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Temperature Control During Homogenization.* (25201)

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Length of time for which biological materials can be subjected to high speed homogenization is frequently limited by sharp rises in temperature which accompany such treatment. These temperature increases are particularly severe with fibrous tissues, and have also been observed during disruption of microorganisms by agitation with fine glass beads in the Waring Blendor(1). Such temperatures not only destroy labile cellular constituents, but constitute a safety hazard when volatile, flammable solvents are used. Temperature control is particularly difficult in homogenizers whose drive unit is located beneath the container, since the container can not be packed in ice or cooled in a water bath. Procedures commonly used for controlling homogenate temperatures in instruments such as the Waring Blendor include pre-cooling the container, working in the cold room, alternately homogenizing and chilling the container, and dropping ice into the homogenate. These measures frequently are not only either slow or inconvenient, but are ineffective when prolonged homogenization is required. With apparatus modifications and procedures described below, it is possible to maintain low temperatures during extended homogenization. The necessary equipment modifications may be made on standard homogenizer containers; these alterations are simple, inexpensive, and require only materials and tools available to most laboratory shops. Although methods and results described below are for the Waring Blendor, it should be feasible to adapt them to other makes of homogenizers. Evidence for the effectiveness of apparatus and procedures is presented in this report.

Methods. Temperature control in glass Waring Blendor containers is readily obtained by use of an internal cooling coil of the type shown in Fig. 1. During construction, care must be taken (a) not to collapse the tubing when winding the coil and (b) to position the coil so that it clears the blades by a safe distance. When coil is in use, water, a few degrees cooler than the temperature desired in the homogenate, is circulated through it by a small centrifugal pump. *Temperature control in semi-micro containers.* While the internal cooling coils described above are suitable for large glass and metal homogenizer containers, they cannot be used in smaller semi-micro units. Temperature control in these containers, however, is easily obtained by installation of water jackets. Fig. 2 shows a metal semi-micro container for the Waring Blendor fitted with cooling jacket made from 3-inch diame-

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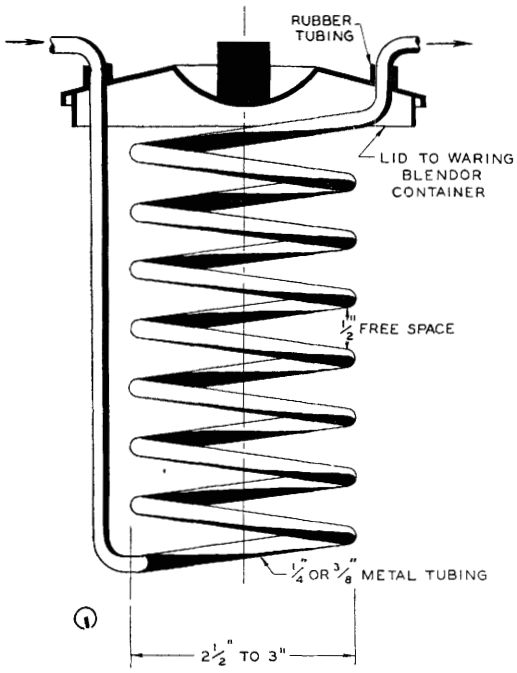

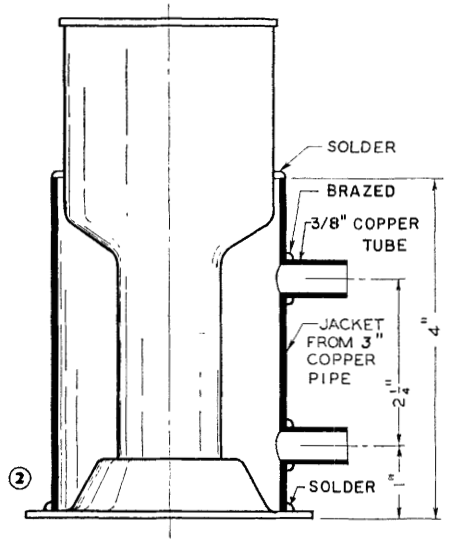

FIG. 1. Metal cooling coil for use in glass containers for Waring Blendor.

FIG. 2. Cooling jacket for metal semi-micro Waring Blendor container.

ter copper pipe. The shape of container renders it unnecessary to construct either a top or bottom for the jacket. Inlet and outlet tubes of 3/8-inch diameter are first installed in the copper pipe and fastened either by soldering or silver brazing. Because the container is itself fabricated with a brazed joint, it is not advisable to attempt to braze the jacket to the container, since the heat required might damage this joint. The jacket, with inlet and outlet tubes in place, is therefore joined to the container with ordinary soft solder and carefully controlled heat. Blade assembly and gaskets are removed prior to soldering. During homogenization, cooling is accomplished by circulation of cold water with a small centrifugal pump.

Results. With either the jacketed semi-micro container or the glass container with internal cooling coil, we have consistently kept homogenates at 2°C to 7°C by circulating water at 0°C. Both modified containers have proved their superiority over unaltered containers in maintaining low temperatures, even when the unaltered containers were pre-cooled to −10°C and operated in the cold room. Efficiency of the jacketed container is indicated by the data in Fig. 3. The internal cooling coils were similarly effective: in a representative experiment, the temperature of a homogenate of cotton leaves (75 g in 350 ml water) rose from 3.2°C to only 6.5°C during

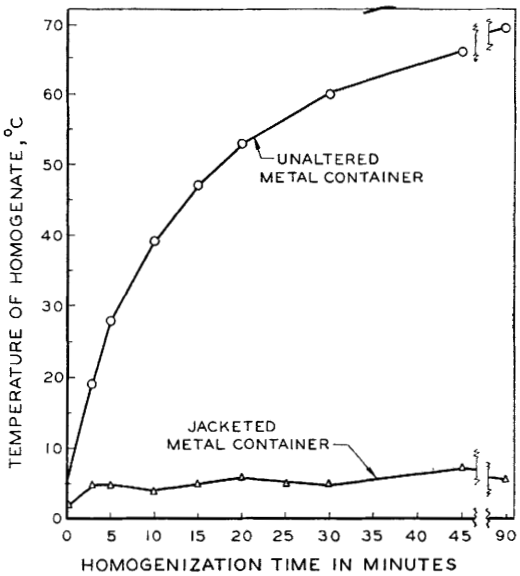

FIG. 3. Temperatures observed during disruption of yeast cells by agitation with small glass beads in jacketed and unaltered metal semi-micro containers for Waring Blendor. Cooling water at 0°C was circulated through the jacketed container. The unaltered container was pre-chilled in the freezer and kept in cold room at 11°C during agitation. Mixture consisted of 100 g of glass beads, 50 g yeast, and 100 ml water.

3 hours of homogenization, while the temperature of an identical sample in a container without cooling coil increased from 0.5°C to 90°C after only 1 hour.

Internal cooling coils did not interfere with effective homogenization, and proved to be more efficient in controlling temperatures than water jackets on the glass containers. Jackets for these containers constructed from large polyethylene bottles and cemented in place with epoxy resin were only moderately successful because of poor heat transfer through the thick glass walls. When the cooling coil is used during homogenization in the presence of abrasive materials (such as small glass beads) there is an etching effect on the lowermost helix, and this section should be protected by a sleeve of plastic tubing. Cooling coils of ⅜-inch tubing were found to be more satisfactory than those of smaller size because of the faster flow rate of cooling water which they permit. We have used both aluminum and copper tubing; stainless steel may be employed where conditions require it.

Summary. Effective cooling during prolonged homogenization at 15,000 r.p.m. in the Waring Blendor was obtained by simple, inexpensive modifications to standard Waring Blendor containers. Homogenates in glass containers were cooled by insertion of coils made of metal tubing through which ice water was pumped; metal semi-micro containers were modified by installation of water jackets for circulation of a coolant.

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Viral Sensitivity of Cell Cultures Maintained with Skim Milk Medium. (25202)

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Development of a serum-free medium containing skim milk, which effectively maintains a number of cell cultures(1), prompted this study. Previous data showed that heat sterilization of milk in preparation for this medium completely destroyed any antibodies that might be present(1). The viral sensitivity of cell cultures in presence of skim milk maintenance medium was compared to sensitivity of the same cell lines on an established medium.

Material and methods. Virus. Virus strains and host cell lines are listed in Table I. *Cell cultures* employed were prepared in the cell culture laboratory, Division of Biologics Standards, Nat. Inst. of Health by Mr. George Gardner. Primary monkey kidney cells(2) were propagated in medium 199(3) containing 2% calf serum* and antibiotics† and maintained in medium 199 containing 20% skim milk at 30°C until inoculated with virus. All other cell lines were held at 36°C. HeLa cells(4) were propagated and maintained in medium 199 containing 10% calf serum. Primary rabbit kidney cells(5) were propagated in lactalbumin hydrolysate medium(6) containing 10% calf serum and maintained in basal medium Eagle (BME) (7) containing 10% calf serum. HEP II cells(8) were propagated and maintained in BME containing 20% calf serum. *Viral titrations* were performed in duplicate in each cell line. Cell cultures of 1 titration were fed medium 199 containing 20% skim milk. Cell cultures of second titration were fed as follows: monkey and rabbit kidney cells—medium 199; HeLa cells—tryptose phosphate broth medium (TPB)(9) consisting of 70% medium 199, 25% tryptose phosphate broth and 5% chicken serum; HEP II cells—BME

* All serum used for cell culture medium was inactivated at 56° for 30 min.

† The following concentration of antibiotics was used: 100 units of penicillin/ml, 100 μg of streptomycin/ml, 100 μg of mycostatin/ml (not used in maintenance medium).