

absent. Accidental coincidences between laboratory and solar wavelengths can easily lead to spurious identifications, but if technetium exists in nature, the identifications tentatively suggested in Table 2 do not seem unreasonable.

The longest-lived Tc isotope known to date has a very short half-life, so far as elements found in the sun are concerned. The half-life of  $^{99}\text{Tc}$  produced at Oak Ridge National Laboratory by irradiating Mo with neutrons is  $9.4 \times 10^5$  years (4). A later determination from the Argonne National Laboratory is  $2.12 \times 10^5$  years (5). If a longer-lived isotope having a half-life of the order of  $4 \times 10^8$  years or more does not exist in nature, the suggested solar identifications are subject to serious doubt. The possibility that Mo can be transmuted into Tc in the sun is an interesting speculation. Lines of Mo I and Mo II are known to be present, although not conspicuous, in the solar spectrum. These considerations raise the interesting question as to whether Tc is as rare in the earth's crust as has been previously supposed. It appears that the possibility is not ruled out that the half-life of  $^{97}\text{Tc}$  or  $^{98}\text{Tc}$  may exceed  $10^6$  years (6).

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## A Simple, Inexpensive Microhomogenizer

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Quantitative assays of enzyme activity in tissue homogenates require that the respective homogenates be uniform as to completeness of cell lysis. The ordinary Pyrex glass homogenizer, consisting of an outer tube and a fitted, motor-driven pestle, has proved unsatisfactory in this respect in micro-analytical experiments involving many separate homogenizations. This is due primarily to the fact that the closely ground surfaces rapidly wear away, increasing the clearance and rendering the shearing action progressively less efficient. When one deals with minute amounts of tissue, these differences in the degree of cellular disintegration, however slight, may be quite significant, inasmuch as they are reflected as relatively large differences in enzyme activity, when the latter are projected against the small weights of the tissue assayed. The problem is more acute, of course, with tissues such as prostate, muscle, and

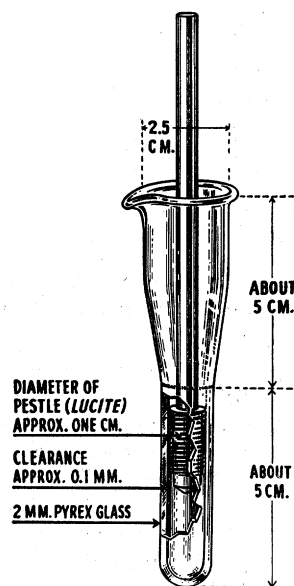


FIG. 1.

uterus than with more friable cellular tissues like liver, kidney, or spleen. Nevertheless, even in the latter variety, wear becomes an important factor when many homogenizations have to be done.

Early in 1948 efforts were made to develop a micro-homogenizer, to be used primarily for enzyme studies on rodent prostate.<sup>1</sup> Several varieties of all-Pyrex apparatus were either purchased or constructed in the laboratory, but all proved unsatisfactory because of rapid wear of the shearing surfaces after 10–20 homogenizations. Also, as has been pointed out elsewhere (1), the presence of powdered glass in the homogenates prevented the use of dry weights for reference. A homogenizer was finally constructed of a glass homogenizing chamber and a Lucite pestle (Fig. 1).

A thick-walled (2-mm) Pyrex test tube is used, approximately 10 cm long, with an inside diameter of 1 cm. The upper 5 cm are cut away and replaced by a glass cup 5 cm in length, which is fashioned from a Pyrex test tube measuring 2.5 cm in diameter. The pestle is made from  $\frac{1}{2}$ -in. Lucite stock. It is easily machined to fit snugly into the test tube and measures 4.5–5.0 cm in length. The upper end is threaded onto a 6-mm aluminum rod about 12 cm long. The pestle is then ground into its final form with fine carborundum powder, using a slow stirring motor. The grinding is judged satisfactory when the dry tube drops slowly off the stationary pestle, indicating a clearance of about 0.1 mm.

The original homogenizer has been in active use since its construction three years ago. There has been no apparent change in the amount of clearance between the shearing surfaces or in the efficiency of homogenization. This is in marked contrast to the

<sup>1</sup> The author wishes to express his thanks to Charles Tesar and A. G. Morrow, of the Johns Hopkins Hospital, for their help in this work.

rapid deterioration of all-Pyrex homogenizers because of the abrasive action of the surfaces on each other. The original pestle was designed in the manner described above because it was anticipated that it would wear away while the tube remained intact; it was therefore threaded onto a metal rod from which it could easily be unscrewed when worn, and replaced by an identical pestle. In actual fact, however, it has demonstrated surprising endurance, and there is thus no reason why pestle and rod cannot be machined in one piece from Lucite.

Homogenates made with the original apparatus have remained uniformly acellular, microscopically. Reproducibility of assays of enzyme activity has been excellent. The time required for complete homogenization is somewhat longer than that of a new, all-Pyrex homogenizer, but it remains approximately constant, whereas the latter suffers a progressive loss of efficiency. For cellular tissues like liver, complete homogenization of 100 mg in 2 ml water at 500 rpm can be effected in 15 sec; prostate or uterus require 40–45 sec. In our work we have used wet weights for reference most often, but there is no objection to the use of dry weights in view of the absence of formation of powdered glass.

The homogenizer is ideally suited for minute amounts of tissue, such as cell colonies growing in culture. Here, where tissue weights are impracticable to measure, we have referred our results to mg total nitrogen.

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## Autoxidation in Lactating Mammary Gland Tissue<sup>1</sup>

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In the course of study of fatty acid oxidase in lactating guinea pig mammary gland tissue a phenomenon has arisen similar to the oxidative system described by Ellman and McLaren (1) for frozen poultry adipose tissue and erroneously called lipoxidase. The system described by Munoz and Leloir (2) and Lehninger (3) for liver, and by Grafflin and Green (4) for kidney, which oxidizes fatty acids, cannot be demonstrated to be in operation in mammary gland tissue under the conditions used by these investigators when fumarate or malate is added as the "sparking" cooxidant. However, if such a system is allowed to incubate for several hours (4–8 for various tissue preparations) at 37.5° C, a period of rapid oxidation is initiated, which continues at a steady rate

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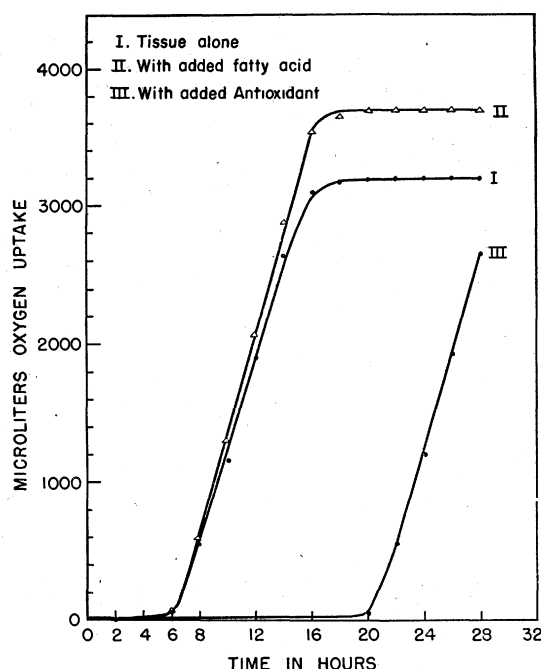


FIG. 1. The system contained the following in final concentration: 1.0% tissue homogenate, 0.002 M  $MgSO_4$ , 0.001 M Na ATP, 0.01 M phosphate buffer, pH 7.4; also, some flasks contained 1.5  $\mu M$  fatty acid (octanoate, laurate, palmitate, or stearate as the sodium salt); others contained an antioxidant (pyrogallol, hydroquinone, or thymol) in a final concentration of 0.001 M. Water was added to make a final volume of 3 ml.

for an additional 10–12 hr. The later phases of this oxidation are prolonged and intensified by the addition of numerous fatty acids. A definite similarity can thus be noted between this oxidative system in mammary gland tissue and that of Ellman and McLaren in adipose tissue. This is not surprising in view of the relatively large fat-tissue content of the mamma.

In the opinion of the authors the oxidative capacity of these tissue preparations does not indicate an enzymatically controlled oxidation and especially not the fatty acid oxidase system. Fatty acid oxidase has been demonstrated by numerous investigators (2–4) to be a very labile enzyme system which will not withstand freezing and thawing, storage, or long-time incubation. Also, these authors and others (5) have shown that to demonstrate the presence of a fatty acid oxidase, tissue preparations must be prepared in an isotonic medium and provided with some member of the citric acid cycle as a "sparking" reaction. None of these criteria was fulfilled by the system of Ellman and McLaren.

The question then arises as to the proper interpretation of this oxidation observed in adipose tissue (1) and mammary gland tissue. There is ample evidence from this laboratory to indicate that the reaction is simply autoxidation. Using standard Warburg procedures and mammary gland tissue homogenates, and measuring the oxygen uptake continuously for 22–30 hr, the curve shown in Fig. 1 was obtained. The kinetics of this curve correspond closely to those