HIGH BIOLOGICAL ACTIVITY IN CHLOROPLASTS FROM *EUGLENA GRACILIS* PREPARED WITH A NEW GAS PRESSURE DEVICE

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The algae *Euglena gracilis* has been extensively used as an experimental material in photosynthetic and developmental studies. However, attempts by several authors to prepare highly active chloroplasts, particularly in terms of their ATP synthesizing capacity, have not as yet produced an effective technique [1, 2]. Thus, the best rate of phosphorylation published to date is 50 μmoles ATP/mg chl. × hr [3] compared to above 2000 [4] of the same units for higher plants.

We wish to report the design of a new device with which the highest activities reported to date could be routinely observed. The device is now commercially available under the name "Yeda Press" from Yeda Research and Development Co., POB 26, Rehovoth, Israel. In preliminary experiments, it was also found useful in breaking other biological materials while retaining high activity.

In the Yeda Press (fig. 1) compressed gas from a standard high-pressure tank is applied to the material in question at any desired pressure and the sample is released through a needle valve. The sudden decompression causes controlled disruption of the biological material. The principle of operation is similar to that employed in other commercially available pressure cells. However, two factors seem to contribute to the more consistent and higher activity observed in the Yeda Press. These are the maintenance of constant pressure throughout the procedure and the employment of inert gases (Nitrogen or argon) during the disrupting procedure.

Table 1 illustrates the extent of cell disruption observed with several biological materials in the Yeda Press under variable conditions. As can be seen many types of cells may be disrupted at relatively low pressure.

The activity of chloroplasts isolated from *Euglena gracilis* in the Yeda Press is illustrated in table 2. As can be seen these chloroplasts possess considerably higher activity than hitherto observed, although still lower than the corresponding activities of higher plant chloroplasts.

It should also be noted that the chloroplasts obtained by the technique described contained considerably higher quantities of cyt. 552 than those obtained by other techniques [7]. Thus, a higher extent of chemically or light-induced cytochrome

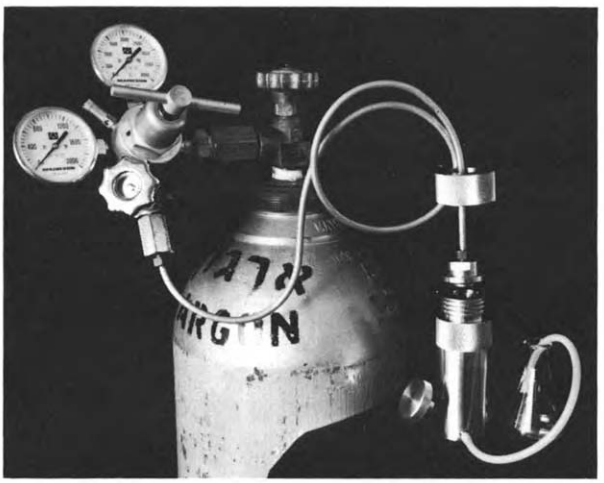

Fig. 1. A photograph of the Yeda Press. The instrument is shown connected to the pressure tank as in actual operation, but not closed, to illustrate the component parts.

Table 1
Degree of breakage of several biological materials in the Yeda Press.

Material used	Gas employed	Pressure in PSI	Times passed through press	Percent broken
Red blood cells	Air	500	1	>95
Euglena gracilis	Air	500	1	50
Euglena gracilis	Air	500	2	72
Euglena gracilis	Nitrogen	500	2	95
Euglena gracilis	Nitrogen	1000	2	>95
Euglena gracilis	Argon	500	2	95
Euglena gracilis	Argon	1000	2	>95
Rhodospirillum rubrum	Nitrogen	500	2	22
Rhodospirillum rubrum	Nitrogen	1500	2	60
Escherichia coli	Nitrogen	1500	2	52
Bacillus subtilis	Air	1500	2	< 1
Chlorella pyrenoidosa	Air	1500	2	< 1
Anacystis nidulans	Air	1500	2	50
Phormidium luridum	Nitrogen	1500	2	>95

Cells were placed in the instrument in their usual growth or maintenance medium in a concentration of approximately 5–10% packed cells. All disruptions were carried out at 0° in a precooled press.
The degree of breakage was determined as follows: in algae and red cells by direct counting in a hemocytometer under a microscope; in *R. rubrum* by determination of the bacterio-chlorophyll as previously described [5]. In other bacteria, by determining the protein content of the supernatant [6] after a 12,000 *g* centrifugation, compared to the protein content of a similar supernatant broken in a French Press twice at 12,000 PSI (the latter used to represent 100% breakage).

Table 2
Photosynthetic activity of *Euglena* chloroplasts isolated in the Yeda Press.

Reaction measured	Specific activity (μmoles electrons or ATP/ mg chlorophyll × hr)
NADP photoreduction by water	80
$K_3Fe(CN)_6$ photoreduction by water	200
$K_3Fe(CN)_6$ dependent phosphorylation	26
Phenazine methosulfate dependent phosphorylation	140

Euglena cells were grown heterotrophically in the light in Hutner's medium [9]. They were washed and suspended in a solution, containing 0.2 M sucrose, 0.1 M NaCl, 0.05 M tris, pH 7.8, and broken twice in the Yeda Press at 500 PSI under argon. The suspension was centrifuged at 300 *g* for 5 min; the pellet discarded, and the supernatant recentrifuged at 1000 *g* for 10 min. The pellet was resuspended in the above solution and used as the chloroplast preparation.
The reaction mixture contained, in a total volume of 3 ml, in μmoles: KCl, 60; and chloroplasts containing 40 μg of chlorophyll and the following additions, NADP: NADP, 3; phosphate, pH 6.5, 150; and a saturating amount of ferredoxin. Ferricyanide: $K_3Fe(CN)_6$, 1.5; tricine, pH 7.8, 45; magnesium chloride, 12; potassium phosphate, pH 7.8, 16 (containing 3×10^6 cpm P^{32}); ADP, 2; glucose, 18; and hexokinase (Sigma, type III), 4 units. Phenazine methosulphate: phenazine methosulphate, 0.1, and all the additions as for ferricyanide, except for ferricyanide itself. The reaction mixtures were illuminated at 160,000 lux at 20° for 2 min. 0.3 ml of 70% $HClO_4$ was added, following by placing the tube at 100° for 8 min, cooling to 0°, centrifuging, and assaying the supernatant for organic P^{32} as previously described [10]. The acid-heat treatment was introduced in order to hydrolyze the ADP^{32} formed via the enzyme ADP-ribose phosphorylase [11, 12].
$K_3Fe(CN)_6$ was assayed as previously described [13]. NADP reduction was monitored directly in a Cary 14 spectrophotometer.

oxidation was observed in a dual-wavelength spectrophotometer in isolated chloroplasts [8], and reasonable rates of photoreduction of NADP from water (table 2) could be observed even in the absence of added cytochrome-552 [7].

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