurred not only in fertilized eggs but also in activated and in enucleated eggs, and to about the same extent in each. Corollary experiments confirmed that the incorporation of isotope is a true indication of net protein synthesis: (i) When puromycin was injected into eggs (fertilized, activated, or enucleated), and then isotope was injected, incorporation was greatly diminished (*16*). (ii) When injection of the isotope was followed, after 90 minutes, by injection of an excess of unlabeled leucine, all the label incorporated in the first 90 minutes remained in the fraction precipitated by hot perchloric acid (*17*).

After enucleation, an exovate containing the egg chromatin and spindle apparatus formed on the outer surface of the vitelline membrane. Although this exovate was completely detached from the egg surface, several groups of eggs were divested of their vitelline membranes before homogenization in order to determine whether the presence of the extracellular nucleus would alter the results. There was no significant difference in detectable counts when the vitelline membrane and attached exovate were removed.

The observation that activated and, particularly, enucleated *R. pipiens* eggs can synthesize as much protein as their fertilized counterparts do suggests first that fertilization and subsequent cleavage divisions are not prerequisites for continued protein synthesis. Moreover, the results obtained from enucleated eggs indicate that protein synthesis can take place in the absence of continuous nuclear control, and thus would not be due to the continued production or release of new "messenger RNA" by the nucleus. This does not eliminate, however, the possibility of direct nuclear participation within a restricted period of time. Removal of the egg nucleus cannot be accomplished until 10 to 15 minutes after activation, and the possibility exists that the nucleus, during this time, might very well activate, or control in some way, the whole complex of events which occur during the succeeding hours.

Denny and Tyler (*18*) and Brachet *et al.* (*19*) have shown that protein synthesis can take place in artificially activated, enucleate fragments of sea-urchin eggs. These results also show that continuous control by the nucleus is not a prerequisite for all protein synthesis. Among possibilities considered to explain these findings are: (i) the transcription of extranuclear DNA to create conventional "messenger RNA" for translation into protein. (ii) the existence in the egg cytoplasm of "stable messenger RNA," which is translated into protein without the par-

ticipation of an RNA-synthesizing system. The latter alternative seems most likely in sea urchins, (*20*) but whether either of these mechanisms is operative in amphibians is not yet known.

The existence of extranuclear DNA in amphibian eggs has been suggested (*4, 21*). But the synthesis of "DNA-like RNA" does not appear to begin until relatively late in cleavage (*6*), although other classes of RNA may be synthesized shortly after fertilization (*5*). While there has been no clear-cut demonstration of the existence of "stable messenger" in amphibians, results with actinomycin D as an inhibitor of DNA-directed RNA synthesis have strongly suggested that early protein synthesis can occur without the synthesis of RNA (*7*).

Thus, it seems that protein synthesis can occur in the amphibian egg in the absence of continuous nuclear influence. Whether the same proteins are being synthesized in the nucleate and anucleate eggs is not yet known, and the nature of the factors controlling this early protein synthesis remains unclear.

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References and Notes

1. R. Briggs, E. Green, T. J. King, *J. Exp. Zool.* **116**, 455 (1951).
2. J. A. Moore, *Exp. Cell Res. Suppl.* **6**, 179 (1958).
3. R. Briggs, J. Signoret, R. R. Humphrey, *Develop. Biol.* **10**, 233 (1964).
4. J. Brachet, *Biochemistry of Development* (Pergamon, New York, 1960).
5. M. Decroly, M. Cape, J. Brachet, *Biochim. Biophys. Acta* **87**, 34 (1964).
6. D. Brown, *J. Exp. Zool.* **157**, 101 (1964).
7. J. Brachet, H. Denis, F. deVitry, *Develop. Biol.* **9**, 398 (1964).
8. H. Tiedemann and H. Tiedemann, *Naturwiss.* **41**, 535 (1954).
9. K. R. Porter, *Biol. Bull.* **77**, 233 (1939).
10. W. Shumway, *Anat. Rec.* **78**, 139 (1940).
11. M. Steinberg, *Carnegie Inst. Wash. Yearbook* **56**, 347 (1957).
12. New England Nuclear Corp., Boston, Mass.
13. Packard Instrument Co., LaGrange, Ill.
14. G. A. Bruno and J. E. Christian, *Anal. Chem.* **33**, 1216 (1961).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
16. L. D. Smith and R. E. Ecker, in preparation.
17. R. E. Ecker and L. D. Smith, in preparation.
18. P. C. Denny and A. Tyler, *Biochem. Biophys. Res. Commun.* **14**, 245 (1964).
19. J. Brachet, A. Ficq, R. Tencer, *Exp. Cell Res.* **32**, 168 (1963).
20. P. R. Gross, *J. Exp. Zool.* **157**, 21 (1964); E. Baltus, J. Quertier, A. Ficq, J. Brachet, *Biochim. Biophys. Acta* **95**, 408 (1965); A. Monroy, R. Maggio, A. M. Rinaldi, *Proc. Nat. Acad. Sci. U.S.* **54**, 107 (1965).
21. E. J. Finamore and E. Volkin, *Exp. Cell Res.* **15**, 405 (1958); J. Brachet and A. Ficq., *ibid.* **38**, 153 (1965).
22. Supported by the AEC. We thank Joan Stachura for technical assistance.

6 August 1965

Brain Transplantation: Prolonged Survival of Brain after Carotid-Jugular Interposition

Abstract. *Six isolated canine brains were successfully transplanted for 6 hours to 2 days to the cervical vasculature of dogs. Viability was shown by electrocortical activity and significant uptakes of oxygen and glucose, with production of carbon dioxide. Cerebral blood flows, temperatures, and pressures of the cerebral homograft were continuously monitored by way of an implantable recording module.*

To extend significantly the viable longevity and improve the biological performance of the isolated brain, permanent vascular implantation in a suitable recipient would be required. Whereas Demikhov (*1*) has demonstrated the feasibility of transplantation of the upper portion of the canine body, including the head, and Guthrie (*2*) has successfully revascularized canine heads for short periods, the more difficult surgical maneuver of transplanting the brain as an isolated organ has not been accomplished. The solution to the inherent biological difficulties of neurogenically and vascularly isolating the brain, upon which cerebral transplantation would depend, has recently been described (*3*). The theoretical possibilities and implications of transfer of cerebral tissue have also been reviewed recently (*4*).

We now describe our experience with transplantation of the isolated canine brain into a recipient dog; we utilized the circulatory environment of the neck. To provide for measuring function during transplantation a survey system has been implanted along with the isolated brain to permit continuous monitoring of the electrocortical activity, cerebral blood flow, temperature, and discontinuous measurement of arterial-venous oxygen A-V_{O_2} and venous-arterial carbon dioxide V-A_{CO_2} and glucose consumption of the isolated brain.

With the dog under sodium pentobarbital anesthesia, the brain was surgically isolated within the skull by meticulous removal of all contiguous tissues. By preserving the integrity of the cranium, support and protection are afforded the brain and its enveloping membranes (*5*). To insure absolute hemostasis during the prolonged period

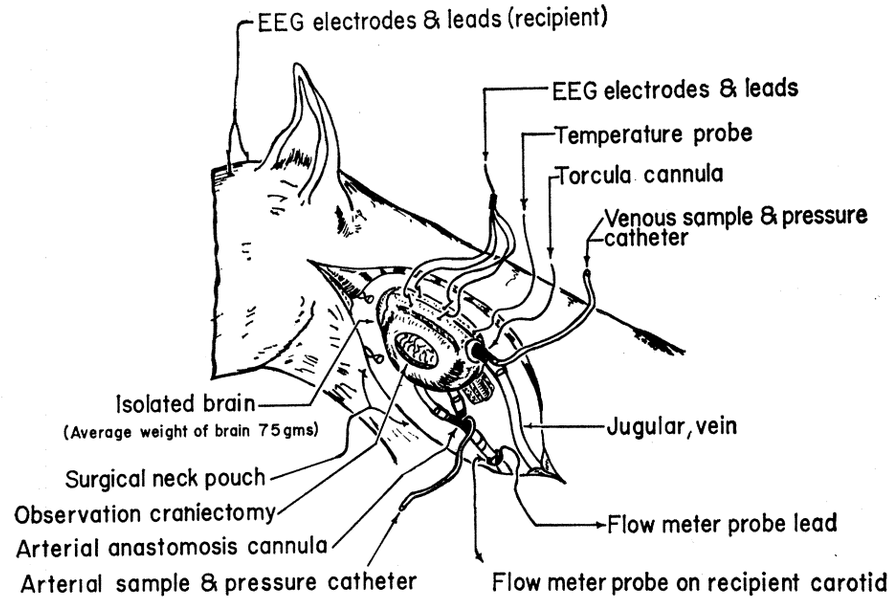

Fig. 1. Isolated brain in position between the carotid artery and jugular vein of the recipient. A small polyethylene tube is connected to the metal side arm of the torcula cannula and provides a means of sampling venous blood from the brain and a means of measuring venous pressure in brain. A similar sampling catheter is connected to the side arm of the arterial cannula. Together with the connections to a 3-mm implantable electromagnetic flow meter probe placed around the recipient carotid artery supplying the brain transplant, the torcular and carotid sampling catheters are brought outside the skin through a small surgical incision. Similarly, the EEG leads and thermoprobe connections are led to the exterior.