reactions of destruction and synthesis.

Glucose was autoclaved under alkaline conditions to form a substance with very slight activity when tested alone on the unheated medium (Fig. 2). A 4 mg supplement/10 ml for instance, furnished a net culture turbidity of only 0.07 O.D. units. However, in the presence of this 4 mg supplement of autoclaved glucose, the growth response curve for GG, which without supplementation deviated from the yeast extract curve, became parallel to that of yeast extract. Evidence for synergisms between autoclaved glucose and low levels of GG (unheated) was striking. Toxicity at high levels of GG was again noted.

Discussion. The need for heat activation of the culture media employed for lactic acid bacteria was discovered by Orla-Jensen(8). Heat activation was found to involve substances like glucose, methylglyoxal, furfural, arabinose and xylose with the remainder of the culture medium, autolyzed yeast or even alkaline tap water. Snell *et al.*(9) have reported that pyruvate could replace the substance formed by heating the culture medium for *L. bulgaricus*. Under the present conditions however, pyruvate was totally without effect for *L. gayoni*. This suggests that the heat activation problem may be much more complex than is even indicated in these papers.

The possibility that these two postulated growth factors may be the ultimate solution to the "gayoni factor" is favored by the fact that the heated GG curve and the unheated GG plus heated glucose curve were parallel to the yeast extract curves (semi-log scale). However, definite assurance of the microbiological equivalence of these factors to yeast extract can not yet be given because of the superior total growth that is possible at high concentrations of yeast extract. The inadequacy of GG plus heated glucose may be due to toxic substances, to a marginal deficiency of some component of the culture medium, to other growth factors, or other more active forms of existing factors.

Summary. 1. For rapid initiation of growth of *L. gayoni*, the culture medium required heating. 2. This heat activation reaction, involving glucose, salts A and glycine, appeared to proceed through an intermediate, N-D-glucosylglycine, and then into at least two heat stable growth factors. 3. One of these factors could also be derived by heating glucose under alkaline conditions.

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Oxidative Dissimilation in Pantothenate-Deficient *Acetobacter suboxydans* Cells.* (20047)

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Work has been focused in this laboratory on the metabolism of *Acetobacter suboxydans*,

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because this organism preferentially uses conjugated forms of pantothenic acid [a pantothenic acid conjugate (PAC(1); coenzyme A (2,3)] over the free vitamin. Attempts to localize the action of pantothenic acid by checking reactions which are known to be coenzyme A dependent(4,5) were unsuccessful. Although hot water extracts of *A. suboxydans* cells contained coenzyme A, oxidation of acetate or any of the Krebs cycle intermediates failed. The organism was unable to form citrate or to acetylate sulfanilamide, even in the presence of external sources of coenzyme A and adenosine triphosphate.

However, preliminary experiments on the oxidation of glycerol revealed that oxygen consumption beyond one atom per molecule was occasionally accelerated by the addition of crude enzyme A to the well-washed resting cells. The results were not consistent, perhaps due to the difficulty of removing coenzyme A by the washing procedure, and the oxidation of various substrates was therefore studied in pantothenate-deficient cells. The present paper reports the retardation of some of these oxidations during pantothenate deficiency.

Experimental. A strain of *A. suboxydans,* ATCC No. 621 was used for the experiments. *Materials.* PAC(1), in a crude state containing some coenzyme A, was used as the pantothenic acid source for cell growth. This concentrate was used in preference to the free vitamin since the growth response was more nearly a linear function of the added nutrilite when these conjugates were used(1). A coenzyme A preparation of 75% purity, obtained from the Pabst Laboratories, was employed in the oxidative studies. *Pantothenic acid analysis.* This was carried out with *Lactobacillus arabinosus*(7) after enzymatic digestion(8). *Preparation of cells. A. suboxydans* cells were grown on 3 different levels of PAC in a synthetic medium described earlier(6). The inoculum was grown in 1500 ml of the medium with aeration for 43 hours. The cells were washed twice with sterile saline, and then transferred in equal amounts to three 5-gallon jars, each containing 8 liters of medium. To the mixtures (labeled A, B and C), the PAC-coenzyme A preparation was added at concentrations of 0.6, 5, and 50 γ pantothenic acid equivalent per liter of medium. These concentrations in a small scale preliminary experiment gave respectively 20, 67, and 100% of the maximum turbidity of culture. The jars were incubated for 48 hours with heavy aeration, at summer temperatures (25-30°C). After centrifugation and 3 washings (one in 0.067 M potassium phosphate buffer of pH 6.0, two in distilled water), the cells were dried *in vacuo* from the frozen state. They were then investigated for their dissimilation of various substrates by standard Warburg manometric technics.

Results and discussion. The yield of dry cells for samples A, B, and C were respectively 0.5, 1.1, and 0.9 g. Testing of the cells showed that cells C were not appreciably different from B in their oxidative capacity for either ethanol, glycerol or sorbitol. The experiments described below were therefore carried out only with the cells from the 2 lower levels of pantothenate, namely samples A and B.

As shown in Fig. 1, *alcohol was oxidized* rapidly by both cells A and B, with no significant difference in rate. The oxidation ceased after 80 minutes, at an oxygen consumption of 95% of the theoretical. The alcohol oxidation was thus independent of changes in the pantothenate content of the cells under the conditions used.

For *oxidation of glucose* the velocities also were the same for the two preparations, up to a consumption of one atom of oxygen per molecule substrate. At that point, however, the oxidation in the deficient cells leveled off sharply. With sorbitol, the initial oxidation rate was lower in the deficient cells, and the process virtually ceased when one atom of oxygen had been consumed. Sorbose was not oxidized when supplied as the exogenous substrate (data not shown in Fig. 1). These results indicated that coenzyme A was needed for the second step of glucose or sorbitol dissimilation, and possibly in the first step of sorbitol oxidation.

Similar behavior was exhibited toward *glycerol.* The reaction velocities were the same for cells A and B up to one atom of oxygen consumed per molecule of substrate.

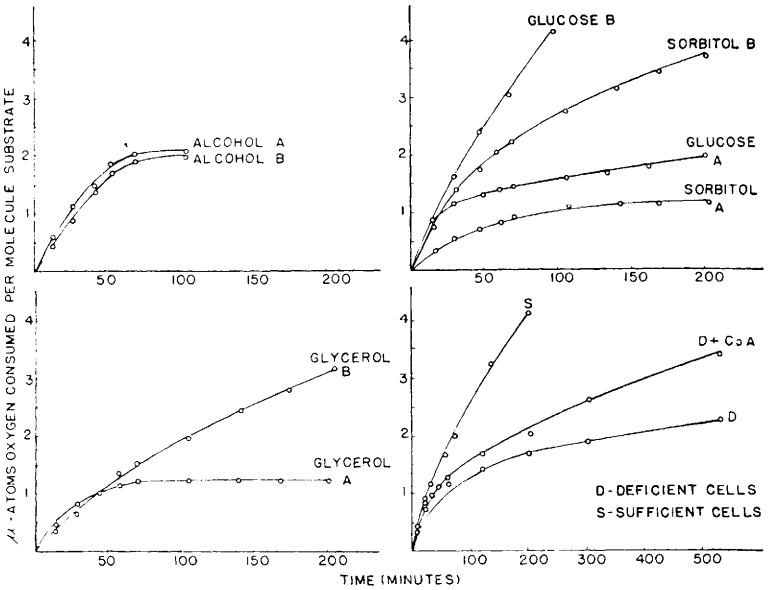

FIG. 1. Effect of pantothenate deficiency on oxidations in resting cells of *A. suboxydans*. Each vessel contained 20 μ moles sorbitol or glucose, or 40 micromoles of glycerol or alcohol; .05 M Sörensen's phosphate buffer at pH 6, .01 M $MgCl_2$, .3 ml of .5% diphosphopyridine nucleotide, and .2 ml of 20% KOH. 10 mg (dry wt) of *A. suboxydans* cells were used, and water added to 3 ml. The fourth group of curves (lower right) show the effect of coenzyme A on glycerol oxidation by resting *A. suboxydans* cells. Cells D (pantothenate deficient) contained 6.6 γ calcium pantothenate/g; cells S (pantothenate sufficient) contained 27 γ/g. Supplements were added as described in Fig. 1.

The rate of oxygen uptake by cells A then diminished sharply, whereas cells B proceeded to consume oxygen rapidly for several hours. This indicated an inability on the part of the pantothenate-deficient cells to oxidize dihydroxyacetone to any significant extent. A separate experiment with dihydroxyacetone as substrate was then conducted and confirmed this finding.

The fourth group of curves in Fig. 1 demonstrate the effect of exogenous coenzyme A upon glycerol oxidation, by a second sample of pantothenate-deficient cells. Although these cells possessed somewhat greater capacity for dihydroxyacetone oxidation than those described in the foregoing paragraphs, the addition of 500 γ coenzyme A per flask more than doubled the rate of oxygen consumption; for the first atom of oxygen consumed, the rate increase was 130%. It thus appears that the dissimilation of dihydroxyacetone in pantothenate-deficient cells is coenzyme A-linked, although it has not yet been possible to prepare a coenzyme A-dependent apoenzyme from the dihydroxyacetone oxidase. Further experiments are in progress to investigate these reactions in cell free systems.

Summary. 1. Resting cells of *A. suboxydans*, grown with deficient levels of pantothenic acid, were investigated for their ability to oxidize a series of substrates. These cells showed retarded ability to oxidize dihydroxyacetone, sorbose, and gluconic acid, and somewhat poorer ability to convert sorbitol to sorbose. 2. Addition of coenzyme A to the deficient cells significantly improved their ability to oxidize dihydroxyacetone, thus suggesting that the further dissimilation of this substrate is coenzyme A-linked. 3. The oxidation of alcohol, of glycerol to dihydroxyacetone, and of glucose to gluconic acid proceeded with equal velocity in deficient and normal cells, indicating non-involvement of coenzyme A in these reactions.

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Factors Associated with the Incidence of Infantile Diarrhea in Mice.* (20048)

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Infantile diarrhea in man and other mammals is classified as an infectious disease but searches for common, specific agents have failed. Etiology of infantile diarrhea in mice has been attributed to a Salmonella microorganism in one instance(1) and a virus in another instance(2). Both reports further indicate that a variety of factors such as temperature, diet, season, infection, and physiological condition of parents and offspring may, singly or cumulatively, be influential in determining the severity of an epidemic. Infantile diarrhea is prevalent in many colonies of experimental mice and presents a serious handicap for standardizing experimental animals.

Methods and materials. A colony of C3H mice was observed for symptoms of diarrhea in suckling animals between September 14, 1949 and January 17, 1950. More than 900 animals were born during this period of which almost 30% displayed severe diarrhea before they were 18 days old. Symptoms of diarrhea varied from a mild condition recognizable only by the finding of fecal material adhering to the under surface of the tail, to the passage of large amounts of yellowish, watery, feces. The intensity of the disease varied within litters, but the onset of symptoms usually occurred between 8 and 14 days. The mice were kept under conditions typical in our laboratory, *i.e.,* 75°F, exhaust ventilation and water and Purina Fox Checkers *ad libitum.* Clean dry pens of wood were provided on Tuesday of each week. Individual pairs of breeders were kept together at all times. Notation concerning the presence of diarrhea was made on the parent's pedigree card. The subsequent analysis of data was made on the basis of litters as units. Correlations were made (a) between litters born just before and just after clean pens were supplied (b) between litters born to females of different ages (c) between litters of different parity (d) between litters of different size and (e) between litters born in 3 successive 5-week periods. Significance of differences in these correlations was tested by the χ^2 test for homogeneity.

Results. Data were obtained from 76 pairs of C3H mice and 900 young born to them during the period of 4 months (Table I). Thirty-seven of the pairs gave birth to 62 litters, including 350 young, none of which showed appreciable symptoms of diarrhea. The remaining 39 pairs had 92 litters, which included 550 young, of which 46 litters (50%) were discarded due to severe diarrhea. The overall incidence was about 30% of the young born. Although the incidence of diarrhea remains high the colony of animals still prospers. The following tabulations and cor-

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