BIOMAGNETISM AND FERRITIN

FINAL REPORT

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This report follows the semi-annual report of May 12, 1966, the continuation proposal of July 30, 1966 and the current status report of September 23, 1966. A copy of the latter, which consisted of a detailed letter to Dr. Huertas, is enclosed. For the remaining three months of the year, very little new can be recorded.

We continued the work outlined in the above reports, but without the availability of fertile eggs of Rana pipiens, due to the season of the year, no answers to our fundamental questions have been obtainable. However, we did arrive at several important conclusions in this period.

1. The best way to prepare eggs for determination of iron content in various parts of the embryo is by first fixing them. Freezing for storage results in so many added difficulties in sectioning that it proved to be worthless. The fixing procedure we used does not dissolve the iron and is as follows:

   Fix in 5% Formalin for 24 hours.
   Wash in double distilled water 6-24 hours.
   Dehydrate, clear and embed as usual.

   This change in procedure was undertaken since we have reason to believe that the fixative previously used (Bouin-Dioxane) may dissolve iron.

2. Our indications to date are that eggs not treated magnetically have a random distribution of iron both in stage 2 and in stage 8. See Table 1.

3. Development of the embryos in the very small set of chambers in the holder that is placed in the magnet and its identically dimensioned twin that serves as a control out of the magnet, is possible, provided (a) the water temperature is kept below 20 degrees centigrade; (b) the flow of nutrient (spring water) is sufficiently fast to carry away any products of the teflon holder, the tygon circulating tubing and the very small and short stainless steel connectors used to complete the system; and (c) the reservoir of spring water which is circulated is large and exchanged with fresh water sufficiently often so as to prevent an accumulation of undesirable products.

4. It appears to be essential that rather large specimens of Rana pipiens be used as the source of fertilized eggs. Injection of small specimens with pituitaries seems to be largely a waste of effort.

The above observations and comments supplement the previous reports. Our main effort in the immediate future is

1. To complete a sufficient number of runs in the magnet and in the control to observe the development of the embryos in these two conditions,

2. To fix and preserve enough of these specimens for subsequent detailed chemical and histological examination.

One of the ways in which the resultant normalcy of the development of the embryos seems to best be demonstrated is by the observation of their motion. Therefore, we plan to keep a motion picture record of our embryos after exposure to the magnetic field compared to the controls. The first completed run through the magnet showed impaired development of the magnetically treated embryos when compared to their controls but no statistically valid conclusion can be drawn from this first run.

TABLE 1

Data for Whole Eggs† and Egg Sections (Cryostat) in µg Fe

September 9, 1966 - December 1, 1966

No. Sections From Egg **	1st Half	2nd Half	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter	Whole Egg
1							0.65
1							0.00
1							0.87
1							0.01
2	0.50	0.46					0.96
2	0.68	0.00					0.68
1							0.00
1							0.00
1							0.00
2	0.00	0.00					0.00
2	0.00	0.00					0.00
1							0.00
1							0.72
1							0.00
1							0.57
2	0.05	0.00					0.05
2	0.00	0.00					0.00
*4			0.24	0.16	0.00	0.42	0.82
*4			0.61	0.20	1.20	0.00	2.01
*4			0.35	0.83	0.31	0.90	2.39
*4			0.42	0.53	0.57	0.68	2.20
1							1.83
1							0.50
1							0.00
4			0.24	0.46	0.65	0.20	1.55

* Stage 8 eggs (all others Stage 2)

** Eggs cut in approximately equal sections starting at vegetal pole.

† These eggs were obtained from General Biological Supply House because we had no supply at the time. Their detailed history and particularly their exposure to iron contamination, therefore, is unknown and was not under our control.

September 23, 1966

Dr. Jorge Huertas
National Aeronautics and Space Administration
Ames Research Center
Moffett Field, California 94035

Dear Dr. Huertas:

I just wanted to let you know the current status of our work, as you requested.

(1) Work on getting the bull frogs to ovulate was discontinued as we were unable to get any fertilized eggs up to the end of August. We will start again with Rana pipiens, for which we have a successful technique worked out, when their season begins.

(2) We have worked out a micro method for the determination of the iron content of frog embryos and of sections of frog embryos using bathophenanthroline as described in the appendix. This allows us to determine as little as 0.1 µg of iron, 1/10th the total of the average iron content of an egg.

(3) We have started a survey of the distribution of iron in the frog embryos, Shumway stages 3 and 20, and sections of these consisting of approximately one-half of the egg. These are Rana pipiens embryos which had previously been fixed in neutral formalin. The results are shown in Tables 1 and 2. They confirm that embryos on the average contain about 1 microgram of iron. The variation in the results from embryo to embryo and from one-half of an embryo to another are, of course, the items of greatest interest. As yet there are not enough results to draw any conclusions, but we have a large supply of embryos and are in the process of analyzing them to get appropriate statistical information on the distribution of iron in them.

(4) Further tests with the electron probe of ferritin containing rat intestine sections did not indicate the ability of this method to see any ferritin. Perhaps none was present in large enough clumps. The resulting pictures are included in the small glassine envelope. I would appreciate your returning them to me.

I think that this just about brings you up to date. By the time Rana pipiens come into season again, we should have the chemistry survey pretty much completed and will then devote full time to growth in the gradient field.

I hope this information is in the form most useful to you.

Best regards,

Sincerely yours,

(signature)

Peter W. Neurath, D.Sc.
Chief Biophysicist

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TABLE 1

DATA FOR WHOLE EGGS

Date	Shumway Stage	Absorbance	µg Fe (from curve
September 8	#3 - 2 cell	.016	0.38
September 8	#3 - 2 cell	.024	0.73
September 8	#20 - hatching	.024	0.73
September 21	#3 - 2 cell	.007	0.00
September 21	#20 - hatching	.002	0.00
September 21	#20 - hatching	.049	1.85

TABLE 2

DATA FOR HALF EGGS

Date	Half*	Absorbance	µg Fe (from curve)	Total µg Fe in Egg
September 12	a	.010	0.1	0.1
September 12	b	.004	0.0	
September 12	a	.005	0.0	0.86
September 12	b	.027	0.86	
September 13	a	.006	0.0	0.46
September 13	b	.018	0.46	
September 13	a	.020	0.55	2.09
September 13	b	.042	1.54	
September 21	a	.016	0.37	0.37
September 21	b	.001	0.0	
September 21	a	.019	0.51	1.77
September 21	b	.036	1.26	

* Stage 3 eggs were cut along the axis of the division between the two cells.