Pressure Induction of Triploids in the Axolotl

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In 1942, Fankhauser and Humphrey reported that cold shock could be used to induce triploidy in the axolotl. Eggs were refrigerated (1-3°C) immediately after fertilization for a period which varied from 9-24 hours. Though 80% of the larvae obtained were triploids, only 20% of the treated embryos survived to hatching.

In 1964, Rott and Betina succeeded in producing triploids by heat shocking fertilized eggs 30 min after oviposition, for a period of 30 min. Of the surviving larvae, 80% (shocked at 33°C) and 74% (shocked at 36°C) were triploid. The authors did not indicate what percentage survived to hatching, but mortality was probably high. In our own experiments we found that 10 min at 36° reduced survival to 8% (Trottier and Armstrong, 1976). Our results also indicate that the optimum time after oviposition for the heat shock is 60 min. When we shocked eggs for 7.5 min, we obtained 37% triploids and the survival rate was 45%.

The cytological effects of heat and cold shock are quite different. According to Sladecek and Lanzova (1959), cold shock causes a temporary collapse of the spindle of the 2nd maturation division, which results in suppression of 2nd polar body formation, and consequently a restitution female pronucleus is formed. Heat shock, on the other hand, causes submersion of the 2nd maturation spindle under the egg surface resulting in the formation of two female pronuclei which later fuse.

Another method of producing triploid animals involves the use of high pressure, which appears to cause dissolution of the 2nd maturation spindle, thus effectively inhibiting anaphase movements. Dasgupta (1962) pressure treated Rana pipiens eggs 5 min after insemination at 5000 psi for 6 min. Of the treated eggs, 60% cleaved, and 78% of those developed normally. Of the normal embryos, 85% were found to be triploid by chromosome counts.

Recently, we have used pressure treatment for induction of triploidy in the axolotl. Our initial experiments were carried out with an Aminco French pressure cell (American Instrument Co., Silver Spring MD; Cat. no. 4-3398), and a Wabash hydraulic press. However, we had no way of directly determining the pressure within the cell. Though we intended to use the same pressure and duration of treatment as Dasgupta, we later found that we had been using 6000 psi, and all our subsequent experiments were done at 6000 psi for 6 min.

We next tried attaching a hydraulic pressure gauge to a modified base-plate. While this arrangement gave satisfactory results, pressure experiments were usually a two-man operation as one person was required to continually man the hydraulic press during a run. To avoid this, we constructed the pressure cell illustrated in the accompanying diagram. A full parts list is given at the end of this article.

The base plate K fits into the bottom of the French pressure cell, which is placed in the hydraulic press. A simple cylinder with a tight fitting plunger could be substituted. The cell, G, is placed on a wooden stand adjacent to the press. All the components are connected with $\frac{1}{4}$" OD stainless steel high pressure tubing, which seems to allow enough flexibility for the slight up and down movement of the press.

In operation, the system is filled with water or saline. Eggs are placed in a metal cup, which is lowered into the chamber. The top is screwed on with the valve open so that air and excess water are expelled through A. The top valve is then closed. With the other valve open, the system is pressurized, then that valve, too, is closed. At the end of a run, the pressure is released by opening either valve. If all of the connections have been made properly, the cell should maintain 20,000 psi for several hours. Most of the fittings and the valves are rated for 30,000 psi or greater, but we have had problems with the connection to the hydraulic gauge. The equipment is really over-designed for the relatively low pressures and durations required to produce triploids, but it is being used for other pressure experiments as well.

When eggs were pressure treated at various times after oviposition, the results shown in Table 1 were obtained. The eggs were actually collected at 20 min intervals, and the time to pressurization was measured from the time of collection. Ploidy was determined by counting nucleoli in tail-tip squashes at hatching.

Table 1

Pressure induction of triploids at various times after oviposition

	Time of pressure treatment (min)						
	40	50	60	70	80	90	control
# eggs treated	109	141	136	129	113	106	1319
# fertile	104	133	135	129	106	88	1251
# hatched	27	28	73	71	70	38	1042
% survival	26.0	21.1	54.1	55.0	66.0	44.2	83.3
# triploid	3	20	53	42	39	14	-
% triploid	11.1	71.4	72.6	59.2	55.7	36.8	-

From this data, it may be seen that the optimum time of treatment is 60 min after collection, or an average of 70 min after oviposition, which corresponds to the time of completion of the 2nd maturation division (Fankhauser and Griffiths, 1939; Sladecek and Lanzova, 1959). The timing is also the same as that obtained for heat shock in our laboratory. However, the overall percentage of triploids produced by pressure treatment is more than double that by heat shock: 39.3% (72.6% of the 54.1% surviving) as opposed to 16.7% (37% of 45%), and is comparable to Dasgupta's result of 39.8% triploids (85% of 46.8%).

References:

Dasgupta, S. 1962 J. Exp. Zool. 151: 105-116.

Fankhauser, G., and R.B. Griffiths 1939 Proc. Natl. Acad. Sci. 25: 233-238.

Fankhauser, G., and R.R. Humphrey 1942 Biol. Bull. 83: 367-374.

Rott, N.N., and M.I. Betina 1964 Tsitologiia 6: 95-98. (in Russian)

Sladecek, F., and J. Lanzova 1959 Folia Biol. 5: 379-393.

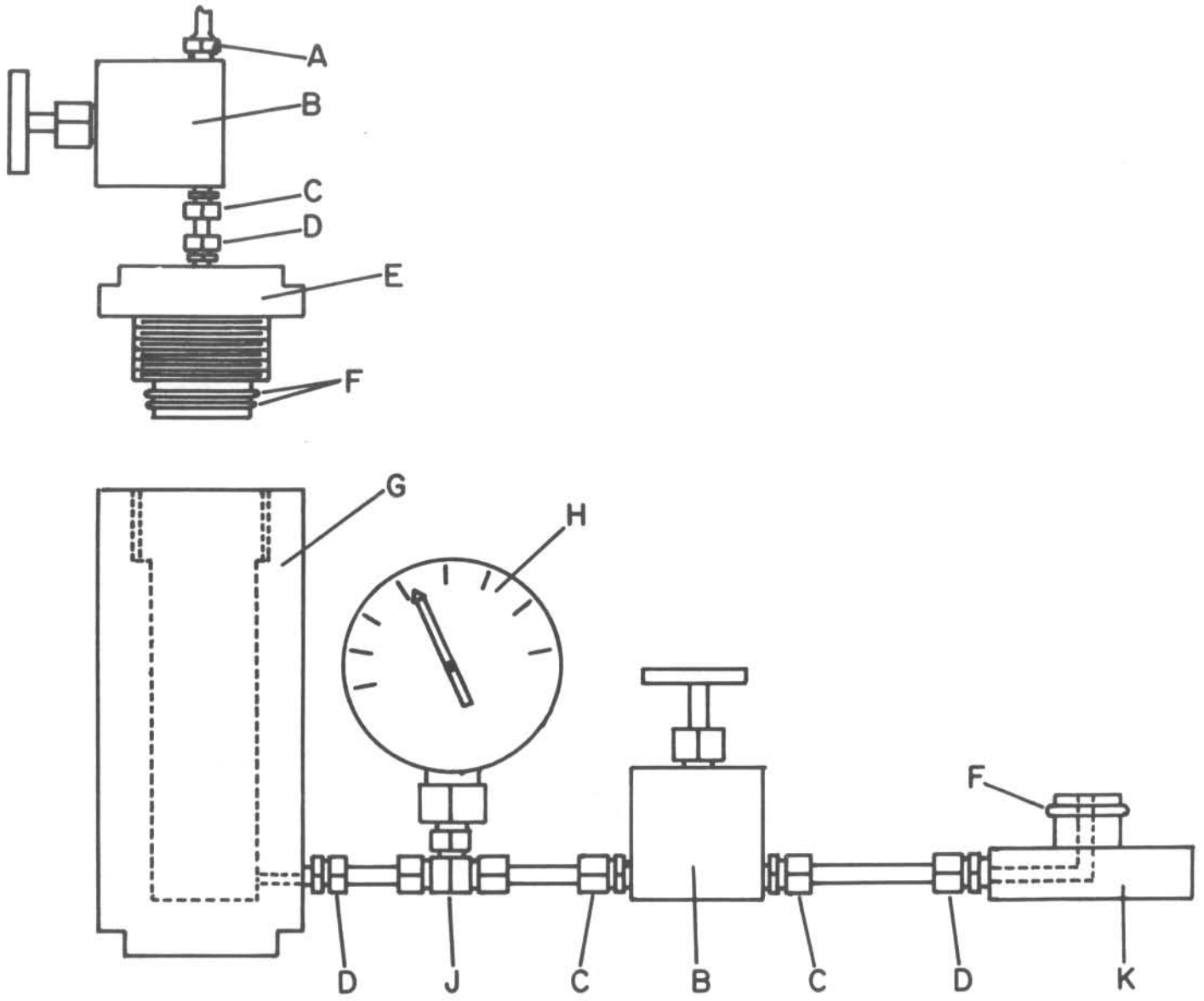
Trottier, T.M., and J.B. Armstrong 1976 Genetics 83: 783-792.

Parts list:

A. Coned tube stub adapter, Sno-Trik* SS-44M-A-441
B. High pressure valve, Sno-Trik SS-445-FP
C. High pressure connector, Sno-Trik SS-440-1-44M
D. Connector, Sno-Trik SS-440-1-4
E. Cap
F. Rubber O-rings
G. Pressure cell
H. 20,000 psi hydraulic pressure gauge (Marsh Instruments)
J. Union tee, Sno-Trik SS-440-3
K. Base plate for French pressure cell

E, G and K were all machined by our Physics Dept. shop from 3 in diameter stainless steel. The dimensions are probably not too critical as long as the walls are reasonably heavy, and a good seal is made by the O-rings, which should be greased lightly.

* Sno-Trik Co., 32550 Old S. Miles Rd., Solon OH 44139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