cate that this involution is due to a restricted output of ACTH by the anterior pituitary (4-6). B) *Thyroids.* The morphological picture of the thyroid in the cortisone-treated cats appears to be indicative of a higher than normal level of thyrotrophic activation. This is in agreement with the findings of Higgins *et al.*(7), and Halmi and Barker(8) in rats. C) Nervous system: Castor *et al.*(1) found that ACTH given to rats in 10 mg dosages per day for a period of 10 days resulted in consistent cytological changes which were restricted to the cells of the hypothalamic paraventricular nucleus. Animals treated with similar quantities of cortisone showed changes of a more widespread nature which involved several hypothalamic and thalamic nuclei. The conclusion reached was that the changes effected by ACTH might be interpreted as evidence of altered secretory activity within the cells of the paraventricular nucleus. However, the ACTH used in their experiment was contaminated with posterior lobe hormones, and it is possible that the latter were responsible for the alterations in the paraventricular nuclei (9). Furthermore, the rats of the Castor group that were treated with cortisone were in poor condition at autopsy whereas the animals used in the present experiment were in excellent condition. This tends to favor the interpretation considered by the authors that the morphological changes in the central nervous system after cortisone treatment might possibly be non-specific and due to the general deterioration of the animals rather than to a specific action of cortisone on the nervous system.

Summary. 1. We attempted to ascertain the effects of large doses of cortisone on the histology of the cat and rat brain. In addition, the adrenals and thyroids of the cats were examined. 2. No change was encountered in the nervous tissue of cortisone-treated animals. 3. The adrenals of the cortisone-treated cats demonstrated the compensatory atrophy which is believed to be brought about by a decreased discharge of ACTH from the anterior pituitary. 4. The thyroids of the treated cats presented histological signs of thyrotrophic activation.

1. Castor, C. W., Baker, B. L., Ingle, D. J., and Li, C. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 353.
2. Rioch, D. M., Wislocki, G. B., and O'Leary, J. L., *Res. Publ. Assn. Nerv. Ment. Dis.,* 1940, v20, 3.
3. Ingram, W. R., *ibid.,* 1940, v20, 195.
4. Ingle, D. J., *Am. J. Physiol.,* 1938, v124, 369.
5. Sayers, G., and Sayers, M. A., *Ann. New York Acad. Sci.,* 1949, v50, 522.
6. Stebbins, R. B., *Endocrin.,* 1951, v49, 671.
7. Higgins, G. M., Woods, K. A., and Kendall, E. C., ibid., 1950, v48, 175.
8. Halmi, N. S., and Barker, S. B., *ibid.,* 1952, v51, 127.
9. Baker, B. L., personal communication.

Received November 18, 1953. P.S.E.B.M., 1953, v84.

Large Scale Preparation of Liver Mitochondria with the Waring Blendor.* (20748)

ROBERT F. WITTER, WALTER J. PORIES, AND MARY A. COTTONE.
(Introduced by Alexander L. Dounce.)

From the Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

For studies on the isolation of oxidative enzymes from liver, it was desirable to have a method for the preparation of mitochondria from fairly large amounts of tissue. Methods employing pestle type of homogenizers(1-6) are not convenient for this purpose. The Waring blendor can be used to disintegrate larger amounts of tissue, and procedures utilizing this means of homogenization have been used to prepare mitochondria from skeletal muscle(7), heart(8), and liver(9). However,

* This research was supported by funds granted by the National Institutes of Health, U. S. Public Health Service.

preliminary investigation disclosed that the conditions of homogenization utilized in the first two methods were too drastic for liver and that complete disruption of the tissue did not occur under the conditions of the third procedure. Several workers(10-13) have claimed that good mitochondrial preparations cannot be obtained with the Waring blendor since a large portion of these cell bodies are damaged during the time required for complete rupture of the tissue.

A method is here reported for the production of virtually undamaged mitochondria from 50 to 100 g of liver with the aid of the Waring blendor. The first step in the procedure consists of a thorough maceration of the liver in a special tissue press (designed by A. L. Dounce and N. Naylor). The tissue is reduced to such a finely divided state that the subsequent time and motor speed required for homogenization in isotonic sucrose with the Waring blendor are not sufficient to damage the mitocondria. The latter are then isolated by differential centrifugation and washed twice with isotonic sucrose. Unlike the previous method for liver(9), the use of salt agglutination steps is avoided so that a clean cut separation of the mitochondria can be obtained. Proof that the mitochondria obtained by this method were nearly undamaged by the Waring blendor was obtained by comparison of the ATP-ase, fatty acid oxidase, and succinoxidase activities of these preparations with those of the mitochondria prepared with a pestle type homogenizer(14). Fresh "intact" mitochondria have virtually no ATP-ase activity(2,3,15). The ATP-ase activity is increased by aging the preparations(2,3,15) or by causing mechanical damage to the mitochondria(12). The fatty acid oxidase system, which is located in the mitochondria(1,16,17), is easily inactivated by conditions which damage these cell particulates(18-20). On the other hand, succinoxidase is more stable and remains relatively intact even in the particles which are formed by mechanical disruption of the mitochondria(10). Thus minor damage to the mitochondria would be revealed by a difference between the two types of preparations in respect to ATP-ase or fatty acid oxidase activity, whereas only gross damage would change the succinoxidase levels. Comparative experiments of this type have not heretofore been conducted with mitochondrial suspensions made with the Waring blendor (7-9).

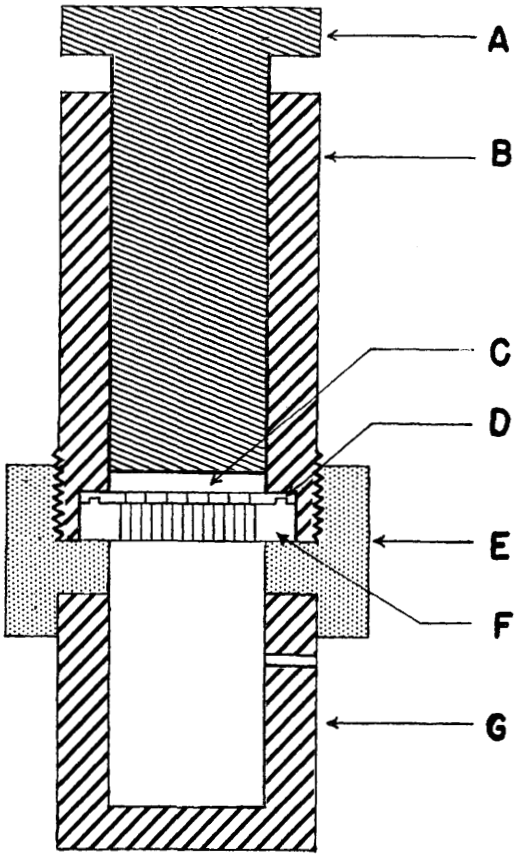

FIG. 1. Tissue press.

Preparation of mitochondria with the Waring blendor. Fifty to 60 g of fresh rat liver are thoroughly chilled in isotonic sucrose at −1°, and the tissue is then removed from the sucrose and pulped in the tissue press (Fig. 1) which has been chilled at 2° for 2 hours. The finely divided liver is weighed and then homogenized with 9 volumes (w/v) of isotonic sucrose at −1°, for 25 seconds at reduced motor speed in a chilled 1 liter Waring blendor cup. The voltage supplied to the motor, which draws 3 amperes, is reduced to 44 volts AC, or 1/3 of the usual amount. After the homogenate has been centrifuged at 1000 x *g* in 250 ml cups to remove the "nuclear fraction", the latter is washed with 150 ml of

isotonic sucrose and resedimented at 1000 x *g*. The combined supernatants from the "nuclear fraction" are centrifuged at 10000 x *g* for 13 minutes in 50 ml centrifuge cups. The supernatant layer is poured off, and the resulting sediment is carefully layered with 2 ml of isotonic sucrose. The "fluffy" layer is loosend by gently swirling the tube and poured off. The remaining mitochondria are suspended in 500 ml of isotonic sucrose and resedimented at 10000 x *g*. The process of removing the "fluffy" layer, resuspending, and resedimenting of the mitochondria is repeated twice, and the latter are then suspended with the aid of a pestle type homogenizer(14) in isotonic sucrose of such volume that 1 ml of suspension contains the equivalent of 0.5 g of fresh liver. Centrifugation at 1000 x *g* is carried out with a refrigerated International centrifuge, whereas centrifugation at 10000 x *g* is brought about with a Servall centrifuge, type SS-1, which is operated in a cold room at 2°. The relative gravitational forces are calculated for the middle of the centrifuge cup. All solutions are kept at −1°, and the temperature should not rise to more than 3° during centrifugation. The motor speed and time of homogenization must not be varied from those given in the directions.

Other methods. The ATP-ase activity was estimated as the amount of orthophosphate (21,22) released per 10 minutes at 30° from 1 ml of reaction mixture containing 0.1 M sucrose, 0.015 M tris (hydroxymethyl) aminomethane, pH, 8.0, 0.003 M ATP, and 0.0075 M magnesium chloride. The assay was run with fresh mitochondria and also with preparations aged in sucrose at 30° for 20 minutes. The fatty acid oxidase or succinoxidase activity was measured by the rate of oxygen uptake at 30° with 0.001 M hexanoate or 0.01 M succinate respectively as substrates in 3 ml of a reaction mixture similar to that described by Lardy and Wellman(3). The phosphate acceptor system in the oxidase experiments consisted of creatine and a fraction of rabbit muscle extract precipitating between 50 and 70% saturation with ammonium sulfate(23). Air was the gas phase in these experiments. Dry weight was determined by heating at 105° for 10 hours after thorough dialysis of the suspension at 0° to remove sucrose. Mitochondria also were prepared with a pestle type homogenizer(14) by a modification of the method of Lardy and Wellman(3).

Tissue Press. In Fig. 1 is given a schematic drawing of the tissue press. The hollow cylinder B is 10.5 cm long and has an inner diameter of 3.9 cm and an outer diameter of 6.3 cm. The plunger A is turned to fit sleeve B closely. Disk C is 0.1 cm thick and is perforated with holes in groups of 3. Each group of holes is arranged in the form of an equilateral triangle with a 0.3 cm edge. Nine of these groups are contained in each 1.2 cm^2 of surface. They are bored in such a manner that they fit over holes 0.4 cm. in diameter on perforated disk D, when the two disks are aligned by means of the small pins E on plate D which project 0.1 cm into corresponding holes on plate C. One end of ring F is threaded to screw onto the bottom end of sleeve B, and the other end of F is recessed by boring so that it fits rather securely by slight friction on cup G. The latter is equipped with an air vent near the top 0.1 cm in diameter. All parts of the press are constructed of stainless steel. To assemble the press plates C and D are aligned by means of pins E and are inserted in the recess in B which has been bored out for this purpose. Then F is screwed onto B and the assembly is set onto G. After the tissue is placed in B, the plunger A is forced down by means of a Carver hydraulic press, and the macerated tissue is collected in G.

Results. Microscopic examination showed that the mitochondrial suspensions prepared with the aid of the Waring blendor were homogeneous and free of nuclei, whole cells, erythrocytes or cell debris.

In Table I it is shown that the succinoxidase, fatty acid oxidase, and ATP-ase activities of these mitochondria compare favorably to those prepared with a pestle-type of homogenizer. It should be noted that the enzymatic activity of the latter preparations is similar to that found by other workers(2,3,15). The ATP-ase activity of the fresh mitochondria prepared with the Waring blendor was usually slightly higher than that of preparations made with

TABLE I. Enzymatic Activity of Mitochondria Prepared in the Waring Blendor or in Pestle Type Homogenizer.

Preparation	Micromoles oxygen/10 min. /mg dry wt		ATPase as micromoles P/mg dry wt	
	Fatty acid oxidase	Succinoxidase	Fresh mitochondria	Mitochondria aged 20 min. at 30°
Waring blendor	.29	.92	.26	.61
" "	.32	1.0	.20	.66
Homogenizer	.33	1.1	.18	.70
"	.30	1.1	.16	.90

the mechanical homogenizer. In agreement with this observation was the finding (not given in Table I) that addition of the phosphate acceptor system was necessary for maximum rate of oxidation of hexanoate or succinate in the preparation made with the mechanical homogenizer but not in the mitochondria prepared with the Waring blendor.

Since disruption of the mitochondria is known to increase ATP-ase activity(24), it is possible that the blades of the Waring blendor do cause slight but measurable damage. On the other hand, the damage cannot be extensive, for the ATP-ase activity of both types of preparations was increased on aging the mitochondria, and the fatty acid oxidase activities of the two preparations were similar.

It thus appears that a rapid and convenient method for the preparation of mitochondria of high enzymatic activity from large amounts of liver has been developed. The procedure should be of particular value to those who wish to utilize the mitochondrial preparations as starting materials for enzyme preparations or to study stable mitochondrial enzyme systems.

Summary. A method is described for the preparation of mitochondria from 50 to 100 g of liver. The tissue is pulped in a special press and homogenized at a reduced motor speed for a short time in the Waring blendor in isotonic sucrose. The mitochondria are isolated by differential centrifugation from isotonic sucrose. The ATP-ase, fatty acid oxidase, and succinoxidase activities of these preparations were shown to be similar to those of mitochondria prepared with a pestle type homogenizer.

The authors wish to express their thanks to Dr. Alexander L. Dounce for the use of his tissue press.

1. Schneider, W. C., *J. Biol. Chem.,* 1948, v176, 259.

2. Kielley, W. W., and Kielley, R. K., *ibid.,* 1951, v191, 485.

3. Lardy, H. A., and Wellman, H., *ibid.,* 1952, v195, 215.

4. Kennedy, E. P., and Lehninger, A. L., *ibid,* 1949, v179, 957.

5. Plaut, G. W., and Plaut, K. A., *ibid.,* 1952, v199, 141.

6. Hogeboom, G. W., Schneider, W. C., and Pallade, G. E., *ibid.,* 1948, v172, 619.

7. Perry, S. V., *Biochem. Biophys. Acta,* 1952, v8, 499.

8. Harman, J. W., and Feigelson, M., *Exp. Cell. Res.,* 1951, v3, 47.

9. Sarkar, N. K., Beinert, H., Fuld, M., and Green, D. E., *Arch. Biochem.,* 1952, v37, 140.

10. Harman, J. W., *Exp. Cell. Res.,* 1950, v1, 382.

11. Potter, V. R., Recknagel, R. O., and Hurlbert, R. B., *Fed. Proc.,* 1951, v10, 646.

12. DeDuve, C., Berthet, J., Berthet, L., and Apelmaus, F., *Nature,* 1951, v167, 389.

13. Beer, C. T., Dickens, F., and Pearson, J., *Biochem. J.,* 1951, v48, 222.

14. Dounce, A. L., and Beyer, G. T., *J. Biol. Chem.,* 1948, v174, 859.

15. Siekevitz, P., and Potter, V. R., *ibid.,* 1953, v201, 1.

16. Kennedy, E. P., and Lehninger, A. L., *ibid,* 1948, v172, 847.

17. ———, *ibid.,* 1948, v179, 957.

18. Leloir, L. F., and Munoz, J. M., *ibid.,* 1944, v153, 53.

19. Lehninger, A. L., *ibid.,* 1945, v157, 363.

20. Grafflin, A. L., and Green, D. E., *ibid.,* 1948, v176, 95.

21. Fiske, C. H., and Subbarow, Y., *ibid.,* 1925, v66, 375.

22. ———, *ibid.,* 1929, v81, 629.

23. Racker, E., *J. Biol. Chem.,* 1947, v167, 843.

24. Kielley, W. W., and Kielley, R. K., *J. Biol. Chem.,* 1953, v200, 213.

Received November 20, 1953. P.S.E.B.M., 1953, v84.