(Fundamentals of) High-pressure Instruments for Innovation and Discovery

Edmund Ting, Sc.D.
Sr. VP Engineering
Pressure BioSciences

The Nobel Prize in Physics 1946

- Percy Bridgman, Harvard
- Invented methods for high pressures experiments
- Reported on albumin, hemoglobin and other biological materials affected by pressure (1914, 1929)

PBI

Bridgman High Pressure

- Used mercury “piston” in small glass tubes to transmit pressure to sample
- Kerosene was used as the hydrostatic pressure media

•Pre-modern metallurgy

•Pre-fracture mechanics

•Pre-computational stress analysis

•Pre-CAD

PBI

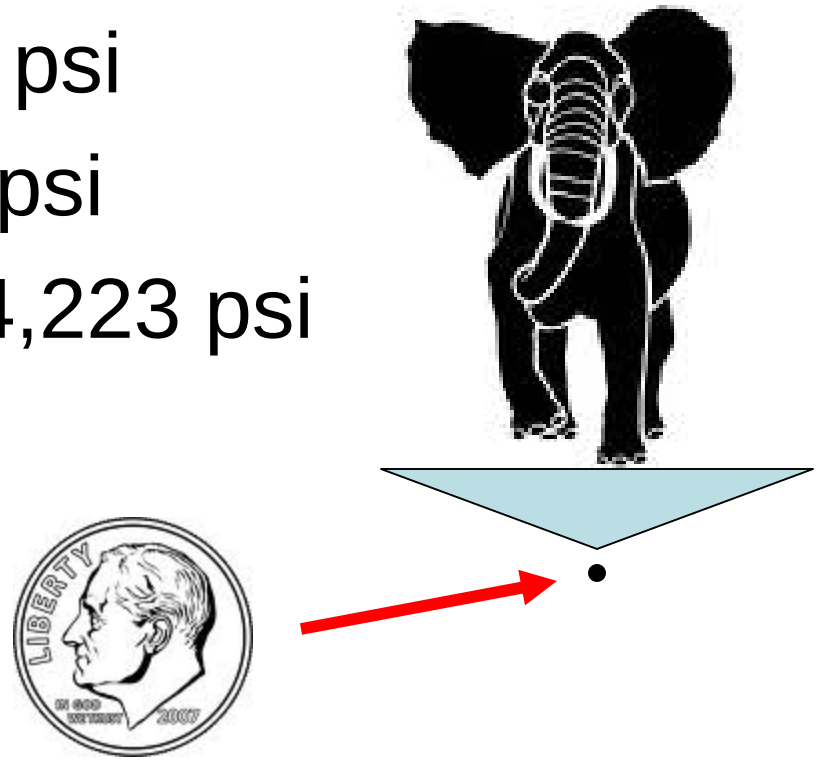
Agenda

- Creating high pressure
- Containing high pressure
- Pictures of small to large equipment
- Fluid compression under high pressure
- Innovation and discovery
- Summary

PBI

Pressure Units

- 1,000 atm = 14,695 psi
- 1,000 bar = 14,503 psi
- 100 MPa = 14,503 psi
- 1,000 kg/cm^2 = 14,223 psi



Pressure = force/area

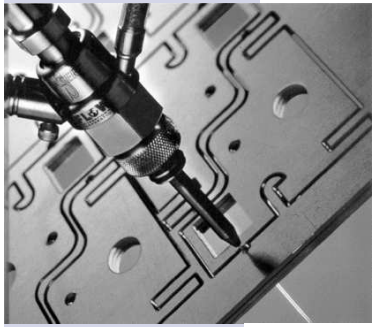
Commercial Applications HP Engineering

WJ Cleaning

25C/ 300 MPa

Gun Barrels

1000C/ 700MPa



WJ Cutting

25C/ 400 MPa

Material Densification

1000C/ 200 MPa

HP Food

4C/ 600 MPa

Crystal growth

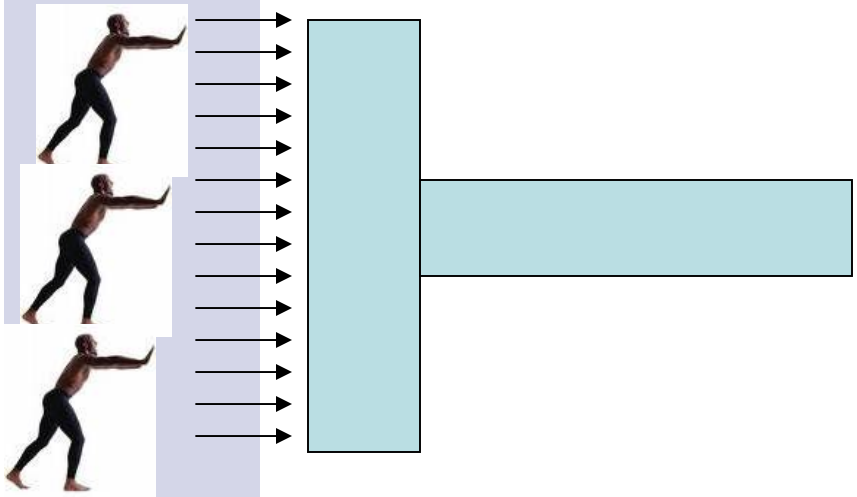
400C/ 200 MPa

PBI

High Pressure Generation

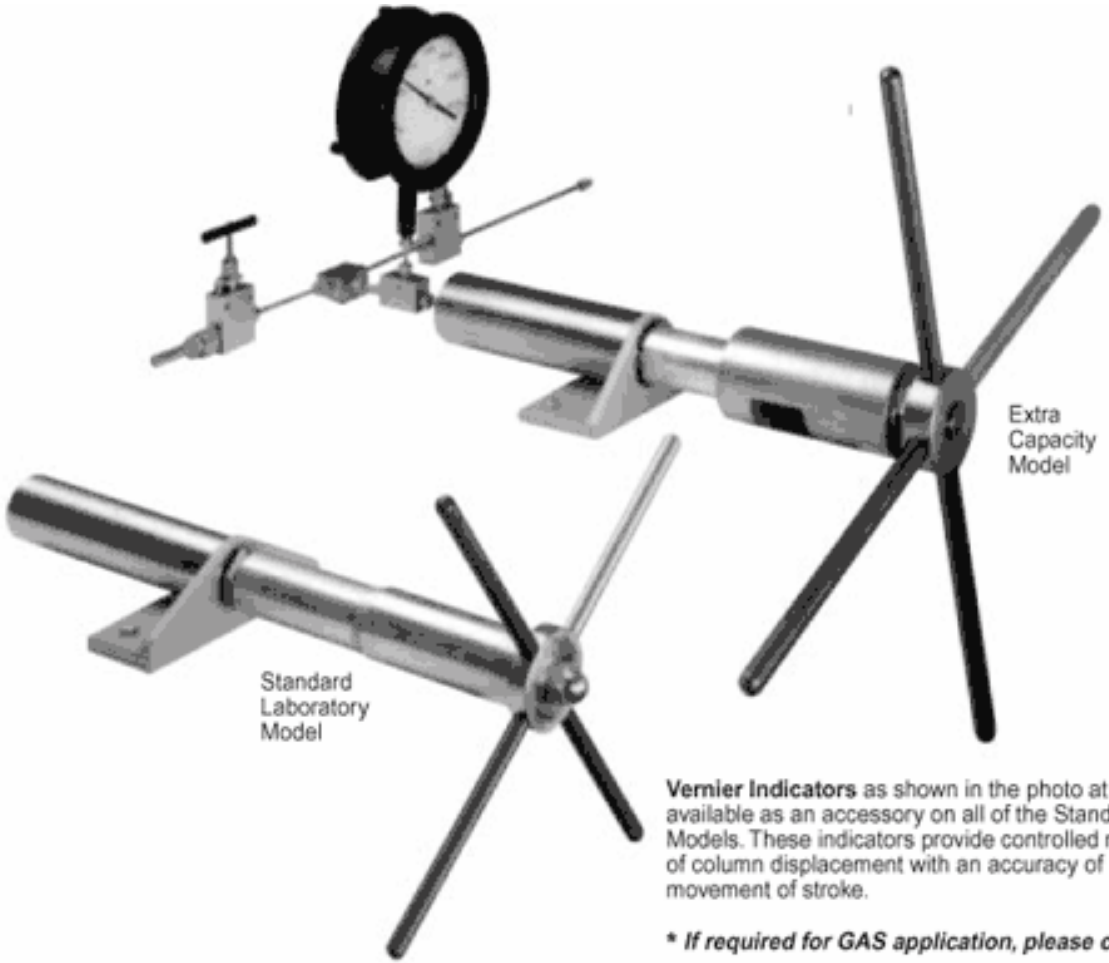
- Pressure amplification
- Force balance

440 to 1



PBI

Human / Lever & Screw



Vernier Indicators as shown in the photo at left are available as an accessory on all of the Standard Laboratory Models. These indicators provide controlled measurement of column displacement with an accuracy of ± 0.003" movement of stroke.

**** If required for GAS application, please consult factory.***

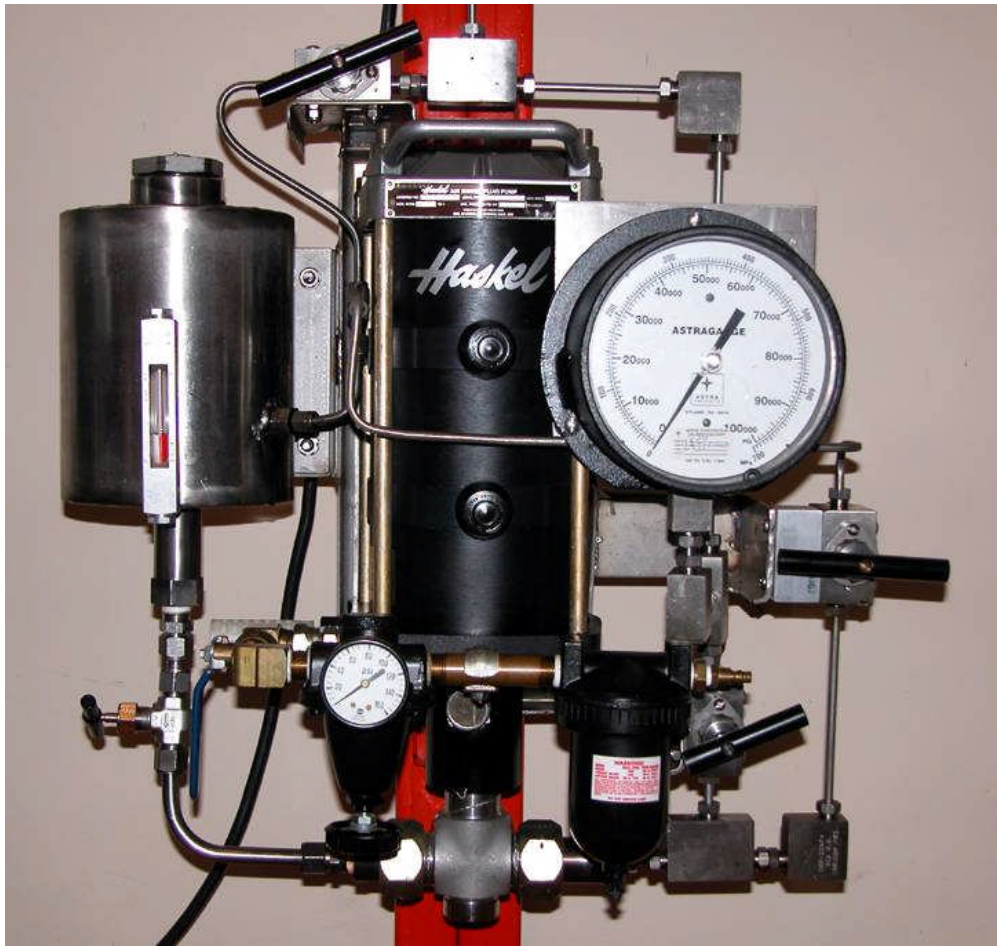
Hand-Hydraulic Pump

- Hand hydraulic pump can produce MPa pressure in small volume

Apparatus used to test pressure effects on proteins

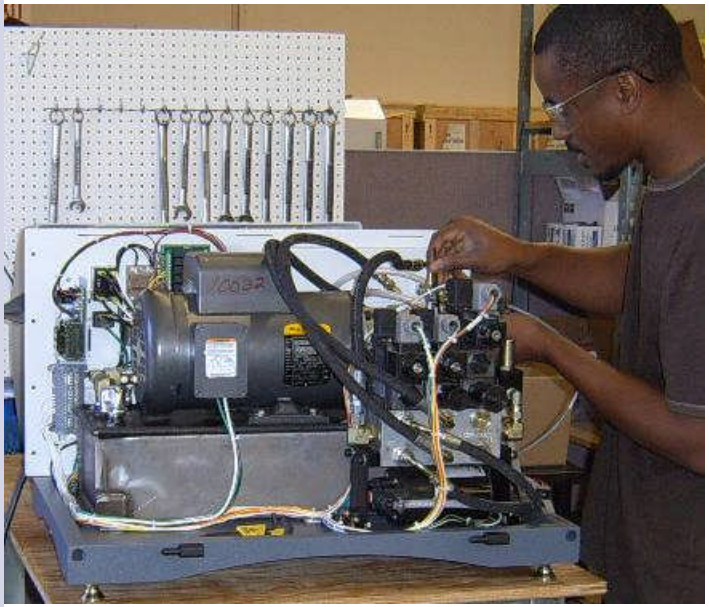
Air Power

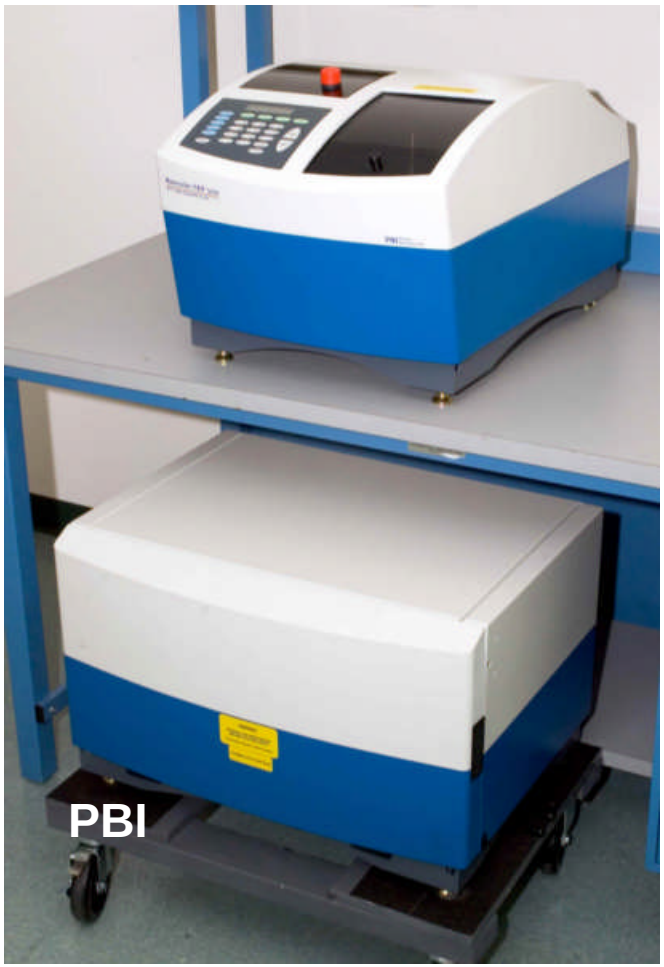
- Pneumatic driven intensifier



Electro Hydraulics

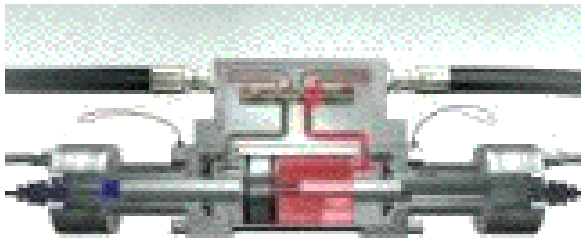
- Electric motor driven hydraulic pump to drive intensifier.



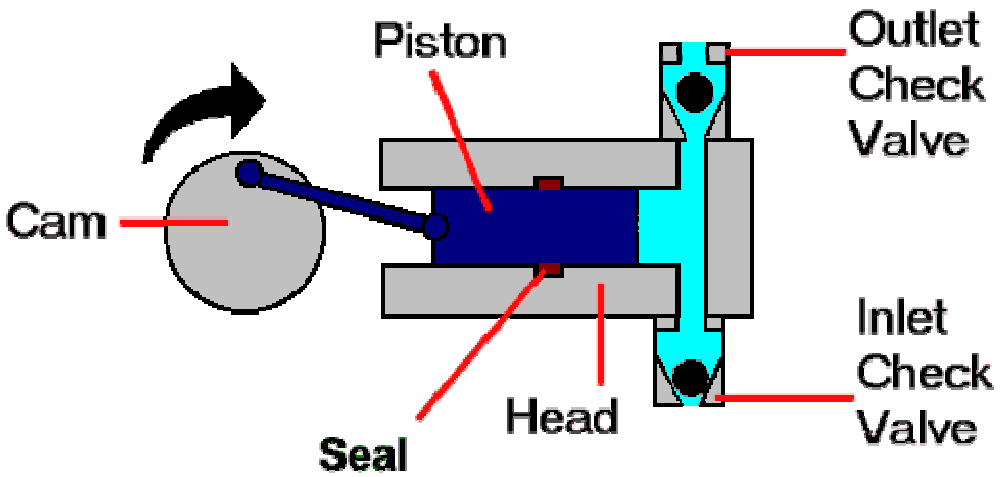


Typical Hydraulic Intensifier

Large or small



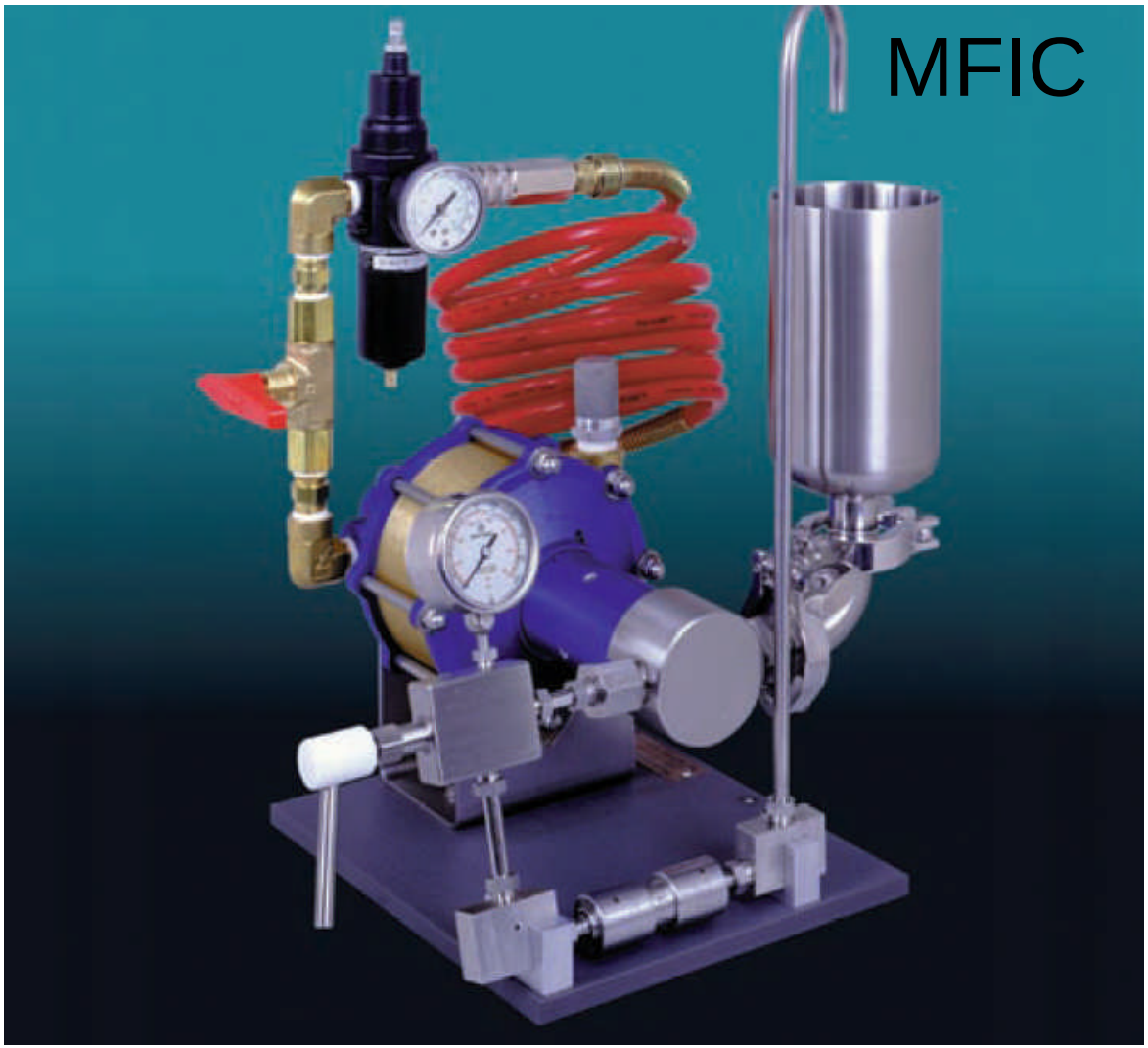
HPLC Crank Pump



PBI

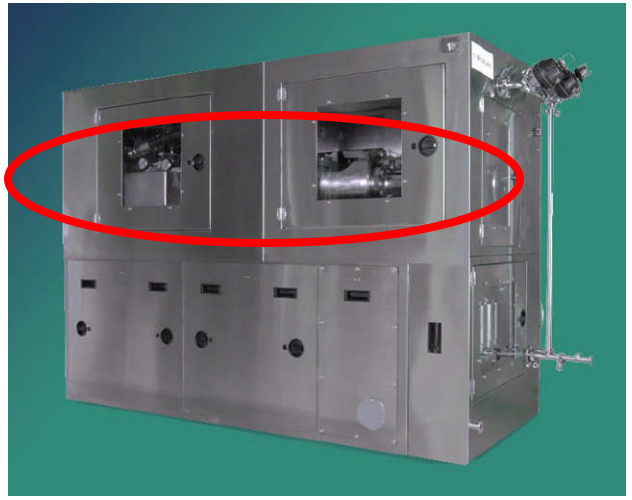
Direct Drive HP Pump

Small Bench Top Air Driven Homogenizer



High Power HP Homogenizer

GEA 22ksi homogenizer



MFIC 100HP 40ksi homogenizer

Pump Power

- 1 gpm at 30,000 psi represents 17.5 HP

$$P = \frac{Q \times \Delta P}{1715 \times Eff_s}.$$

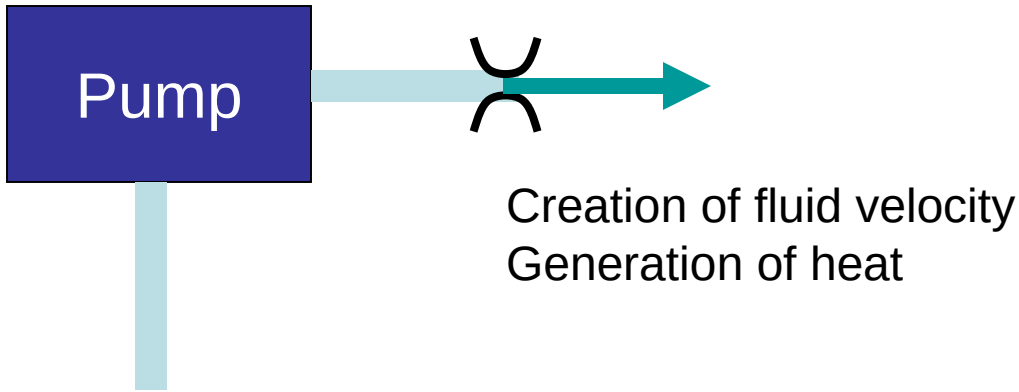
Where, P = Pump Shaft Power (HP)
Q = Flow rate (gpm)
ΔP = Differential pressure (psi)
Eff_s = Pump efficiency

17.5HP=13KW=52 cal/minute

PBI

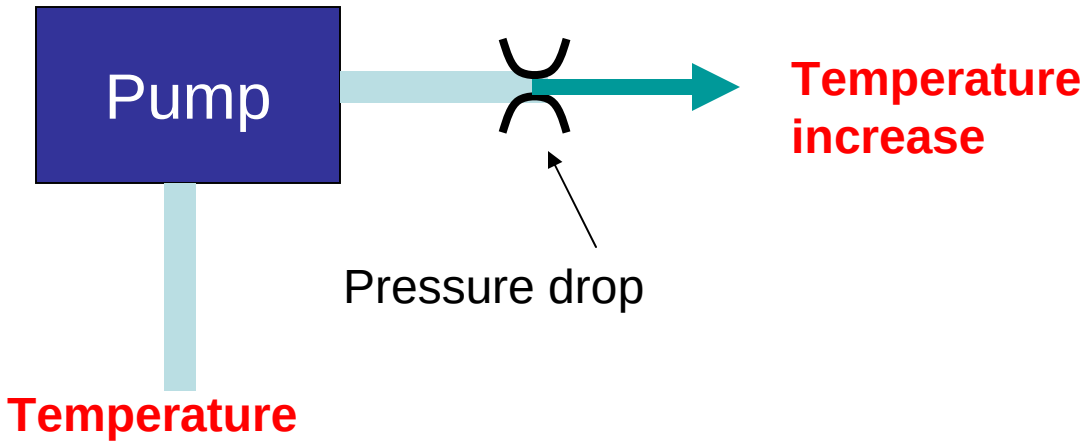
Discharge under Pressure

- A large pressure drop will convert potential energy into kinetic energy, resulting in high fluid shear and heat.

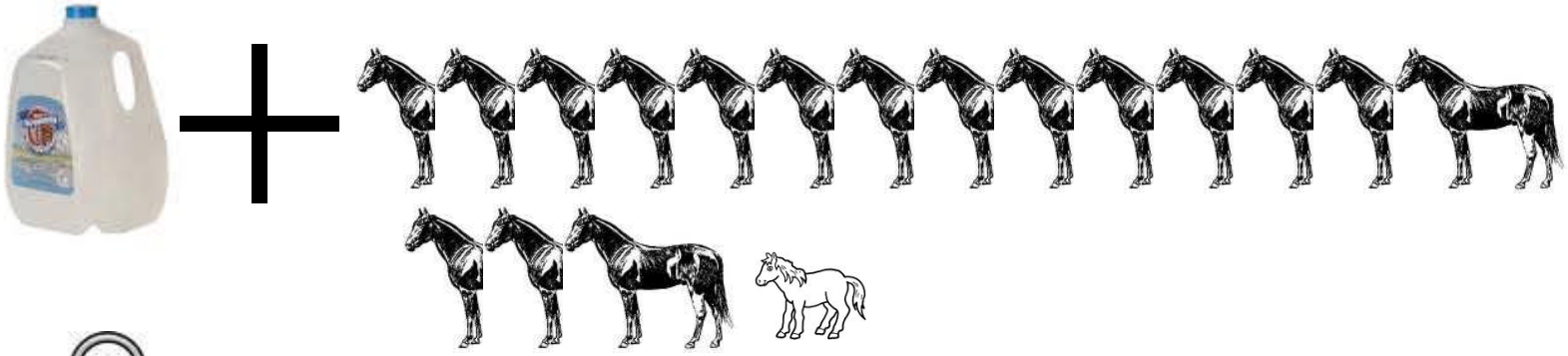


Discharge under Pressure

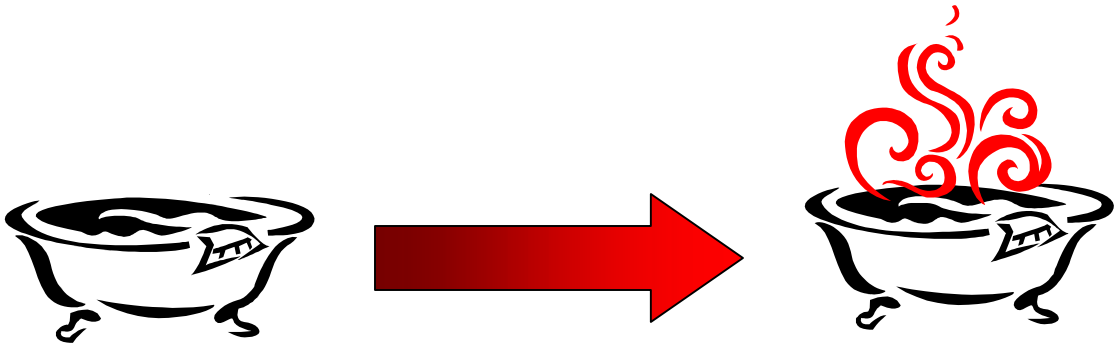
- 1 gal (3785 gm)
- 17.5 HP (13,050 watts)
- In one minute at 35,000 psi
- Energy = 0.2175 kwh = 187,016 cal
- Energy/Mass= 187,016 cal/ 3,785gm
  = 49 degree (K)



Discharge under Pressure



60 seconds



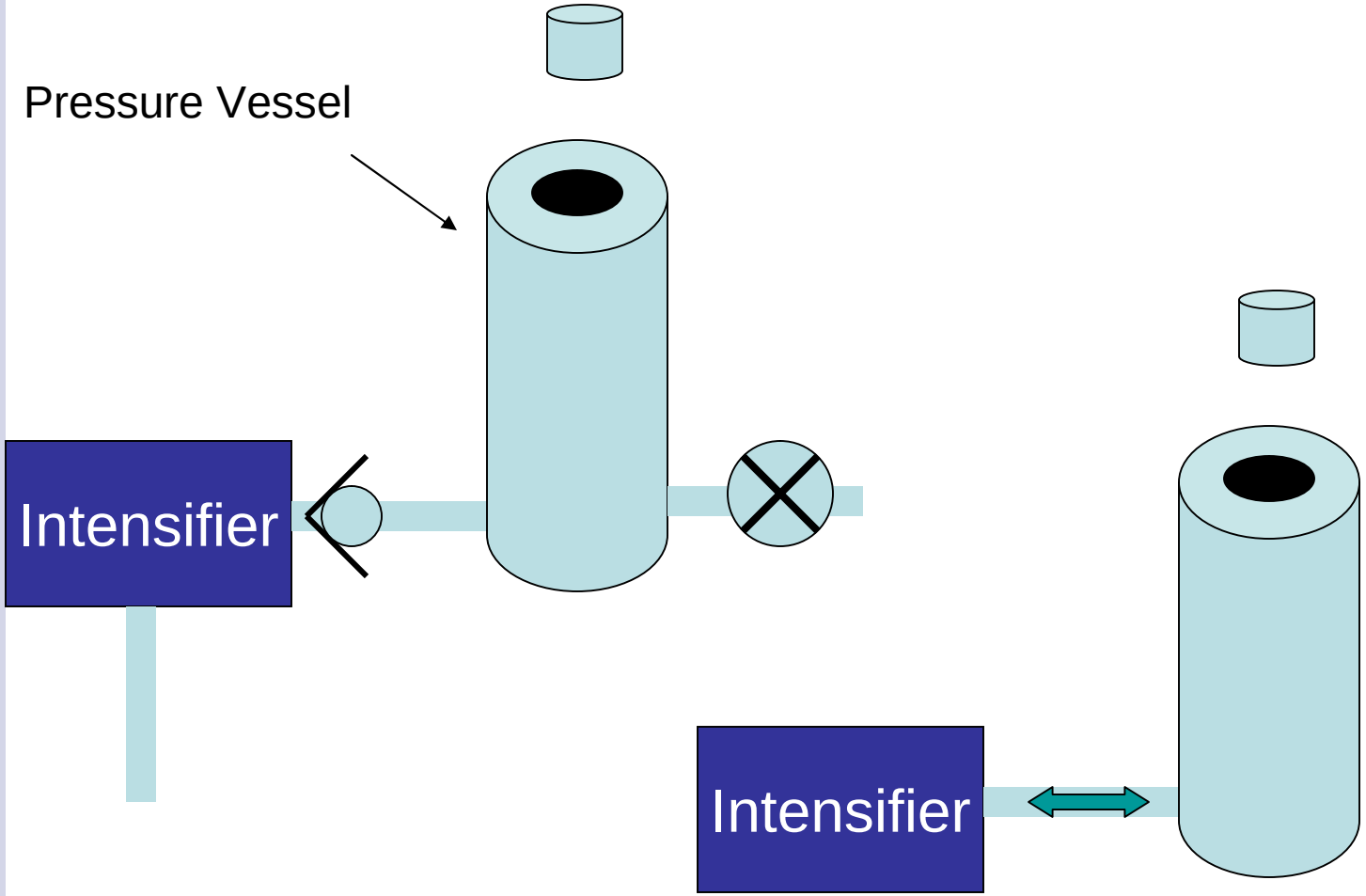
25C + 49C = 74C

Pressure Chambers

- Hydrostatic pressure is confined in a pressure vessel or chamber.

Pressure is transmitted at the speed of sound 1483m/sec

Hydrostatic Pressure Containment



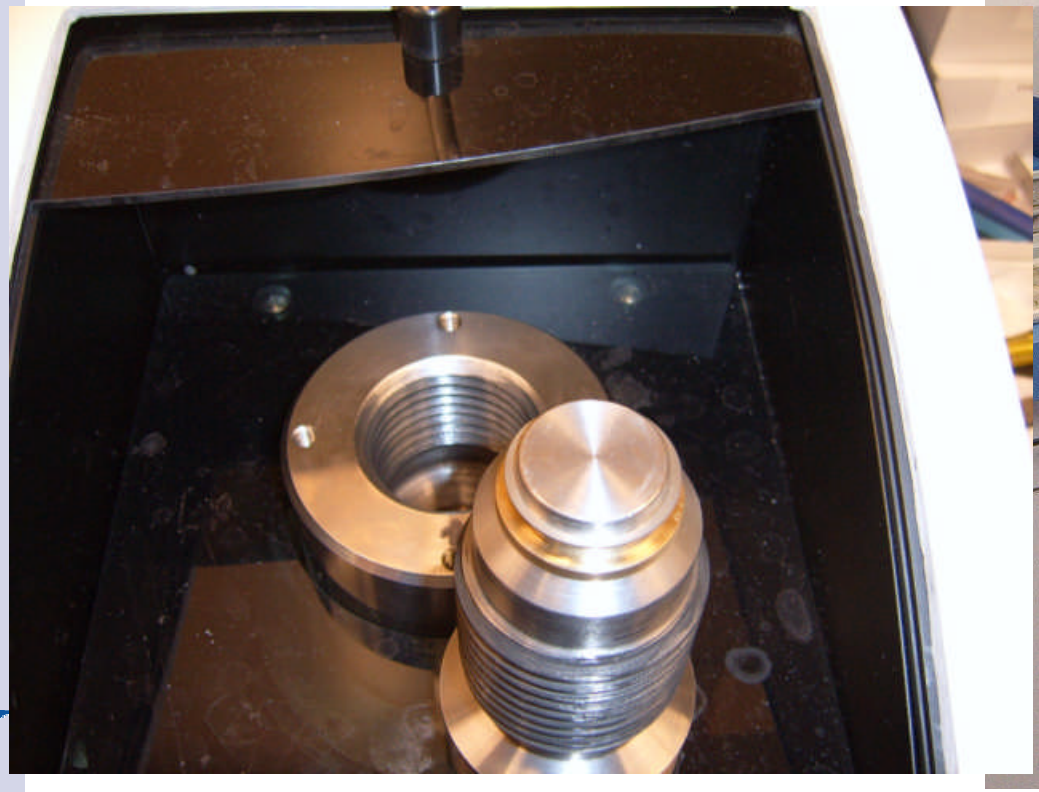
< mL Pressure “Chambers”

- Small diameter HP tubing

Milliliter

Centiliter

Pressure BioSciences
NEP3229



Water Pressure Vessel Energy

$E=P^2V/(2B)$ (PdV mechanical work)

Pressure (P)	Volume (V)
35000 psi	3 in^3 (NEP3229)

B=496,000
(@25C)

Calculated Energy= **3,705 in-lb**

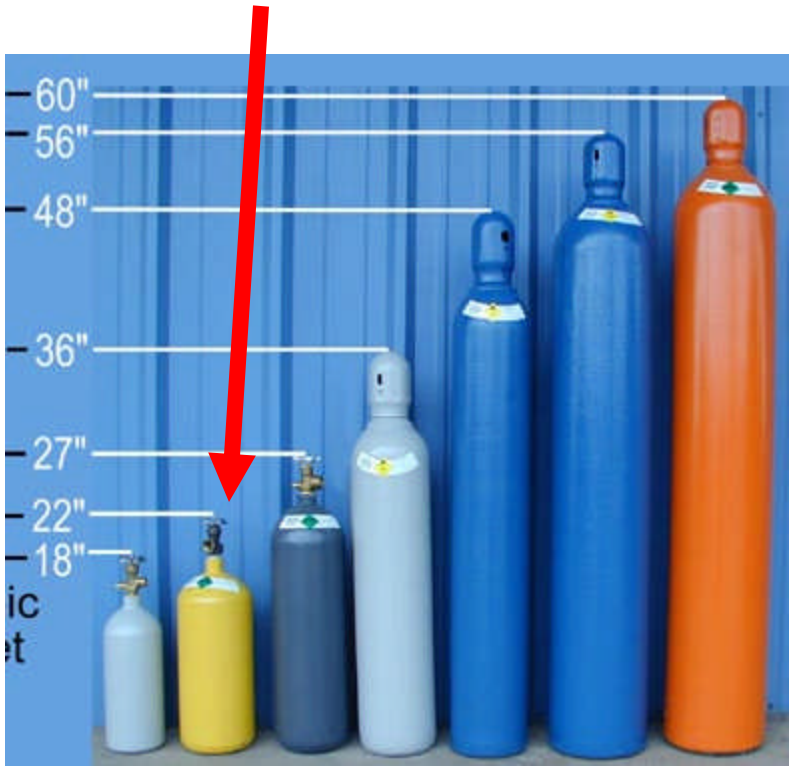
309 ft-lb

420 Joule

1 AA battery = 1000 Joule

Compressed Gas Energy

- 5 liter of air at 200bar (3,000 psi)=
  530,000 joules



Liter

EPSI 2L

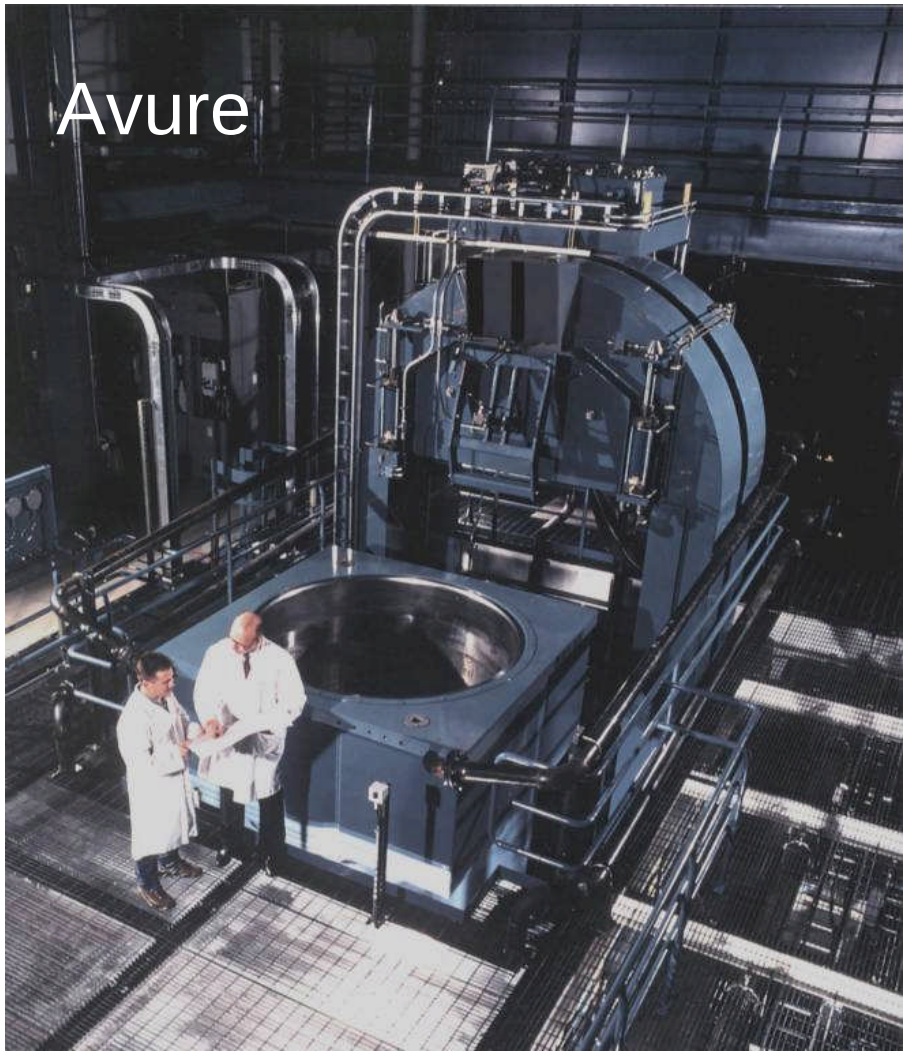
DIY Design

Deciliter

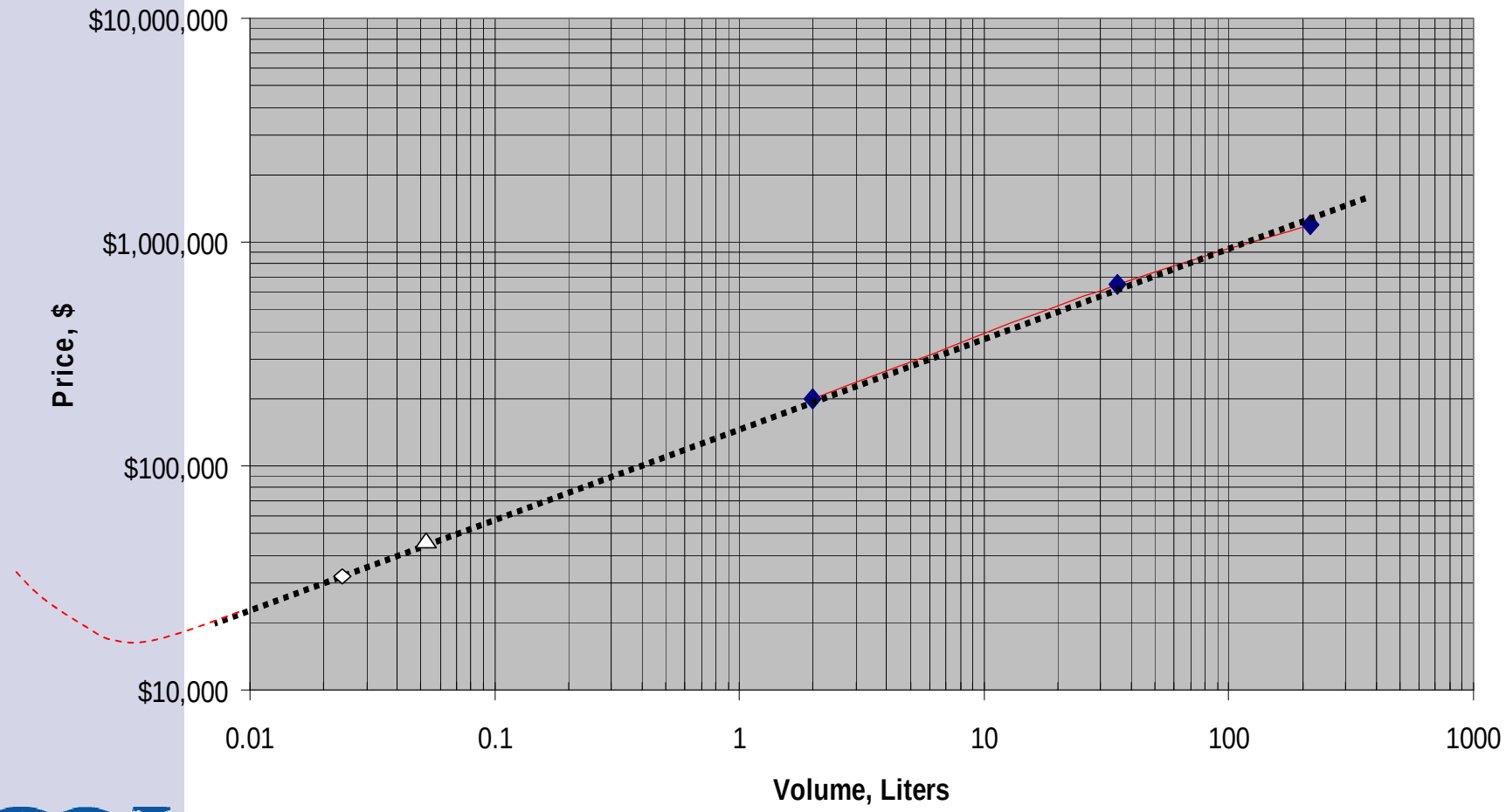
PBI

Hectoliter

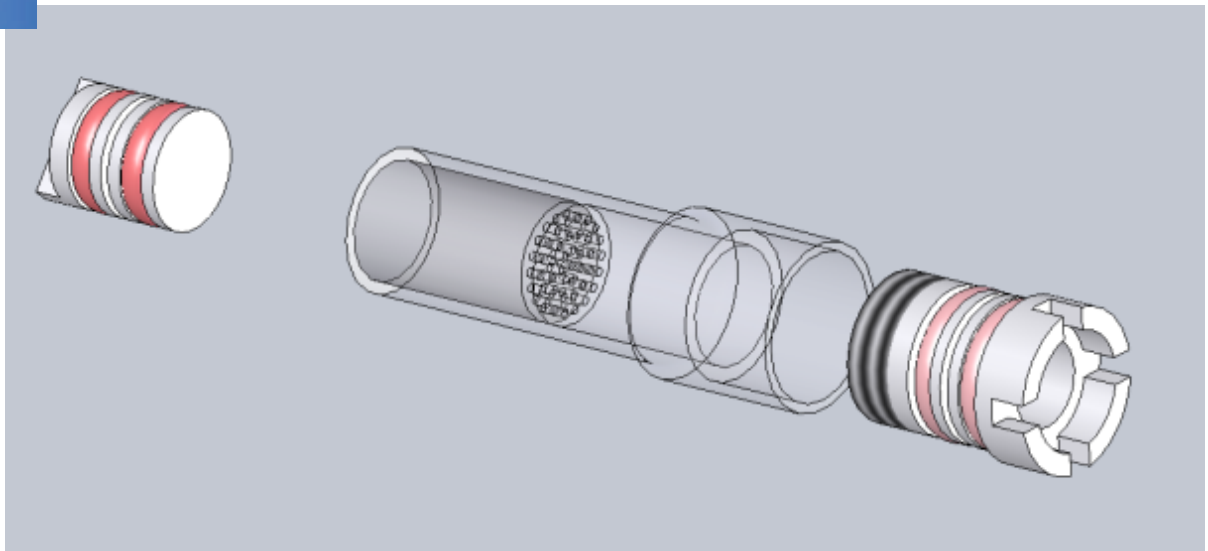
Kiloliter



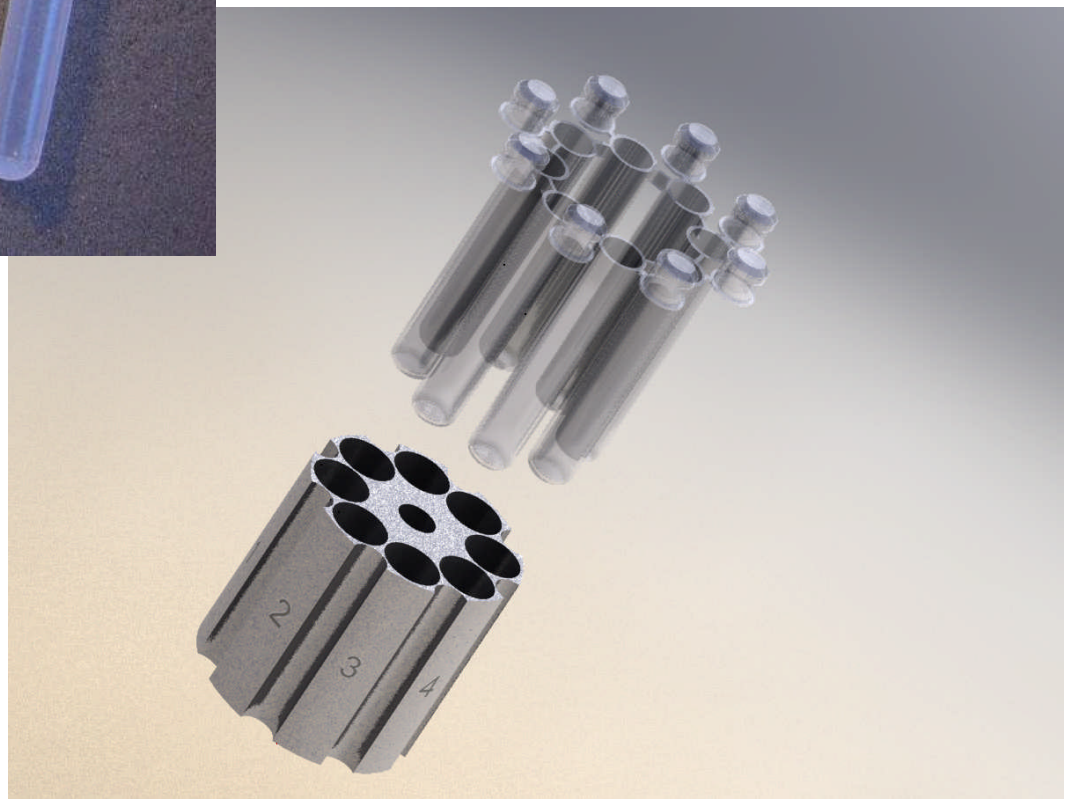
Typical Price of HP Equipment above 30ksi



PULSE tubes



Sample Containers



<50ul

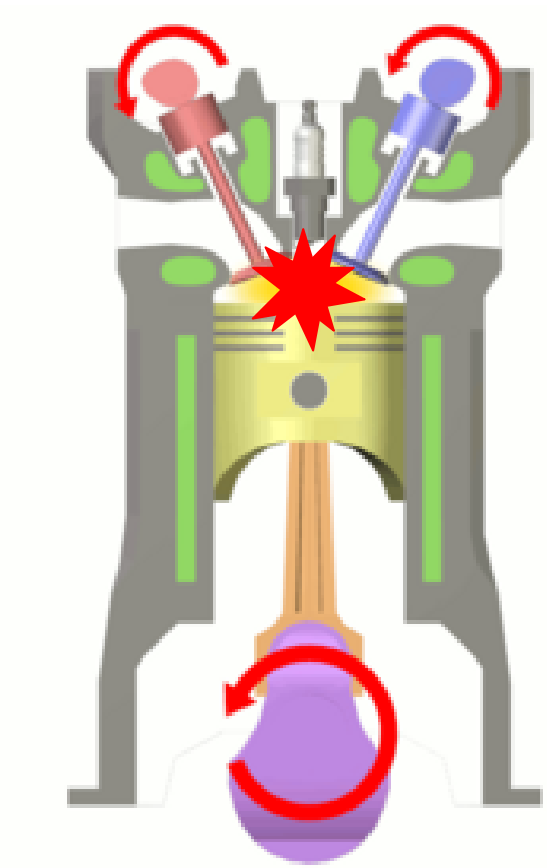
Adiabatic Compression

- Temperature and pressure effects are difficult to separate during rapid pressure increase due to compression heating.
- Adiabatic temperature change can range from 3C/100MPa (water) to over 10C/100MPa (oils) depending on fluid properties.

PBI

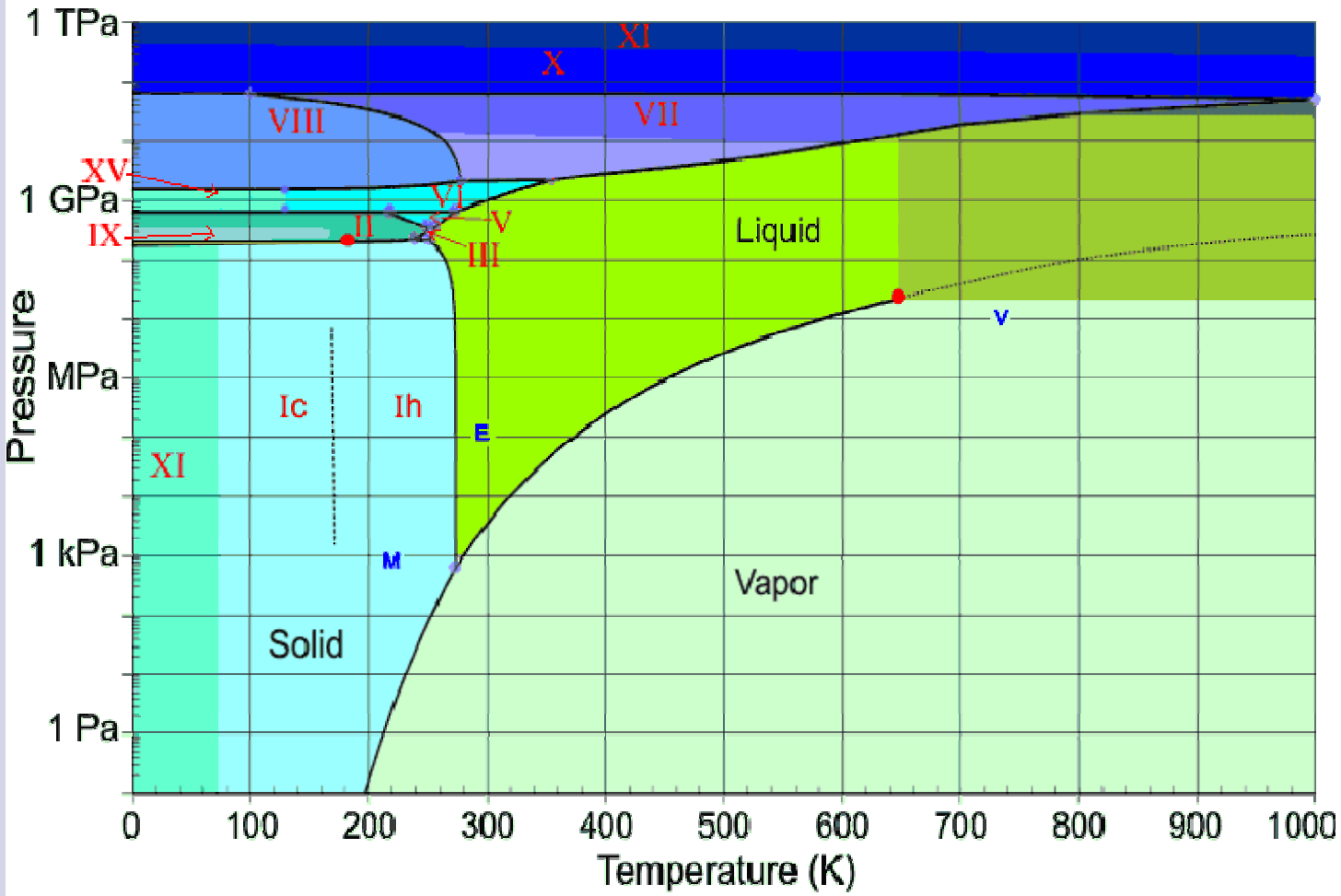
Compression of Air

- PV=nRT



Air is highly compressible so temperature rise is large!

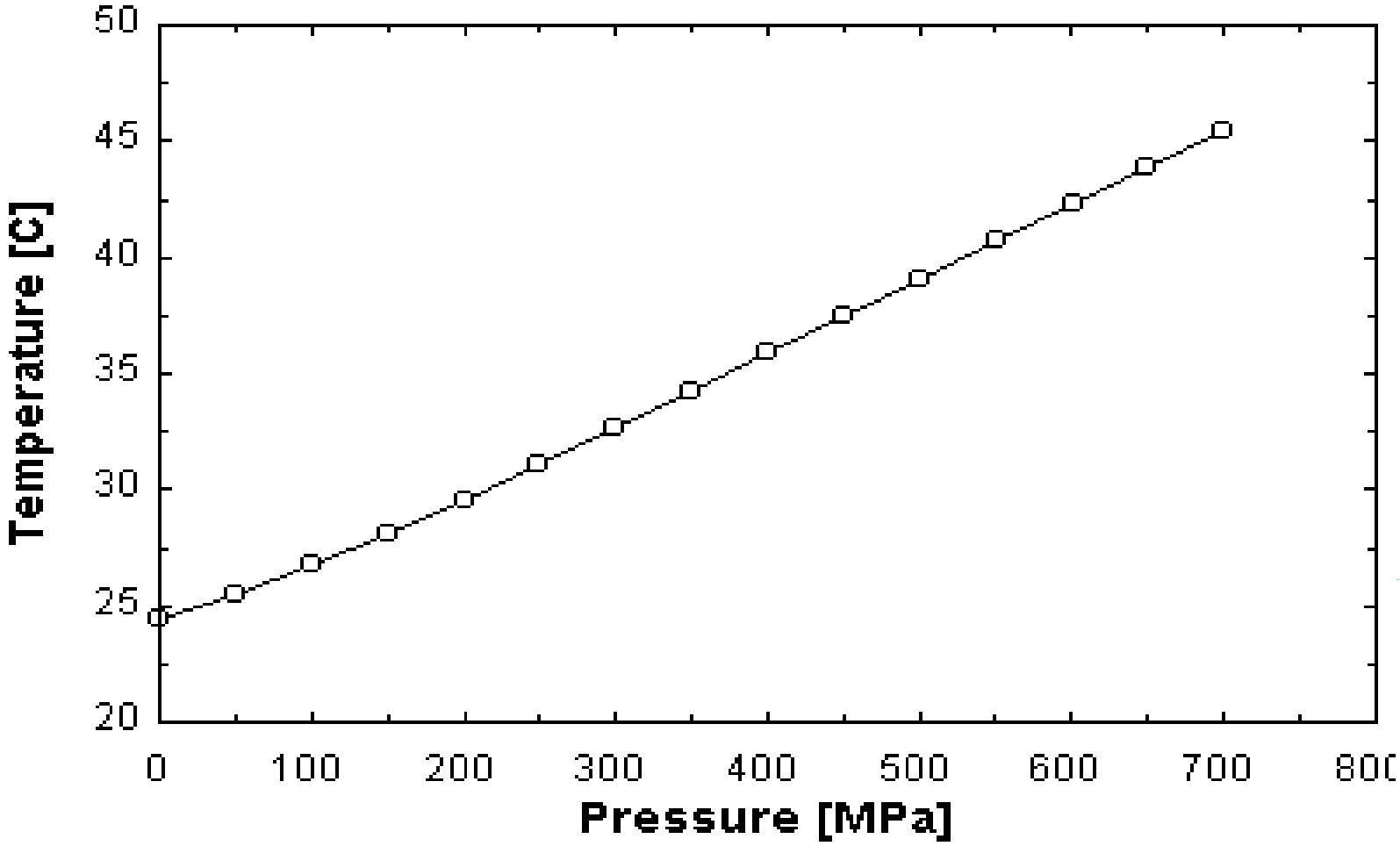
Pressure and Phase H_2O



PBI

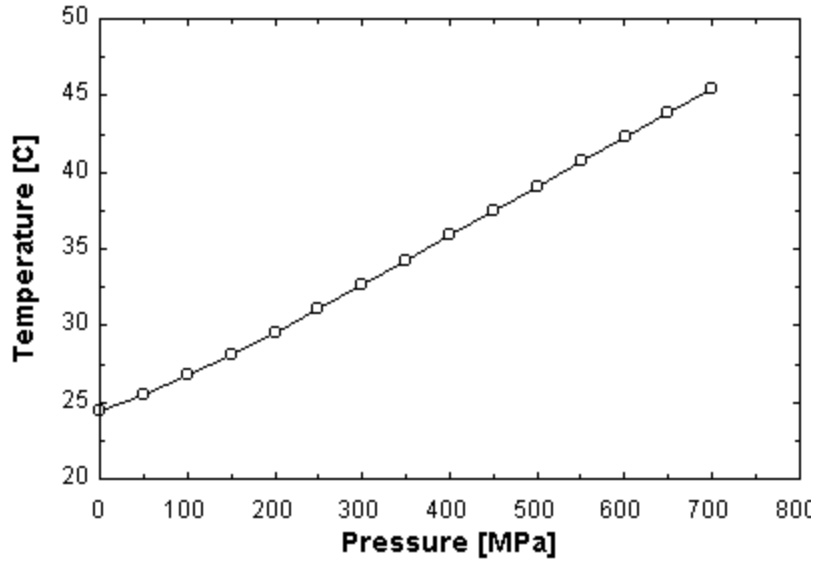
Covalent >>>>>>> ionic, Hydrogen, van der Waals, hydrophobic bonds

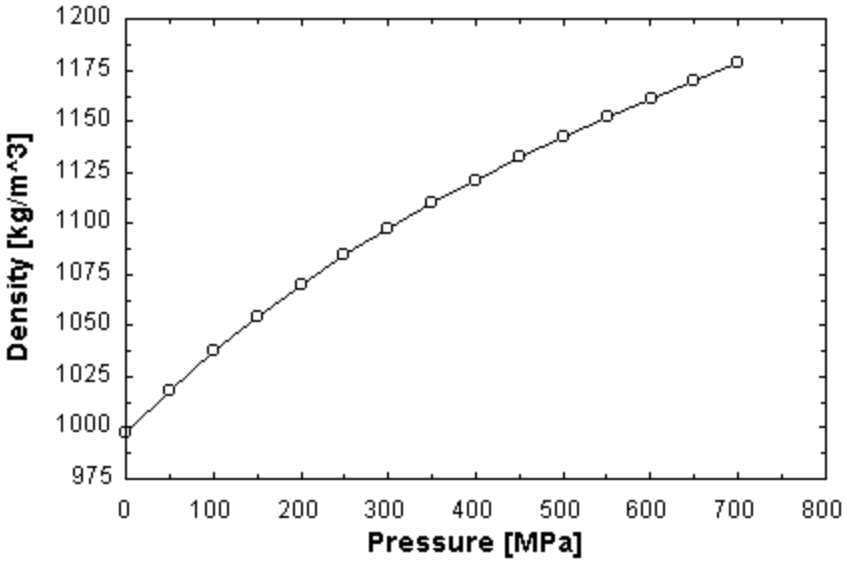
Water Compressibility

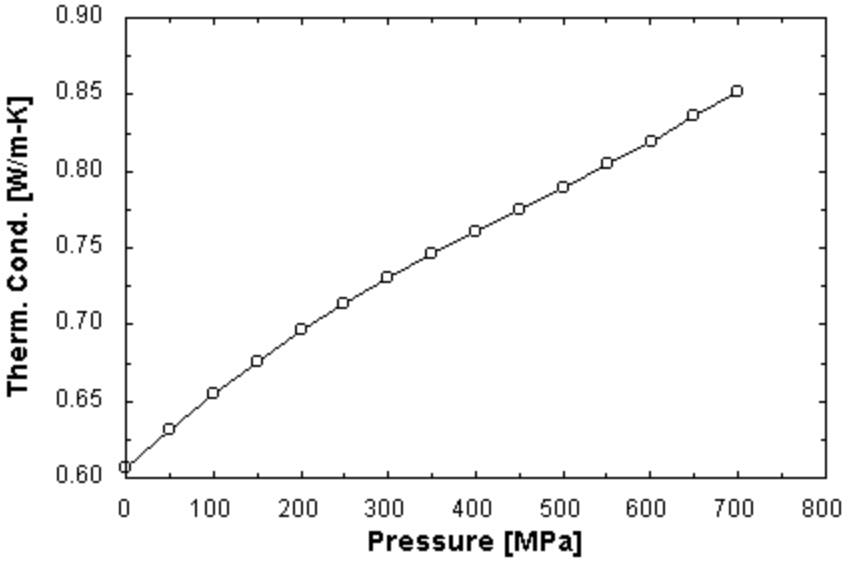


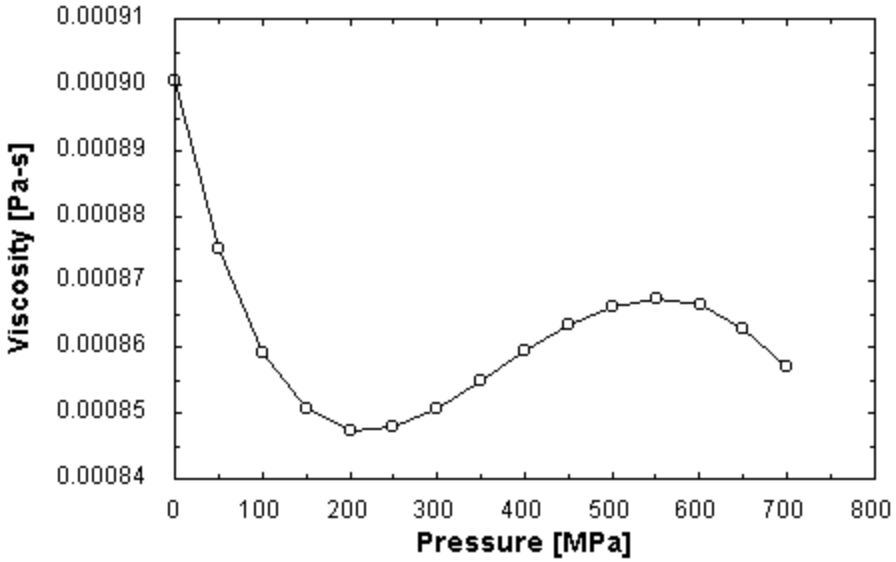
Water is slightly compressible so temperature rise is small!

Thermodynamic Properties of Water vs Pressure under **Adiabatic** Compression from 25°C

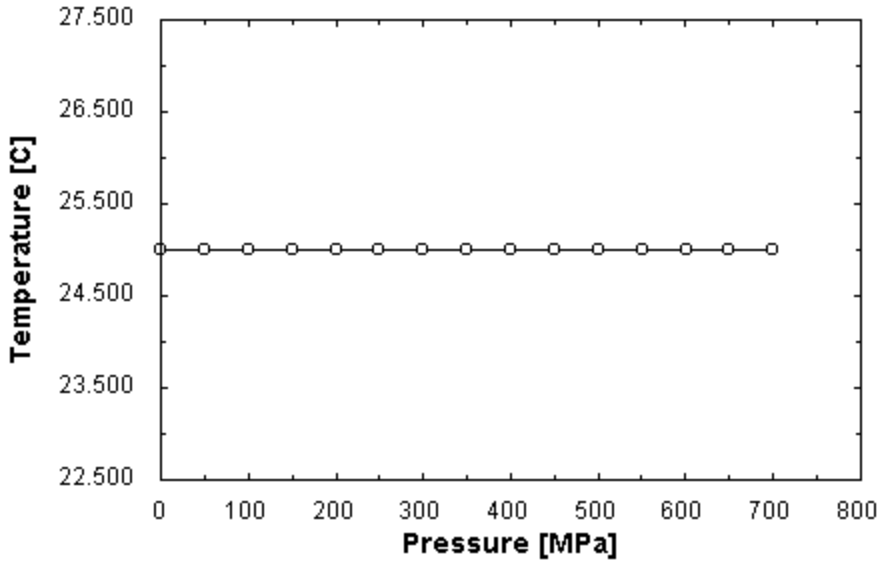


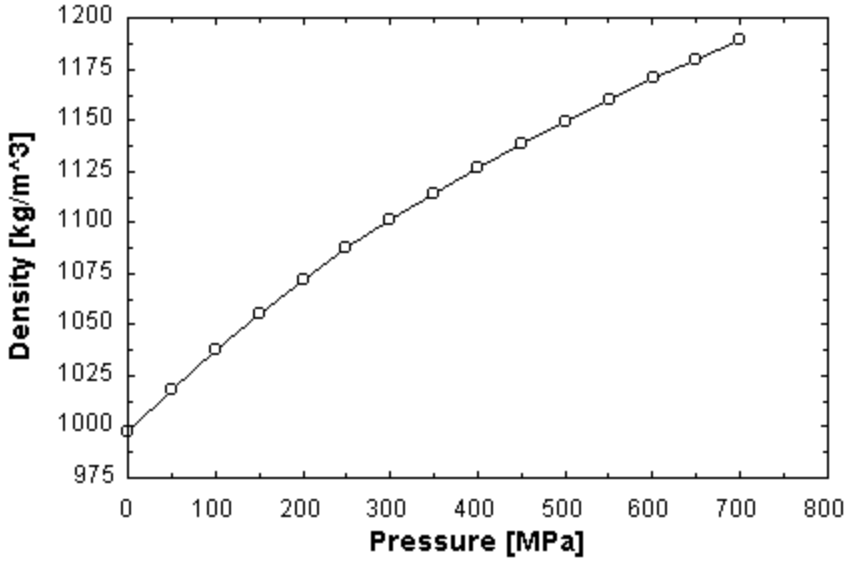


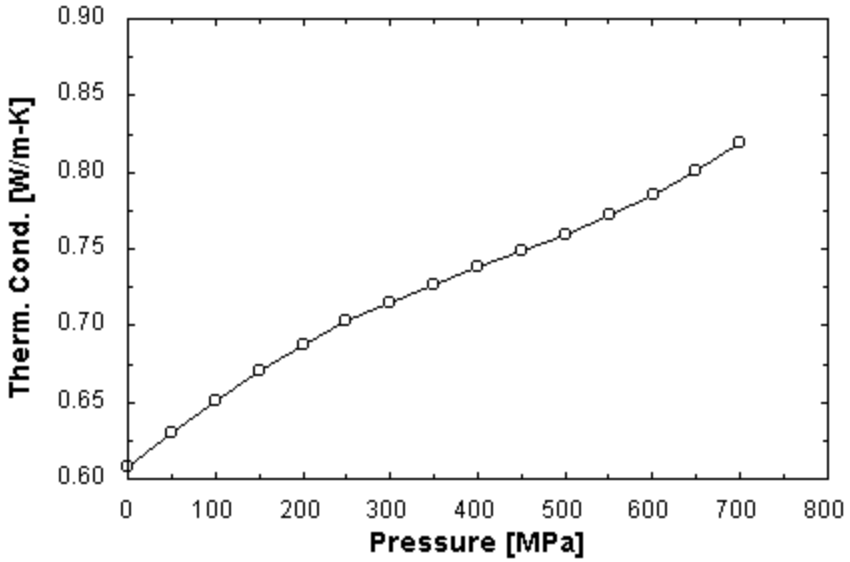


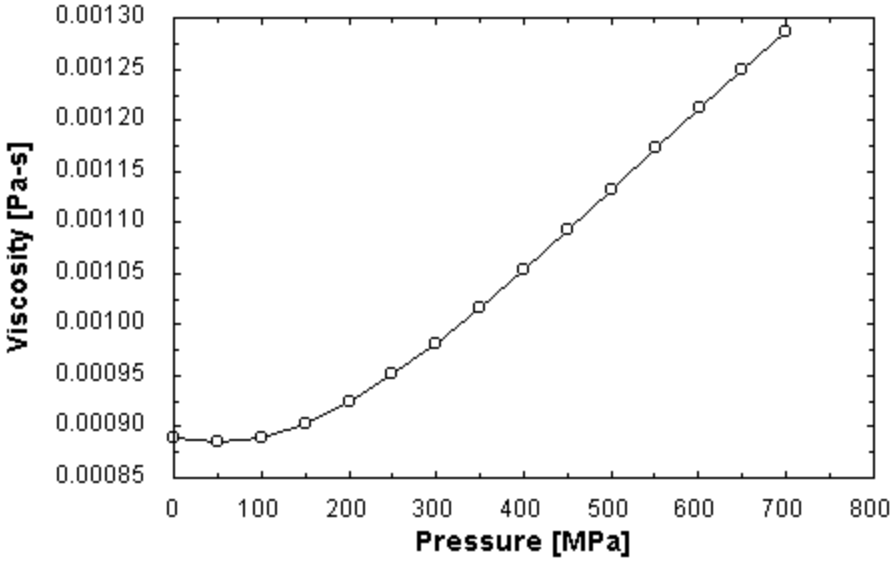


Thermodynamic Properties of Water vs Pressure under **Isothermal** Compression at 25°C









Temperature Change

540MPa (78,000 psi)

Table 4—Comparison between experimental against predicted compression heating values of selected mixtures

Substance	Initial Temperature (°C)	Final Temperature (°C)
100% Glycol	24.2	52.3
75% Glycol	26.3	54.5
50% Glycol	24.5	44.5
25% Glycol	24.4	41.5
0% Glycol +100% water	24.3	40.6
100% Ethanol	24.0	68.5
75% Ethanol	25.0	55.0
50% Ethanol	26.0	50.0
25% Ethanol	21.0	35.0
0% Ethanol + 100% water	24.3	40.6

PBI

Data from: Rasanayagam, Balasubramanism, Ting, Sizer, Bush, and Anderson, JFS, Vol 66, 2003

Temperature Change

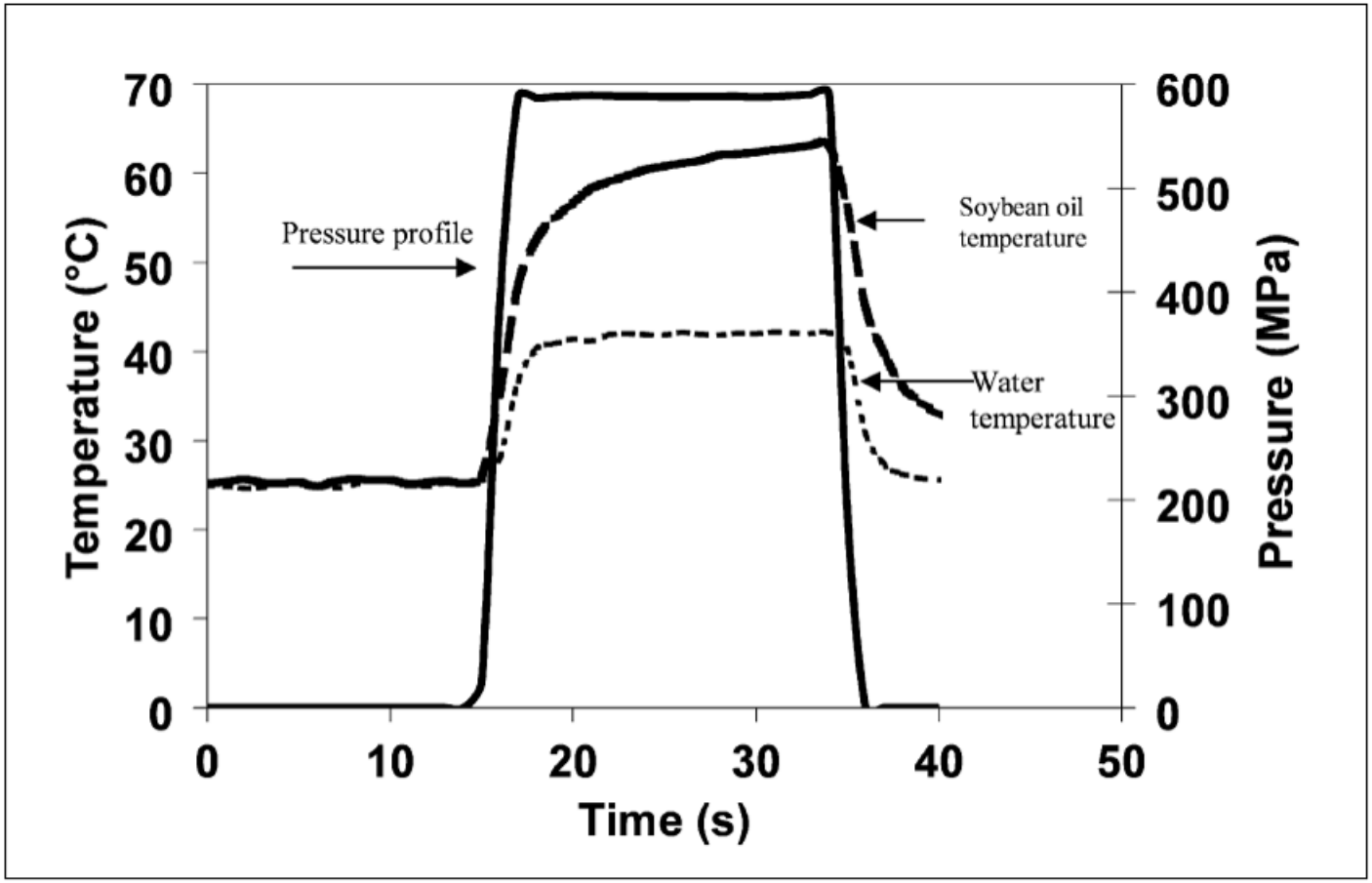


Figure 3—Sample pressure-temperature curves for water and soybean oil tested using compression heating apparatus

Data from: Rasanayagam, Balasubramanism, Ting, Sizer, Bush, Anderson, JFS, Vol 66, 2003

Cooling under Pressure

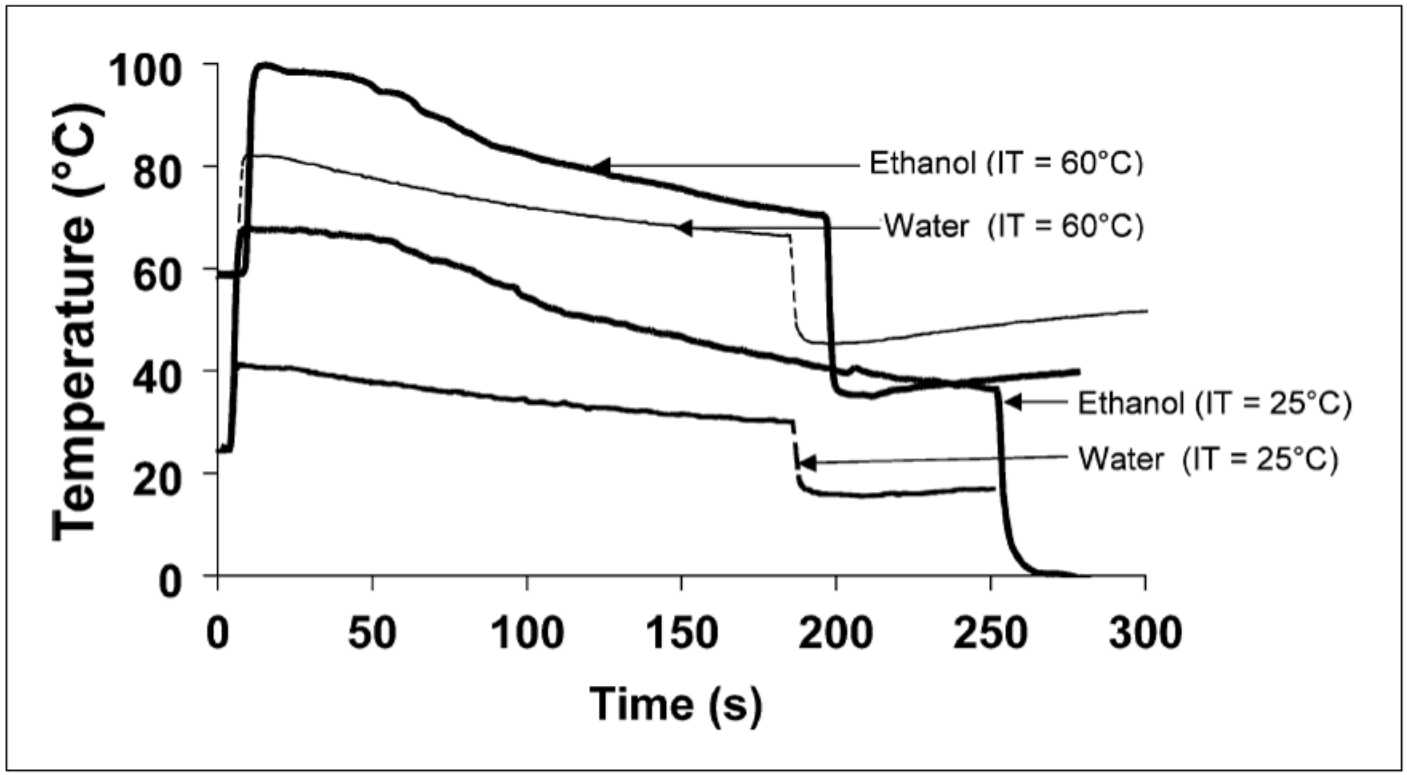
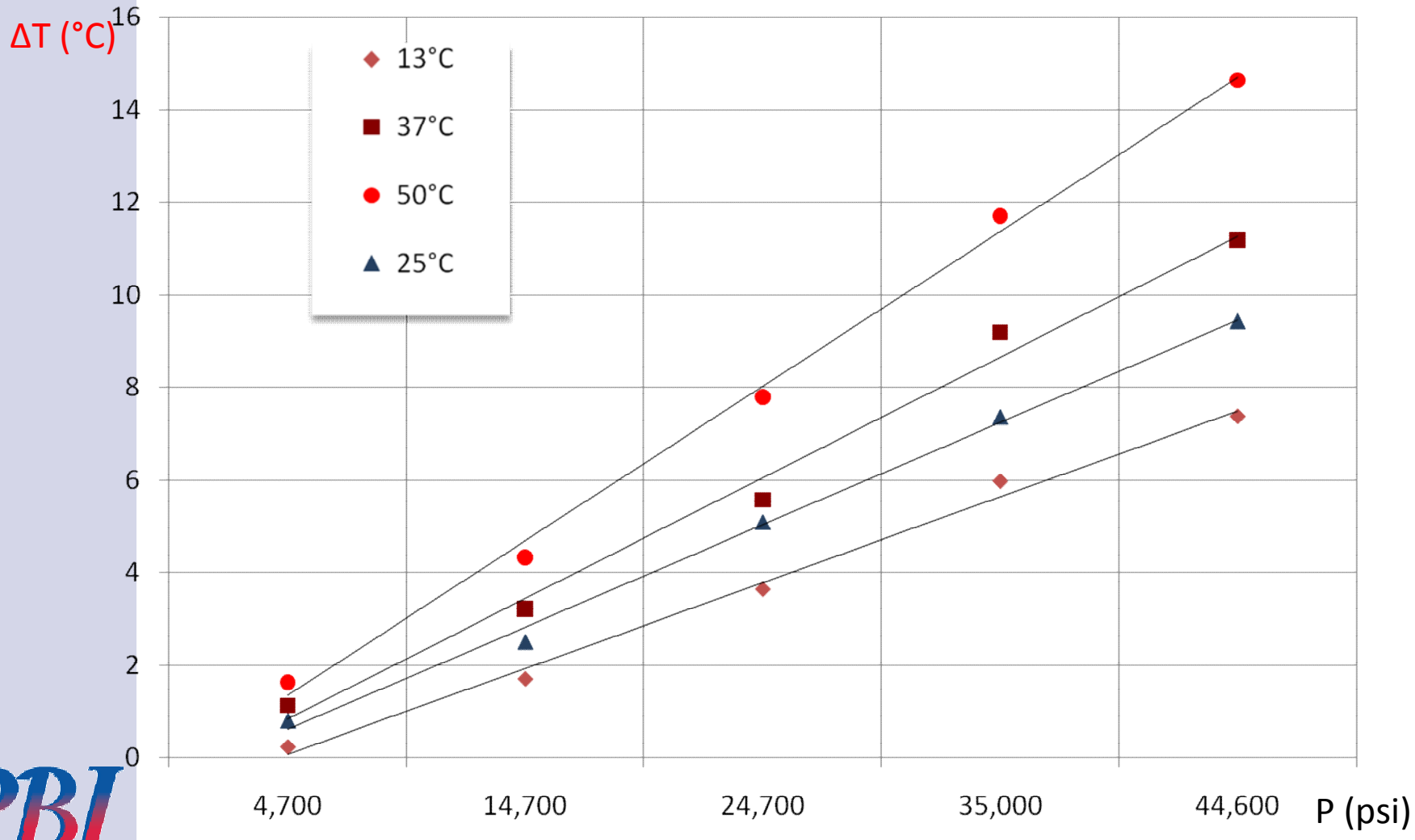


Figure 6—Impact of long holding times on temperature of materials compression heated under pressure (540 MPa)

PBI

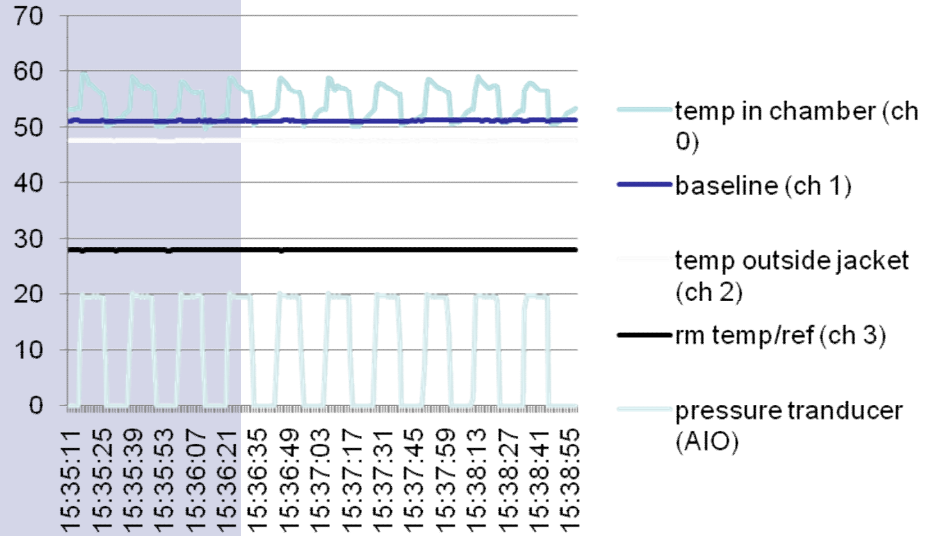
Compression heating effects

ΔT° as a function of pressure at various T_0

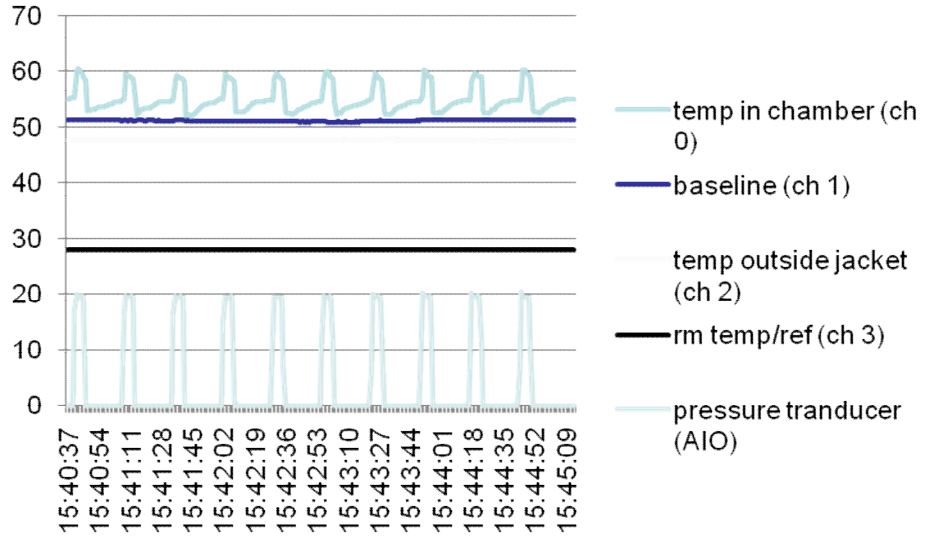


Cycle variability at 51C/ 20,000psi

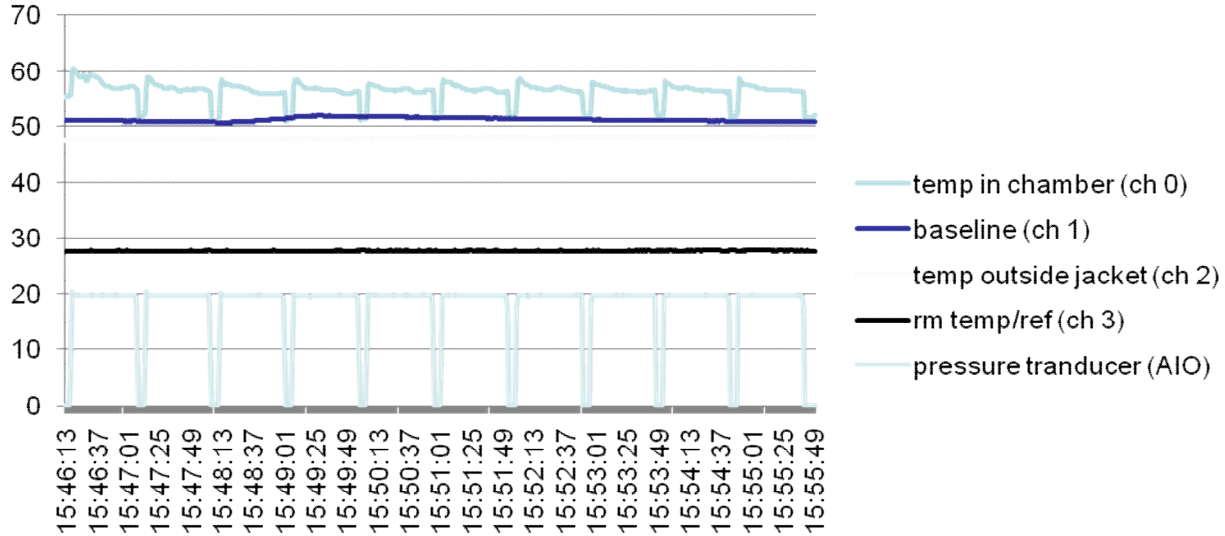
10 on: 10 off



5 on: 20 off



50 on: 5 off



PBI

Temperature Effects

- Under pressure, adiabatic compression increases temperature and this effect should be considered in experiments.
- At lower operating temperature (<30C*) and lower pressure (<20,000 psi*), pressure effects are dominant.
- At higher starting temperature (>50C*) and higher pressure (>30,000*), compression heating may be a significant primary or secondary effect which can be intentionally used to enhanced results.

*** Typically with water solutions**

PBI

Molecular Level Considerations

- Temperature effects are based on vibrational kinetic energy
- Pressure effects that are based on different thermodynamic factors: (ΔV and ΔS) free energy (ΔG) changes.
- Pressure may stabilize or weaken specific bonds at a given temperature.
- Frequently, pressure is synergistic with temperature and chemicals (i.e. water) in destabilizing many proteins (unfold, inactivate).

PBI

Innovation and Discovery

Journal of Immunotoxicology Posted online on 08 Mar 2010.

High hydrostatic pressure treatment generates inactivated mammalian tumor cells with immunogeneic features

E. M. Weiss [1], S. Meister [2,3], C. Janko [2], N. Ebel [4], E. Schlücker [4], R. Meyer-Pittroff [5], R. Fietkau [1], M. Herrmann [2], U. S. Gaipl [1,], B. Frey [1,*]*

1*Department of Radiation Oncology, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany*
2*Department for Internal Medicine 3, and Institute for Clinical Immunology, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany*
3*IZKF Research Group 2, Nikolaus-Fiebiger-Center of Molecular Medicine, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany*
4*Department for Process Technology, and Machinery, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany*
5*Competence Pool Weihenstephan, Technische Universitaet Muenchen, Germany*
*Both these have contributed equally to this work.
Address for Correspondence: Benjamin Frey, Radiation Immunobiology, Department of Radiation Oncology, University Hospital Erlangen, Universitätsstr, 27, 91054 Erlangen, Germany.

Most of the classical therapies for solid tumors have limitations in achieving long-lasting anti-tumor responses. Therefore, treatment of cancer requires additional and multimodal therapeutic strategies. One option is based on the vaccination of cancer patients with autologous inactivated intact tumor cells. The master requirements of cell-based therapeutic tumor vaccines are the: (a) complete inactivation of the tumor cells; (b) preservation of their immunogenicity; and (c) need to remain in accordance with statutory provisions. Physical treatments like freeze-thawing and chemotherapeutics are currently used to inactivate tumor cells for vaccination purposes, but these techniques have methodological, therapeutic, or legal restrictions. For this reason, we have proposed the use of a high hydrostatic pressure (HHP) treatment ($p \geq 100$ MPa) as an alternative method for the inactivation of tumor cells. HHP is a technique that has been known for more than 100 years to successfully inactivate micro-organisms and to alter biomolecules. In the studies here, we show that the treatment of MCF7, B16-F10, and CT26 tumor cells with HHP ≥ 300 MPa results in mainly necrotic tumor cell death forms displaying degraded DNA. Only CT26 cells yielded a notable amount of apoptotic cells after the application of HHP. All tumor cells treated with ≥ 200 MPa lost their ability to form colonies *in vitro*. Furthermore, the pressure-inactivated cells retained their immunogenicity, as tested in a xenogeneic as well as syngeneic mouse models. We conclude that the complete tumor cell inactivation, the degradation of the cell's nuclei, and the retention of the immunogeneic potential of these dead tumor cells induced by HHP favor the use of this technique as a powerful and low-cost technique for the inactivation of tumor cells to be used as a vaccine.

PBI

Conclusions

- The ability to control high pressure enables unique control of molecular stability, phase structures, chemical solubility, and other effects important to bioscience.
- Pressure represents a rich opportunity for continued discovery and commercialization.
- Engineering advances continue to make laboratory high pressure equipment more available and affordable.

PBI

Thank you