Summary. 1. Differences in the vitamin requirements of susceptible strains of *S. mitis*, their artificially-induced resistant variants and a naturally-resistant *S. fecalis*, have been determined. 2. The greatest difference was in the PGA requirements, the *mitis* strains grew in its absence; the resistant mutants required PGA. 3. The induced resistance of the trained *S. mitis* strains appears to be the same as that of the natural resistance of *S. fecalis*, and may be due to the loss of ability to synthesize PGA, the synthesis of which is inhibited by sulfonamides.

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Effect of Method of Homogenization of Beef Muscle Tissue on Activity of Succinic Dehydrogenase System.*† (18070)

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In the present studies, application of the method for determining the succinic dehydrogenase activity of rat tissues(1,2) to studies of the activity of this system in beef muscle was investigated. In previous work the rat muscle tissue usually has been prepared for assay in a Potter-Elvehjem homogenizer. Since the 1-2 g samples used in this procedure would not be sufficient for a representative sample of beef muscle tissue, alternative methods of tissue preparation were investigated. The results obtained with the use of the Waring blendor for varying periods of time, for tissue minced either with scissors or with a Latapie mincer prior to homogenization in the Waring blendor, the use of the Potter-Elvehjem homogenizer, and grinding with sand as techniques for preparing beef and rat tissues to determine succinic dehydrogenase activity are reported in this paper.

Experimental. The method as described by Schneider and Potter(1,2) was used for the manometric determination of succinic dehydrogenase activity. The results are expressed as μl O_2 uptake per hour per 100 mg wet weight of beef muscle or per mg dry weight of rat tissue.

For beef muscle, 10% homogenates of 5 g or 10 g samples were prepared in the Waring blendor (500 ml capacity), and 3 levels of tissue were assayed for each homogenate. Low temperatures were maintained during the homogenization with ice water, and the use of a Varitran afforded constant speed of homogenization. For the first experiments, a 5-minute homogenization period was used with a 5 g sample which was minced with a scissors before homogenization. Although the values for 3 levels of tissue from one homogenate checked well, difficulties of reproducing results with separate homogenates were encountered. The use of larger samples gave no improvement in reproducibility.

Shorter homogenization periods were investigated and were found to result in better reproducibility and higher values. For 3 separate samples of *Longissimus dorsi* and *Semitendinosus* muscle, a 2-minute period was compared to a 5-minute period and the values averaged 145 and 57 μl of O_2 uptake per 100 mg of fresh tissue per hour, respectively. Further studies were undertaken to compare three homogenization times, namely

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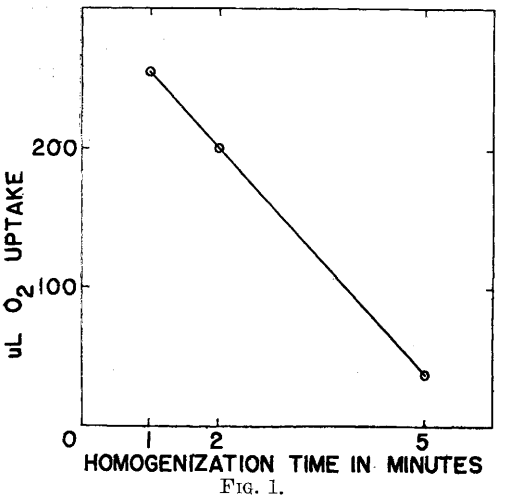

FIG. 1.

Effect of homogenizing beef muscle in the Waring blendor for varying periods of time on the succinic dehydrogenase activity (expressed as O_2 uptake per 100 mg wet weight per hour).

1, 2, and 5-minute periods. Representative results, obtained for several samples of beef round, are shown in Fig. 1. It will be seen that some decrease in activity occurred when the beef muscle was homogenized for 2-minutes as compared to 1 minute and a striking decrease was observed after homogenizing for 5 minutes in the Waring blendor. The 5-minute period was then compared to a 1-minute period using 10 g samples, since it was thought that the increase in volume of homogenate which resulted when the sample size was doubled would decrease the possibility of inactivation of the enzyme system by aeration during homogenization. For 8 *Longissimus dorsi* muscles of beef, the oxygen uptake for 1-minute homogenates averaged 153, and the oxygen uptake for 5-minute homogenates averaged 36. Similar data were obtained for 3 *Semitendinosus* muscles with an average of 164 for the 1-minute period and 42 for the 5-minute period, or a ratio of approximately 4:1.

Scissor minces were not homogenized in the Waring blendor for periods shorter than 1 minute because of inadequate tissue fragmentation and difficulties of sampling the preparation. A finer mince was obtained with the Latapie mincer. This mince was not fine enough for a uniform suspension without further treatment; therefore homogenizing for ½, 1, and 1½-minutes in the Waring blendor was investigated. Considerable loss in activity was noted for homogenization periods that exceeded ½ minute for the 3 samples of beef muscle studied. For example, values of 157, 84, and 46 were observed with the Latapie mince which had been homogenized in the Waring blendor for ½, 1, and 1½ minutes, respectively; while for a 1-minute Waring blendor treatment of a scissor mince of the same sample, the value was 156. Similar results were obtained in other experiments. It was concluded that a 1-minute homogenization (Waring) of the scissor mince was as effective in retaining succinic dehydrogenase activity as homogenizing the Latapie mince in the Waring blendor for ½ minute. Since the homogenization of beef muscle scissor mince for 1 minute in a Waring blendor was the most satisfactory method studied, it was desirable to compare the results for rat tissues obtained with this method with those obtained with the Potter-Elvehjem homogenizer and grinding in sand.

Homogenates of rat tissues were prepared with the Waring blendor for 1, 2, or 5-minute periods and the data obtained compared with those obtained with the Potter-Elvehjem homogenizer and sand grinding technics. Two gram samples of rat muscle were taken, minced with a scissors and homogenized as described. Two per cent homogenates were prepared in all cases. The results are presented in Fig. 2 (2-7 analyses were made for

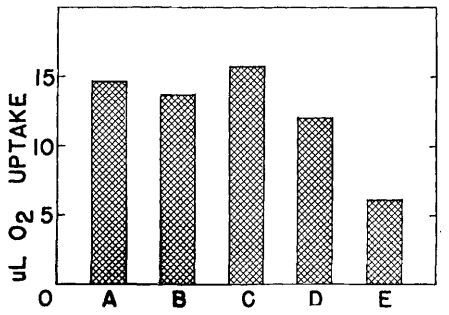

FIG. 2.

Succinic hydrogenase activity of rat muscle per mg dry weight per hour determined after different methods of homogenization of the tissue. A. Ground in sand. B. Potter-Elvehjem homogenizer. C. Homogenized 1 minute in the Waring blendor. D. Homogenized 2 minutes in the Waring blendor. E. Homogenized 5 minutes in the Waring blendor.

each sample treatment). Slightly higher values were obtained with the Waring blendor when a homogenization time of 1 minute was used, as compared to the values obtained by the Potter-Elvehjem homogenizer or grinding with sand. The 1-minute Waring homogenates had higher succinic dehydrogenase activity than 2 and 5-minute homogenates which is in agreement with the data for beef muscle.

Parallel experiments were conducted with rat liver by the use of 1 g samples for homogenization and it was found that the sand-ground and Potter-Elvehjem homogenized samples had somewhat greater succinic dehydrogenase activity than 1-minute Waring homogenates, 63 μl O_2 uptake per mg dry weight per hour, as compared to 45. This may be explained by the fact that liver is easily fragmented and aeration and inactivation probably occur more rapidly during homogenization than during similar treatment of muscle tissue. Longer homogenization (Waring) periods resulted in a decrease in succinic dehydrogenase activity.

Discussion. Quinlan-Watson and Dewey (3) reported considerable inactivation of cytochrome oxidase of guinea pig liver after 1-minute homogenization in the Waring blendor at room temperature. As noted in the present studies, the loss in succinic dehydrogenase activity of rat liver occurred more rapidly than in muscle tissue. Cytochrome c oxidase is present in large excess of succinic dehydrogenase in tissues and some inactivation of cytochome oxidase may not affect the activity of the succinic dehydrogenase system as assayed in the present experiments(1).

The inactivation of wheat germ enzymes by 2 and 5-minute Waring blendor treatment was cited by Stern and Bird(4) since sand-ground suspensions retained more enzymatic activity. In our studies the sand-ground suspensions of rat muscle did not have higher succinic dehydrogenase activity than 1-minute Waring blendor homogenates.

For demonstrating the presence of the enzyme system in tissues, or the necessary components of the system, the use of technics which result in some inactivation of the enzyme, such as homogenization for 2 or 5 minutes with the Waring blendor would probably not negate the validity of the findings. However, for studying the activity and stability of the system in muscle tissue on a quantitative basis, investigations on the stability of the enzyme system during homogenization would appear to be a necessary prerequisite.

Summary. The succinic dehydrogenase activity of beef muscle determined after different methods of homogenization of the tissue was investigated. The amount of beef muscle needed for a representative sample was too large to be conveniently used with the Potter-Elvehjem homogenizer; however, the samples could be prepared for assay by homogenizing in a Waring blendor, provided that the homogenization time is held to a minimum. This was evidenced from studies which showed that homogenizing the minced beef tissues in a Waring blendor for 1 minute or homogenizing Latapie minces for ½ minute resulted in a greater succinic dehydrogenase activity than that obtained when the tissues were homogenized for longer periods. Other experiments with rat tissues showed that the enzyme activity of rat muscle homogenized in the Waring blendor for 1 minute, in a Potter-Elvehjem homogenizer, or ground with sand was essentially the same.

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