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Improved Procedures for Preparation of Tissues for Chemical Analyses.* (22828)

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Many years of work on histochemical characterization of various tissues have required different technics for their preparation for precise chemical analyses. Previously, some soft tissues were minced and aliquots of wet minced tissue were weighed for all analyses: skeletal muscle(1,2), brain(3) and heart(4); some tissues were minced, dried and smashed in a special apparatus for all analyses: liver (5,6), skin(7,8), kidney(9), cartilage(10,11).

The method described here has many advantages: it is saving in time and effort while producing material excellent for sampling; it permits a uniform routine for all soft and hard tissues, especially cartilage; and it provides for numbers of tissues being taken from the same animal simultaneously and handled more satisfactorily.

Procedure. Tissues are taken from anesthetized animals, wiped with dry gauze and dropped quickly into ground glass-stoppered jars. After being cooled in a refrigerator to minimize water loss, the tissues that have visible connective tissue or fat are placed on a tile and adherent structures removed. The tissues are then dropped into glass-stoppered weighing bottles and again cooled before mincing with points of scissors. Tissues such as liver and brain that do not have visible fat or connective tissue are simply cooled and minced. Skin after being trimmed is cut into strips 8×10 cm. Samples are allowed to come to room temperature, and then weighed into vycor crucibles in aliquots depending upon amount of wet tissue available. These samples can range to 10 g of wet tissue. It is preferable to weigh 2 or more samples of the same tissue to check determinations for water and fat. The crucibles are next placed in 100°C thermostatically controlled oven for 48 hours, after which they are cooled in a desiccator over activated alumina oxide and then weighed. After this, the dried tissues are covered with dry ethyl ether and placed in another desiccator containing sufficient ethyl ether to cover bottom of desiccator to about one inch. After remaining in this ether desiccator for 24 hours, the ether layer over tissue containing extracted fat, is removed with Pasteur pipet and new dry ethyl ether added. This process is repeated at least 3 times. After removal of last ether extraction, the crucibles are placed in 100°C oven for approximately 5 hours, then cooled in a desiccator over activated alumina oxide and weighed. This gives a dry, fat-free solid which can be kept in a desiccator until ready to be ground and analyzed.

Grinding the dry tissue. The mills used for grinding dry tissues were either a Zassen-

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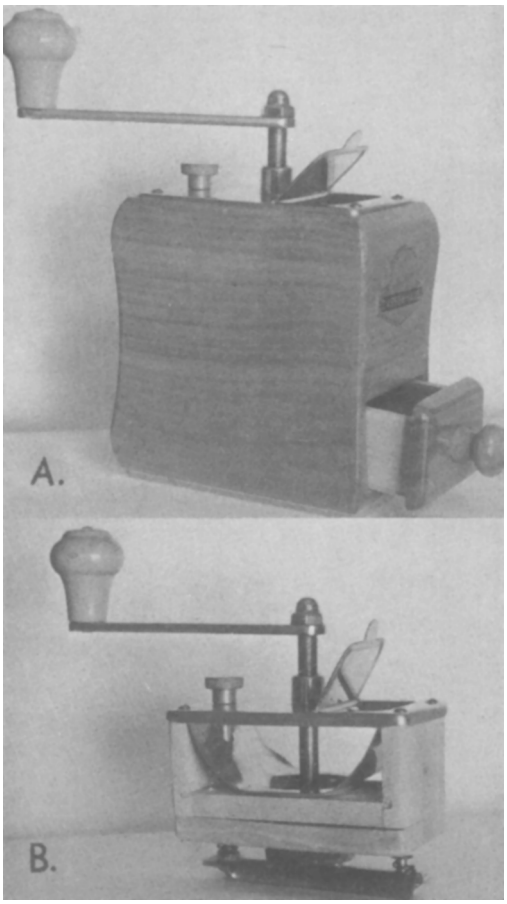

FIG. 1. A. Zassenhaus spice mill showing opened spring door through which tissue is introduced into mill and opened drawer that receives the ground sample. On top is also shown grinding handle and screw for setting size of desired particles. B. Cutting unit lifted out of mill.

haus spice or pepper mill,† depending on size or weight of tissue sample. These mills have hard steel cutters that can be adjusted for size of particles by a turn screw in top of spice mill or in bottom of pepper mill. The set screws on both ends of spice mill can be removed permanently so that the cutting unit can be lifted out readily. Illustrations of spice mill and cutting unit are shown in Fig. 1.

For grinding, dried tissue is scraped quantitatively with stainless steel spatula from the vycor crucibles onto a square of glazed paper and then transferred by brushing into the cutter compartment through a spring door on top of mill. The tissue is ground in a few minutes, requiring no effort. The powder is collected into a small drawer of the mill or in a devised cellophane bag. If the drawer is used, the powder from the drawer is transferred to a square of glazed paper, the cutters are lifted out and the drawer and the mill box are tapped sharply several times on the glazed paper until all particles of tissue have fallen from mill onto paper. The cutters are held over the glazed paper and any powder sticking to them is brushed with the sample for quantitative recovery. If the cellophane bag is used it is prepared as follows: a strip of heavy cellophane $18 \times 6''$ is wrapped around the cutter unit, using the extra for overlap; then bottom part of cellophane envelope is pleated and a hem is folded over to close the end of bag which is then fastened with a paper clip. For grinding, the cutting unit can either be returned to mill box for holding or it can be held firmly with a wood clamp attached to laboratory desk. When grinding is finished, cutter unit with attached cellophane bag is removed and placed on laboratory desk, the paper clip removed and the bag opened flat. Any adhering powder on cutters is brushed with the sample on the cellophane piece. The ground samples can be placed into small screwtop jars for later work, or they can be ground finer immediately.

To grind sample finer, samples from the mills are placed in a mullite mortar and either ground by hand or ground with a Fisher Mortar Grinder. The Fisher Electric Grinder is faster and superior to hand grind, requiring only about 15 minutes for soft tissues, and a little longer for cartilage samples. The powdered tissues are then returned to screw-top jars and, before weighing aliquots for analyses, they are dried in a 100°C oven overnight, cooled and kept in a desiccator over activated alumina oxide.

Summary. Improved procedures for preparation of tissues for chemical analyses have been presented. These procedures have definite advantages over all previous procedures; they are saving in both time and effort; they

† Kirkhams, Glens Falls, N. Y. Mill No. M495.

permit a uniform routine for all types of tissues; they produce homogenous materials that insure adequate sampling, and they enable numbers of tissues to be taken from the same experimental animal and handled satisfactorily.

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The Breeding System in *Stylonychia putrina*. (22829)

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Mating types have been reported in *Paramecium aurelia*(1-4), *P. bursaria*(5-7), *P. caudatum*(9-12), *P. multimicronucleatum*(8, 9), *P. trichium*(13), *P. calkinsi*(14), *Euplotes patella*(15), *Tetrahymena pyriformis*(16-18), *Stylonychia putrina*(19), and *Oxytricha bifaria*(20). In several of these species a number of sexually isolated varieties have been discovered. In some, the number of mating types found in each variety was 2, but in *P. bursaria, P. multimicronucleatum, P. trichium, E. patella, T. pyriformis, S. putrina,* and *O. bifaria* multiple mating types have been found. In one variety of *P. bursaria* there were eight; in *E. patella,* 6; in a variety of *T. pyriformis,* 11; in *S. putrina,* 5; and in *O. bifaria,* 9. The present paper reports another variety and additional mating types in the first variety of *S. putrina* reported in 1952 (19).

Materials and methods. Cultures of the 5 mating types in the first variety reported in 1952 died out in the laboratory. Two clones of progeny from crosses that had been made between clones of some of these 5 mating types were left. In 1952, 1954, and 1955, 155 additional clones of *Stylonychia putrina* were collected in Southern California in 6 different sites, 3 in the mountains and 3 in the valleys. The food was a liquid culture of a species of *Chlamydomonas* to which a loop of *Aerobacter cloacae* was added. The animals were isolated and grown in depression plates and later transferred to vials. The clones were fed every day, often twice a day.

Results. Mating behavior. The mating behavior found was that described previously (19), except that some degree of mating activity was observed in practically all of the 155 clones reported here, while none was observed in more than a third of the 41 clones reported in 1952.

Varieties and mating types. The two clones of progeny mentioned above reacted with some of the clones of a group that is called Variety I in this report. The mating types of Variety I now in existence have not been correlated with those previously reported. In 1954, six mating types were found in this variety. Various crosses were made among clones of these 6 mating types and among the progeny raised were 5 clones belonging to 3 new mating types. Among clones collected in 1955, 6 additional mating types were found, making a total of at least 15 mating types in Variety I. Eleven mating types were found among the Variety II clones, giving a total of 26 mating types for the two varieties.