was somewhat greater in the injected rats being 5.3 g/100 g body weight as compared with 4.6 g/100 g body weight in controls.

Summary. The steroid Δ^1 desoxycorticosterone acetate will maintain adrenalectomized immature rats for 20 days with dosages of 0.25 mg and 0.5 mg and will increase daily body weight 1 and 2 g respectively. Activity is approximately 1/8 to 1/10 that of desoxycorticosterone acetate. Liver water content was unchanged in maintained adrenalectomized rats and the relative liver protein content of adrenalectomized rats receiving Δ^1 desoxycorticosterone acetate was comparable to that of normal rats. This steroid did not alter organ weights in normal immature female rats over a 10-day period except for a tendency toward liver weight increase.

Received July 17, 1950. P.S.E.B.M., 1950, v74.

Growth Requirements of *Streptococcus mitis* and Sulfonamide Resistance.* (18069)

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Previous work in our laboratory showed that trained sulfathiazole-resistant strains of *Streptococcus mitis* differed considerably in other biochemical properties from the original strains(1,2). The resistant strains acquired the ability to grow on 40% bile agar, in 6.5% NaCl, in .1% M.B. milk, and in the presence of increased concentrations of 4 antibiotics. These properties are characteristic of enterococci(3), and were shown by a known strain of *Streptococcus fecalis*, No. 9790, obtained from the American Type Culture Collection.

The growth requirements of an organism are fundamental properties sometimes suggesting a possible mechanism for their response to bacteriostatic agents. Since we had observed that the resistant strains grew readily in a medium containing no riboflavin (Bacto Riboflavin Assay Medium), while the parent strains did not, we have investigated the vitamin requirements of several parent and resistant strains of *S. mitis* and of *S. fecalis*, No. 9790. This paper reports the results of that study.

Experimental. Semi-synthetic medium. This was a modification of the medium used by Smiley, Niven, and Sherman in their study of the growth requirements of *S. salivarius*(4). It was prepared in two stages, Part A being autoclaved prior to addition of Part B. Each batch of medium, upon the addition of the vitamins, was filtered through a Seitz filter.

Organisms used. Five strains of streptococci showing the biochemical characteristics of *Streptococcus mitis*, isolated in our labora-

PART A

Casein hydrolysate, 10% solution*	50 ml
DL-Tryptophane	60 mg
Dipotassium phosphate	6.0 g
Glucose	5.0 g
Sodium thioglycollate	100 mg
" chloride	2.0 g
Magnesium sulfate, cryst.	80 mg
Ferrous sulfate, cryst.	4 mg
Manganese chloride	1.2 mg
Uracil	10 mg
Water to	1,000 ml
pH 7.5	

* Vitamin-free casein hydrolysate (acid) 10% solution prepared by Nutritional Biochemicals Corp., Cleveland, Ohio.

* A portion of the data presented in this paper was taken from a thesis submitted in June, 1950, by Virginia M. Harrison to the University of Colorado in partial fulfillment of the requirements for the degree Master of Science.

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PART B

	Amt per 250 ml of above
Thiamine hydrochloride	100 gamma
Pyridoxine hydrochloride	100 "
Calcium pantothenate	100 "
Riboflavin	100 "
Nicotinic acid	100 "
Biotin	1 "
Pteroylglutamic acid	1 "
p-aminobenzoic acid	10 "
Xanthopterin	1 "

tory, and these strains trained to sulfathiazole resistance, were used. Training was accomplished by repeated transfer in tryptose phosphate broth containing gradually increasing concentrations of sodium sulfathiazole. In addition another *mitis* strain maintained as a stock culture and *S. fecalis* No. 9790 were included. The letter S designates the trained sulfathiazole resistant strains.

Procedure. Tubes containing 5 ml of the medium with all the vitamins or with one of 8 vitamins omitted, were inoculated with 1 drop of a twice-washed suspension from a 24-hour culture in tryptose phosphate broth, made up in saline to a turbidity matching a No. 5 McFarland standard. All tubes were incubated at 37° for 48 hours, after which time the cultures were transferred to Klett-Summerson colorimeter tubes and the turbidity determined. Positive controls contained the complete medium inoculated with the organism under investigation. Blanks containing the uninoculated medium were measured for turbidity and this value subtracted from those tubes showing growth. The percentage of growth achieved in a vitamin-deficient medium was calculated by the following formula:

$$\% \text{ of growth} = \frac{\text{Growth in vitamin-deficient medium—Blank}}{\text{Growth in complete medium—Blank}}$$

Averages of the readings of 2 identical tubes were used to determine each value. The determinations were repeated 3 times for each organism and each vitamin.

Inhibitory concentrations of sulfathiazole were determined by inoculating tubes of the complete medium containing 10, 1, .1, .01, .001, .0001, .00001, .000001, and 0 mg per ml of sulfathiazole. One drop of a 24-hour broth culture was used to inoculate and the reading was made at the appearance of growth in the control tubes. The concentration of sulfathiazole which prevented discernible turbidity was called the inhibiting concentration.

Results and discussion. The inhibiting concentrations of sulfathiazole in the semi-synthetic medium of the 12 strains studied are shown in Table I. All the trained strains are at least 1,000 times as resistant as the parent strains. The concentration necessary for inhibition of these resistant strains is the same as that required for *S. fecalis* No. 9790.

Table II is a summary of the percentage of growth achieved by 5 organisms in the absence of one of 8 vitamins as compared to their growth in the complete medium. The values represent the average of 3 separate determinations, each of which was performed in duplicate. A value which shows less growth in the deficient medium showed this decrease in all 3 determinations. Individual values never varied more than 15% and usually much less.

The 3 *mitis* strains showed no appreciable growth in the absence of calcium pantothenate and nicotinic acid. All were inhibited by the absence of PABA, pyridoxine, and biotin, while 2 of the 3 strains required riboflavin and one thiamine for complete growth. All 3 strains achieved maximum growth in the absence of pteroyl glutamic acid (PGA). The growth response of the trained resistant strain 6 S to all of the vitamins is the same as that for *S. fecalis* No. 9790. Omitting thiamine, riboflavin, and PABA had no effect on the growth of these sulfathiazole resistant strains. Incomplete growth was observed in

TABLE I.
Inhibitory Concentration of Sulfathiazole Expressed as mg/ml.

Organism	Sensitive parent	Resistant mutant
1*	0.0001	1.0
4	0.0001	1.0
6	0.0001	1.0
17	0.0001	1.0
34	0.001	1.0
B	0.0001	—
9790	—	1.0

* 9790 = *S. fecalis;* all others = *S. mitis.*

TABLE II.
Comparison of Percentages of Full Growth Achieved by Each Organism in the Vitamin-deficient Media.

Organism	No thiamine, %	No riboflavin, %	No PABA %	No calcium pantothenate, %	No nicotinic acid, %	No pyridoxine HCl, %	No biotin, %	No PGA, %
34	100.0	23.7	67.5	0	0	27.4	37.7	97.6
B	58.9	26.1	47.6	3.3	0	32.8	47.6	95.2
6	87.1	85.4	67.9	2.6	2.8	33.7	35.4	97.2
6S	97.1	95.5	97.3	1.4	28.3	61.8	34.7	0
9790	97.1	96.5	98.7	1.8	25.8	61.8	44.8	0

Organisms 6, 34 and B = *S. mitis.*
6S = Trained resistant strain from 6.
9790 = *S. fecalis.*

the absence of nicotinic acid, pyridoxine, and biotin, and no growth in the absence of calcium pantothenate, quite like the response shown by the *mitis* strains to these vitamins. The growth response to PGA, however, was quite different than that shown by the *mitis* strains. The strains of *S. mitis* do not need PGA, while the trained resistant strain and the strain of *S. fecalis* do. Niven and Sherman (5) have reported nicotinic acid, pyridoxine, biotin, and pantothenate as necessary to growth for 5 of 5 strains of *S. fecalis* studied, and folic acid necessary for 3 of the 5 strains.

To test the possibility that this loss of ability to synthesize PGA was a property common to other trained sulfathiazole resistant strains of *S. mitis*, 4 more strains and their resistant mutants were examined for their PGA requirements. The results, summarized in Table III, show that these additional strains responded in the same manner

TABLE III.
Comparison of the Percentages of Full Growth Achieved by Each Organism in PGA-deficient Media.

Organism	No PGA, %
1*	98.1
1S	5.3
4	100.0
4S	5.6
17	100.0
17S	5.9
34	100.0
34S	16.3

* Organisms 1, 4, 17, and 34 = *S. mitis.*
Organisms 1S, 4S, 17S, and 34S = trained resistant strains.

as did the first strains tested. It was interesting that a variant of strain 34, trained to only 10 times the resistance of the parent strain, showed the same responses to PGA as the parent strain; but when the resistance was carried on to 1,000 times, this new strain required PGA, as shown in the table.

Lampen and Jones(6) demonstrated that *L. casei* and *S. fecalis R.* require PGA for growth, and that they were virtually unaffected by high concentrations of sulfadiazine. They concluded that sulfonamides block the synthesis of PGA from PABA. Nimmo-Smith, Lascelles, and Woods(7) further substantiated this hypothesis by showing that resting cell suspensions of *Streptobacterium plantarum* synthesized folic acid, and this synthesis was inhibited by sulfonamides. Our data would indicate that susceptible *S. mitis* strains, trained to sulfonamide resistance, become resistant because the resistant strains no longer synthesize PGA, but can utilize it preformed from the medium. The synthesis but not the utilization is blocked by sulfonamides. While this is not a new mechanism, this is the first time, to our knowledge, that it has been shown to be associated with trained sulfa-resistant strains of streptococcus. It is also rather interesting that all of the properties of the resistant strains that we have investigated, including the growth response to vitamins, have been entirely the same as those for *S. fecalis.*

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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.

Received July 21, 1950. P.S.E.B.M., 1950, v74.

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

* This work was supported in part by a contract with The Production and Marketing Administration, U. S. Department of Agriculture, under the Research and Marketing Act.

† Journal Paper No. 27, American Meat Institute Foundation.

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