A rapid steel mill for the disintegration of yeast

An ultra-rapid shaker, described previously[1], may be used for the preparation of respiring cell-free extracts of yeast[2] and other micro-organisms[3], but only limited amounts of extract can be prepared. Up to 2 g wet wt cells are used for each disintegration, and, with five separate capsules, about 20 disintegrations can be carried out in less than 30 min. Even such amounts may be insufficient for certain experiments.

Our mill is based on a machine used by LANG AND SIEBERT[4] for the preparation of nuclei from animal tissues. It resembles an UTTER AND WERKMAN[5] mill, except that the glass cone and socket are replaced by hardened steel parts. This means that much higher speeds can be achieved (1500 r.p.m. instead of 300), greater pressure can be applied and there is no danger of breakage. The machine is shown in Fig. 1.

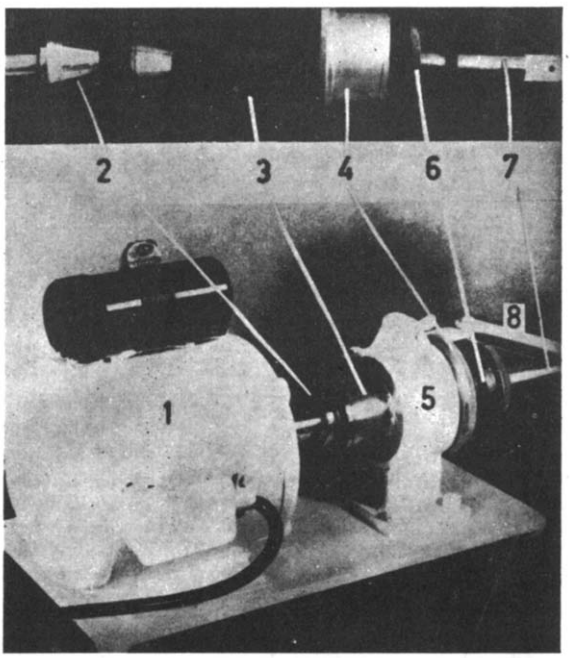

Fig. 1. Rapid stainless steel mill. Lower Part: Assembled, ready for use. Upper Part: Moving constituents exploded. Scale (of upper part only): 1: 2.5. 1. $^1/_4$ h.p., 1400 r.p.m. A.C. single phase motor. 2. 30° -taper cone, Balfour's S.C. 23 steel, heat-treated to 65 Rockwell C. and hard-chromed, attached by two grub screws directly to motor shaft; hollow, for inclusion of coolant. 3. 30° -taper socket, Balfour's S.C. 23 steel, heat treated as above and hard-chromed, integral with cylinder to hold the material to be ground, outside threaded to fit 4. 4. Threaded aluminum outer collar, to fit 5. 5. Cylinder clamp casing, fabricated steel. 6. Locking collars for fixing position of 3. 7. Stainless steel piston, 0.0005″ clearance from cylinder of 3. 8. Steel lever for pushing piston.

A thick yeast paste is prepared with No. 12 Ballotini glass beads and a suitable solution, *e.g.* 0.1 *M* phosphate buffer pH 7 (10 parts wet wt baker's yeast, 30 parts glass beads and 7 parts (by vol.) buffer). Cone and socket are kept in the deep-freeze, and the whole procedure is preferably carried out in the cold room. However, the original trials were done at room temperature and yielded active extracts. Cone and socket assembly are placed in position, and the socket screwed so close to the cone that the mill can only just revolve. The locking-collar is set for this position. The cone assembly is removed, charged with the yeast paste, and put back in position. The motor is switched on, the paste is forced slowly through the mill and the issuing material collected in a small tray underneath. For the extract described in Table I, the resultant material was rapidly cooled in a freezing mixture and put through the mill twice more. About 40% disintegration (by direct cell count) is obtained. The whole procedure takes less than 5 minutes.

The cone and socket are washed with the buffer and the washings and issued material pooled. The cell-free extract is prepared by centrifugation for 10 min at 1000 g[1]. Extracts are examined microscopically to make sure that they are not contaminated with whole cells.

The results in Table I show that such extracts are similar to those prepared with the high-speed shaker[2], but there are some definite differences: Mn^{+2} enables ethanol oxidation to proceed over a much longer period; DPN* also activates; ATP and A5P, which in shaker extracts cause great increases in blank respiration (unpublished results), here inhibit ethanol oxidation; succinate is oxidised over a longer period than in the shaker extracts.

* Abbreviations used are: DPN for diphosphopyridine nucleotide, ATP for adenosine triphosphate, A5P for adenosine-5-phosphate and cyto. *c* for cytochrome *c*.

TABLE I

OXIDATIONS IN CELL-FREE YEAST EXTRACTS PREPARED WITH THE MILL

3 ml extract (= *ca.* 50 mg dry wt) in phosphate buffer pH 7. 400 μM substrate. Cofactors: $MnCl_2$: 10 μM; DPN: 160 μg; Cyto. *c*: 200 μg; ATP and A5P: 4 μM each.

Substrate	*Cofactors*	μlO_2 *taken up for the following periods from the commencement of reading:*			
		0–10 min	*30–40 min*	*60–70 min*	*110–120 min*
None	$MnCl_2$, DPN, cyto. *c*, ATP, A5P	43	29	18	15
Ethanol	None	265	103	35	11
Ethanol	$MnCl_2$	294	165	106	72
Ethanol	DPN	292	175	57	19
Ethanol	DPN, cyto. *c*	242	206	80	16
Ethanol	DPN, cyto. *c*, $MnCl_2$	254	206	120	87
Ethanol	ATP, A5P	157	114	64	29
Succinate	None	248	132	66	45

The machine may be made in any desired size and used for preparing fairly large quantities of respiring yeast extracts. Although the dry weights of extracts are not as quantitatively reproducible as with the shaker, there is good agreement between individual runs on different days. The machine should also be useful for disintegrating other micro-organisms.

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Amino acid metabolism in locust tissues

BARRON AND TAHMISIAN[1] reported the presence of glutamate/alanine transaminase in cockroach muscle. We have now shown that transaminations between a large number of α-keto- and α-amino-acids are catalysed by preparations of fat-body, malpighian tubules and gut wall of the Desert Locust, *Schistocerca gregaria* Forsk., and have found a possible link between carbohydrate and nitrogen metabolism in the occurrence of glutamic dehydrogenase activity in fat-body.

The enzyme preparations were obtained by dissecting out the appropriate tissue under ice-cold insect Ringer solution and homogenizing in ice-cold phosphate buffer, pH 7.4, using a Potter-Elvehjem homogenizer. For detection of transamination, the enzyme preparations were incubated at 37° C with the appropriate substrates (initial conc. $M/20$) and at a tissue concentration of *c.* 30 mg wet wt./ml; the reactions were followed qualitatively by paper chromatography.

As in mammalian tissues, the alanine/glutamate and aspartate/glutamate reactions were found to be the ones most rapidly catalysed by fat-body preparations, and both have been shown to reach an equilibrium position starting with either amino-donor and keto-acid. These two transaminases have also been shown in malpighian tubules and mid-gut wall preparations. Glutamic acid was also formed, but more slowly, when fat-body preparations and α-ketoglutarate were incubated with each of the following amino-acids: glycine, valine, leucine, cysteine (anaerobically), methionine, threonine, serine, phenylalanine, tryptophane, histidine, arginine, lysine, and ornithine. No glutamic acid was detected when proline or hydroxyproline were used. A very active arginase was present in the fat-body preparations, so that the apparent arginine/glutamate