combination in Table II. Variations occurred from series to series but the relative results within a given series were the same for 5 different trials. Such variations are likely to occur in sub-optimal media and are probably related to the vigor of the organism in a given inoculum and its ability to overcome an unfavorable environment.

Summary. The amino acid requirement of *Lactobacillus casei* was determined on a medium containing glucose, sodium acetate, salts, riboflavin, pantothenic acid, nicotinic acid, biotin, pyridoxine, adenine, and concentrates of the eluate factor (folic acid). The simplest amino acid mixture that would yield maximum growth on repeated subculture was: leucine, serine, phenylalanine, glutamic acid, valine, aspartic acid, cystine, arginine, tryptophane, tyrosine threonine, methionine, alanine, isoleucine, lysine, and histidine.

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Effect of Salt and Substrate Concentration upon Activity of Adenosinetriphosphatase.*

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Although the procedure used by some workers for determining adenosinetriphosphatase (ATP-ase) activity[1,2] is adequate for many purposes, such a measurement of the increase in inorganic phosphate at a single time is not necessarily proportional to the enzyme activity. The present studies were undertaken to determine under what conditions the initial rate of formation of inorganic phosphate would be linear, or could be taken as proportional to the enzyme concentration. In the course of the study, it also became evident that the activity of the enzyme was markedly affected by the salt concentration. These observations will also be reported.

Materials. The adenosinetriphosphate (ATP) was prepared from rabbit muscle by the method of Kerr.[3] The dibarium salt of ATP was precipitated twice from water, and was dried over P_2O_5. Although the amount of organic phosphorus was close to the theoretical value, the labile phosphate (readily hydrolyzed with 1 N acid at 100°, and corresponding to 2 moles of phosphate per mole of ATP) indicated that the material was only about 60% pure. The concentrations of ATP given are calculated from the labile phosphate.

For use in the experiments, a weighed amount of the dibarium salt was dissolved in 0.1N HCl and the barium precipitated by the addition of a slight excess of solid Na_2SO_4. The barium sulfate was removed by centrifugation or filtration, the solution neutralized with NaOH, and made up to volume.

The ATP-ase was prepared from rat muscle by the procedure commonly used for the preparation of myosin. Muscle from the hind limb was frozen in dry ice, ground in a Wiley mill, and extracted with 0.5 N KCl. The pH was kept in the neighborhood of 7 to 7.5 during the extraction by the addition of $NaHCO_3$. The extract was centrifuged at a speed of 10,000 RPM in an angle centrifuge. The pH of the supernatant was adjusted to 6.5 with acetic acid, and 7 volumes of cold water added. The myosin precipitate was centrifuged off and washed once with cold water. The myosin was then dissolved with sufficient KCl to make the concentration 0.5 M. The precipitation was repeated twice more, and the myosin was kept in 0.5 M KCl

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1 Needham, D. M., *Biochem. J.*, 1942, **36**, 113.

2 Bailey, Kenneth, *Biochem. J.*, 1942, **36**, 121.

3 Kerr, Stanley E., *J. Biol. Chem.*, 1941, **139**, 121.

till used. All of the steps in the myosin preparation were carried out in the cold room.

Methods. The measurements were made by adding all of the components of the solution except the enzyme and adjusting the

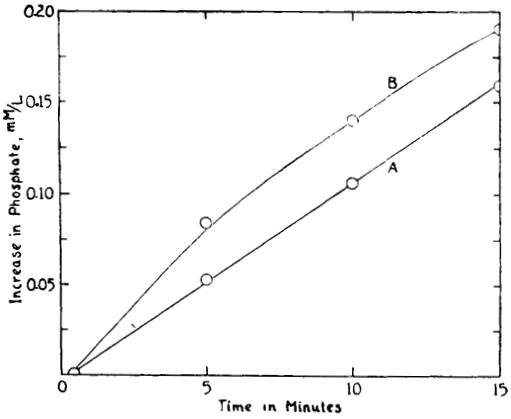

FIG. 1.

Initial rate of increase of inorganic phosphate. 0.45 mM ATP, pH 7.35. Curve A, 0.1 M glycine, 0.123 g myosin per liter. Curve B, no glycine, 0.205 g myosin per liter.

volume with water so that the final volume, after the addition of the myosin, would be 10 ml. The experiments were carried out in test tubes in a water bath at 30 ± 1°C. A sample was taken 20 to 30 sec after the addition of the enzyme, and at intervals of 5, 10 and 15 min. In each case a 2 ml sample was removed with a pipette and delivered into 2 ml of 10% trichloracetic acid. The concentration of inorganic phosphate was determined by the method of Bodansky,[4] using a Beckmann spectrophotometer.

In all cases the solutions contained 0.001 M $CaCl_2$, which has been shown to be necessary for optimum activity of the enzyme.[2] The buffers which were used were diethylbarbituric acid, $NaHCO_3$ and Na_2CO_3, or glycine.

Influence of ATP concentration. Bailey's results show that, within the proper range of concentrations, the increase in inorganic phosphate in 5 min is proportional to the myosin concentration.[2] We have confirmed this finding, but were more particularly interested in determining within what range of concentration the rate of formation of inorganic phosphate would be independent of the ATP concentration.

In this connection it may be pointed out that the evidence from the shapes of the curves is not conclusive. Fig. 1 gives the results of 2 typical experiments in which the ATP concentration was 0.45 mM. Although the inorganic phosphate increases in a linear manner with time in the solution containing glycine, the increase is not linear in the absence of glycine.

Experiments were therefore carried out in which the initial rate of formation of inorganic phosphate was determined when the ATP concentration was varied. The results of measurements at pH 7.35, in the absence of glycine are given in Fig. 2. It is seen that the rate appears to vary with the ATP concentration, but not by an amount comparable with the changes in ATP concentration. In this particular set of experiments, the con-

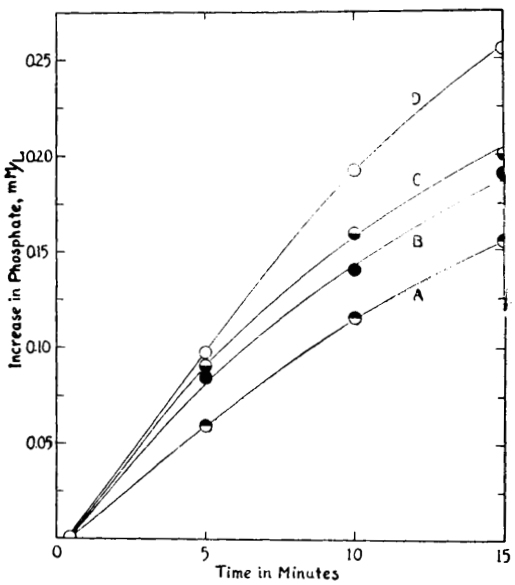

FIG. 2.

Effect of ATP concentration. 0.205 g myosin per liter, pH 7.35, no glycine. ATP concentrations 0.23, 0.45, 0.90, and 1.35 mM in curves A, B, C, and D.

centrations of the stock solutions necessitated a smaller salt concentration as the ATP concentration was increased. We had not anticipated a marked effect of salt as long as sufficient salt was present to keep the myosin in solution, but subsequent experiments in-

[4] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197.

dicated a rather marked effect.

Effect of salt concentration. Experiments were carried out at pH 6.6 and pH 9.1, both without glycine and with 0.1 M glycine. The ATP concentration was 0.45 mM in all cases. The salt concentration was varied by changing the concentration of KCl. The results were calculated as activities relative to an activity of 1 at a total salt concentration of 0.55 M. It will be seen in Fig. 3 that at pH

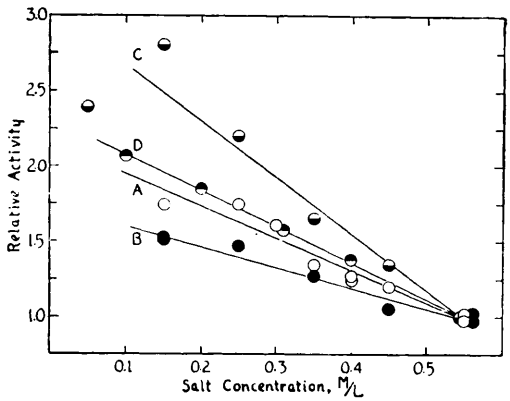

FIG. 3.

Effect of salt concentration. Curve A, no glycine, 0.45 mM ATP, pH 9.1; Curve B, 0.1 M glycine, 0.45 mM ATP, pH 9.1; Curve C, no glycine, 0.45 mM ATP, pH 6.6; Curve D, 0.1 M glycine, 0.45 mM ATP pH 6.6; Activity relative to unit activity at a total salt concentration of 0.55 M.

6.6, in the absence of glycine (curve C), the activity increases about 2.5 times as the salt concentration is decreased from 0.5 M to 0.15 M. In the presence of 0.1 M glycine (curve D), the effect is somewhat less marked. At pH 9.1 the effect is less than at the lower pH, but the effect of glycine (curve B) is again to reduce the effect of salt below that in the absence of glycine (curve A).

It is also worth noting that there is an obvious precipitation of myosin at the lower salt concentrations and pH, but this does not appear to reduce the rate of splitting of phosphate in these experiments.

In Table I are given the results of the experiments in which the ATP concentration was varied, and for which the initial rate of formation of phosphate is corrected for the salt concentration. In all cases the rate has been calculated for a total concentration of 0.5 M salt, except that the concentration of sodium glycinate has not been taken into account. Although the accuracy of the results leaves much to be desired, they do not give any indication that the initial rate is increased by increasing the ATP concentration above 0.23 mM, when the myosin (or ATP-ase) concentration is in the neighborhood of 0.1 to 0.2 g per liter.

Summary. Measurement of the activity of ATP-ase in salt concentrations from 0.15 to 0.55 M show that the activity is decreased as the KCl concentration increases. The magnitude of the effect depends upon the pH and the presence or absence of glycine. The rate of formation of inorganic phosphate is also found to be independent of the substrate concentration when the ATP concentration is 0.23 mM per liter or greater. Observed deviations from a linear rate of formation of phosphate at the higher ATP concentrations must be attributed to inactivation of the enzyme during the course of the experiment.

TABLE I.
Effect of ATP Concentration.

Myosin g/L	ATP mM/L	Glycine M/L	KCl M/L	NaCl M/L	Buffer M/L	Total salt M/L	pH	γ P per hour	γ P per hour, corr.
.205	.23	0	.35	.05	Barbiturate, 0.005	.40	7.35	255	210
”	.45	0	.30	”	”	.35	”	336	242
”	.90	0	.20	”	”	.25	”	300	220
”	1.35	0	.05	”	”	.10	”	390	194
.123	.23	0.1	.25	.05	”	.30	7.25	210	155
”	.45	”	.20	”	”	.25	”	210	146
”	.90	”	.10	”	”	.15	”	264	163
.123	.23	0.1	.50	0	Glycine	.50	9.15	378	378
”	.45	”	”	0	”	”	”	437	437
”	.90	”	”	0	”	”	”	384	384