antiserum disclosed the presence only of Tamm-Horsfall mucoprotein. Thus, the purification had not completely removed Tamm-Horsfall mucoprotein and no other component was detectable. The purified material contained 5.9% N (Dumas) and 18% hexuronic acid; the theoretical values for pure chondroitin sulfate are 2.7% and 37.3% respectively.

No hexuronic acid was found in experiments *b* to *d*.

In the first experiment, the persistent presence of Tamm-Horsfall mucoprotein suggested that it is complexed with the acid mucopolysaccharides, and analogous to chondromucoprotein. The absence of hexuronic acid from the materials examined in experiments *b* to *d* demonstrates that Tamm-Horsfall mucoprotein in urinary CTAB precipitates, is actually co-precipitated, none being bound to urinary acid mucopolysaccharides.

Summary. Tamm-Horsfall mucoprotein, found with urinary acid mucopolysaccharides prepared by precipitation with cethyltrimethylammonium bromide, is present adventitiously as a coprecipitated impurity rather than as bound moiety. The nature of any actual proteinaceous moiety is unknown. None is immunologically detectable with antisera to proteins of human serum or urine.

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Growth Requirements of *Saccharomyces cerevisiae* as a Result of Incubation at Increased Temperature.* (28752)

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An earlier report from this laboratory(1) showed that *Saccharomyces cerevisiae* (Fleischmann strain 139) grew poorly or not at all in a chemically defined medium at 38°C while growth was excellent in the same medium at 30°C. Addition of yeast extract to the defined medium permitted growth at the increased temperature of incubation, as reported also by Sherman(2). Other workers (3-6) have associated specific substances as required growth factors at increased incubation temperatures, and further efforts on our part demonstrated that pantothenic acid could largely replace the effect of yeast extract. Pantoic acid also was found to be effective but only at concentrations 100 to 10,000 times as great as pantothenate.

The present paper considers the growth requirements of a mutant isolated from the original Fleischmann strain of *S. cerevisiae*.

Materials and methods. The experimental procedure was described previously(1). Inoculum per tube was 0.05 ml of a washed cell suspension having a turbidity of 30 in a Klett-Summerson photoelectric colorimeter (660 mμ) using water as a blank.

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TABLE I. Response of *Saccharomyces cerevisiae* to Different Nutritional Environments at 30°C and 38°C.

Additions*					*Saccharomyces cerevisiae*, strain 139-2		139-15	
Pantothenate†	Vitamins‡	Casamino acids§	Purines + pyrimidines‖	Yeast extract¶	Turbidity (24 hr) 30°C	38°C	30°C	38°C
—	—	—	—	—	250	29	224	10
+	—	—	—	—	249	138	227	8
—	+	—	—	—	249	35	233	7
+	+	—	—	—	252	165	235	7
—	—	+	—	—	37	62	21	15
+	—	+	—	—	324	228	268	210
—	—	—	+	—	267	28	240	11
+	—	—	+	—	260	119	232	11
—	+	+	—	—	11	7	13	5
+	+	+	—	—	284	236	249	212
—	+	—	+	—	262	35	246	11
+	+	—	+	—	261	168	246	9
—	—	+	+	—	46	27	13	7
+	—	+	+	—	335	234	268	210
—	+	+	+	—	12	12	8	7
+	+	+	+	—	292	250	262	232
—	—	—	—	+	345	258	283	216

* 0.5 ml added to 2.0 ml double strength synthetic medium; final volume of 5.0 ml by addition of water.

† 10^0 μmoles/ml.

‡ 200 μg each of PABA, niacin, riboflavin and thiamine, 400 μg pyridoxine, and 100 μg folic acid/ml.

§ A 10.0% solution of Norite A-purified, Difco Vitamin-Free Casamino Acids.

‖ 50 μg each of adenine, uracil, guanine, and xanthine/ml.

¶ 0.1 g/ml.

S. cerevisiae 139-2 and 139-15 were derived from isolated colonies obtained by spreading a diluted suspension of the stock culture of *S. cerevisiae* (Fleischmann strain 139) on synthetic medium agar and incubating for 24 hours at 30°C.

The 10.0% casamino acids solution was prepared by further purifying Difco Vitamin-Free Casamino Acids with Norite A. The 10.0% 18 known amino acids was compounded on the basis of the amino acid composition of casein given by Tristram(7).

Results. Several groups of substances were tested, with and without pantothenate, for their ability to permit growth of the 2 strains of *S. cerevisiae* at 38°C. The results (Table I) revealed that addition of pantothenate to the defined medium supported growth of strain 139-2 at the elevated temperature, but that yeast extract or pantothenate plus casamino acids were required for growth of strain 139-15. In view of its response to pantothenate, as well as other experimental observations, it is felt that strain 139-2 represents the original Fleischmann 139 strain. Strain 139-15 represents the mutant organism since there appears to be a nutritional requirement in addition to the need for pantothenate at increased incubation temperatures.

After determining that a mixture of 18 known amino acids could replace the 10.0% solution of casamino acids, work was undertaken to ascertain the specific amino acid(s) required by the mutant at 38°C. The 18 amino acids were divided into several groups and each group was tested with and without pantothenate at the same concentration at which it appeared in the mixture of 18 known amino acids. However, no group showed any capacity to permit growth recovery of strain 139-15 in presence of pantothenate at the increased temperature of incubation. Since this might have been due to the fact that more than one amino acid was needed and inadvertently the amino acids required were in different groups, the groups were tested in combinations. The results of these tests were also largely negative. However, it was noted that with certain combinations in the presence of pantothenate a significant degree of re-

TABLE II. Response of *Saccharomyces cerevisiae* (Strain 139-15) to Amino Acids and Sodium Chloride at 30°C and 38°C.

Additions*		Incub. temp		
Pantothenate	Others	30°C	38°C	% growth recovery
—	——	209†	14	6.7
+	——	216	51	23.6
—	18 known AA	8	7	——
+	"	268	208	77.6
—	Alanine	11	7	——
+	"	220	116	52.7
—	Glycine	50	15	——
+	"	182	66	36.3
—	Lysine	236	19	8.0
+	"	202	137	67.8
—	Proline	232	15	6.5
+	"	232	145	62.5
—	Serine	33	16	——
+	"	240	160	66.7
—	Threonine	30	15	——
+	"	224	133	59.4
—	NaCl	212	26	12.3
+	"	212	148	69.8

* Pantothenate, 10^{-1} μmoles/ml, 0.5 ml/tube. Double strength synthetic medium (without aspartate), 2.0 ml/tube. Aqueous aliquots containing 0.42 mmoles of test substance. Final volume of 5.0 ml/tube by addition of water.

† Turbidity after 25 hr of incubation.

covery was effected. Closer inspection suggested that recovery of growth was almost invariably associated with groups which afforded the highest absolute concentration; *i.e.*, the groups which contributed the largest amounts to the final solute concentration in an experimental tube. On this basis it seemed reasonable to assume that any one of several amino acids individually could promote the growth of strain 139-15 in the presence of pantothenate at 38°C provided that the concentration was equivalent to that of the addition of 18 known amino acids (0.42 mmoles/tube) and barring toxic effects of the amino acid itself. In addition, if it was mainly a matter of concentration, sodium chloride might also be active.

The data in Table II show that several different amino acids and sodium chloride were capable of permitting the growth of strain 139-15 at 38°C in the presence of pantothenate to a significant degree when the concentration was equivalent to that of the 10.0% solution of 18 known amino acids.

Further experiments (Table III) demonstrated that in addition to the compounds already noted at least one other inorganic substance, ammonium sulfate, several sugars, both utilizable (sucrose) and non-utilizable (lactose, mannitol, rhamnose, and xylose), and citrate were capable of mitigating the effect of increased incubation temperature on *S. cerevisiae* strain 139-15. Moreover, it is clear that the effect of solute concentration is a graded one and that increased growth recovery corresponds to increasing solute concentration. The fact that rhamnose and xylose did not permit as great a recovery as did lactose and mannitol may indicate some condition of metabolic interference at the high concentrations used.

Tween 20 and Tween 80 were tested in concentrations from 0.01 to 100 mg/tube. These substances gave maximum recoveries of about 22% and 25% respectively at a concentration of 0.5 mg and 5.0 mg/tube respectively. Higher or lower concentrations gave less response. Results with sodium desoxycholate indicated that this compound was capable of exercising some degree of growth recovery. However, there are inherent difficulties in working with this substance at the

TABLE III. Growth Recovery in *Saccharomyces cerevisiae* (Strain 139-15) in Presence of Several Different Substances and Pantothenate.

	mmoles added/tube				
Additions†	.6	.4	.2	.02	.01
18 known AA	—	78.9*	—	—	—
Alanine	67.7	52.2	34.4	16.0	17.2
Glycine	51 6	46.6	29.0	19.2	16.2
Serine	52.8	50.8	41.4	28.4	25.2
Sucrose	53.9	42.2	21.0	8.0	11.1
Lactose	56.6	40.6	26.2	12.8	17.4
Mannitol	57.8	43.3	31.8	17.6	14.6
Rhamnose	25.9	19.6	12.4	5.5	5.2
Xylose	29.5	25.8	19.6	10.4	10.7
Citrate	79.4	53.4	21.7	7.6	5.6
NaCl	70.8	57.6	43.2	14.2	14.4
$(NH_4)_2SO_4$	91.2	84.0	44.7	16.7	13.2

* Percentages represent avg of 2 separate experiments except for "18 known amino acids" which is the avg of 4 experiments.

† A proper aliquot containing the amount of substance cited plus pantothenate (0.5 ml of a 10^{-1} μmoles/ml solution) plus 2.0 ml of double strength synthetic medium (without aspartate). Final volume was 5.0 ml by addition of water.

Growth determined turbidimetrically after approximately 24 hr of incubation.

pH of the synthetic medium (4.0) due to its marked insolubility in acid solutions. Thus, while growth recoveries in the order of 44% were noted, precise concentrations cannot be given.

Since the effect of high solute concentration on cell morphology is well known, some attention was directed toward microscopic studies of the microorganisms grown under various conditions of nutrition and temperature. No marked changes were observed by phase microscopy of wet mounts of either strain under any conditions of growth. Subtle differences were noted, however, particularly with strain 139-15 at 38°C. Additions of pantothenate and/or high concentrations of amino acids or sodium chloride appeared to remove or at least reduce the slight but noticeable changes apparently induced by the increased temperature. This was somewhat more consistent for strain 139-2 than for strain 139-15. The changes at 38°C consisted of apparent reduction in size of the large intracellular vacuoles, cellular disorganization (more granular appearance), and less tendency for buds to break off from the mother cell. The overall size of the cells and the cell walls appeared to remain relatively normal regardless of the condition of growth. Similar observations have been reported by Kuraishi(8).

A variety of staining techniques applied to both strains harvested from synthetic medium after growth at 30°C or 38°C with or without pantothenate essentially confirmed the observations already noted. Cell wall stains gave no indication of changes having taken place in that structure regardless of strain, temperature, or presence or absence of pantothenate.

Staining techniques for polar bodies or metachromatic granules (Laybourn stain, Ljubinsky stain) gave evidence of cell anomalies precipitated by increased incubation temperature. Again the effect of pantothenate appeared to be to reduce if not remove these irregularities. The full import of these observations is not presently understood, but there appears to be a correlation between the physiological and cytological manifestations of the temperature effects.

Discussion. The results of experiments designed to elucidate the nature of the additional requirements of a mutant derived from *S. cerevisiae* (Fleischmann strain 139) revealed that high concentrations of several chemicals would permit growth in the presence of pantothenate at increased incubation temperature. The fact that ammonium sulfate replaces the mixture of amino acids demonstrates that organic nitrogen is not required. Furthermore, since addition of substances such as sodium chloride, lactose, and mannitol at appropriate concentrations serves to permit growth of the mutant at 38°C it is clear that the supplement need not be metabolically utilizable. It does not appear to be necessary that the substance be ionized since several carbohydrates function well in allowing growth recovery of the mutant. Finally, although ammonium sulfate and sucrose can be utilized by this organism, the concentrations involved suggest a role beyond that of simple nutritional substrates.

A common denominator that might explain the heterogeneity of the substances that serve to permit growth of the mutant is osmotic pressure. This is in consonance with the observations of Ritchie and Jacobsohn(9). The superior effectiveness of ionized substances (sodium chloride, ammonium sulfate, citrate) as compared to non-ionized substances is perhaps due to their greater effect on the osmotic pressure.

The mechanism by which the increased osmotic pressure functions to permit growth of the mutant strain at the increased temperature of incubation is not presently known. A possible explanation for the observed phenomenon is that the permeability of the mutant has been altered. Kovac(10) has noted that sodium desoxycholate affects the permeability of yeast, increasing at higher temperatures, and enhanced by lowering the pH and by increasing ionic strength. In view of this observation the 44% recovery of growth at the increased temperature of incubation made possible by the desoxycholate supplement takes on added meaning. Another possibility is that the effect of the higher osmotic pressure might be exercised on the

membrane(s) of one or more of the intracellular particles.

Finally, other aspects merit consideration with respect to the requirement for high solute concentration. For example, the function could be to stabilize cellular deoxyribonucleic acid, ribonucleic acid or protein(s). Anomalies might exist in certain structural proteins of the mutant which predispose them to greater lability at increased temperature of incubation. This may be the cause of the cellular disorganization observed microscopically and might also explain the partial if not complete remission which ensues on addition of pantothenate and/or amino acids and sodium chloride. It is pertinent that Kuraishi(8) has observed that the production rate of dead cells due to pantothenate deficiency is decreased in the presence of 0.2 M sodium chloride or 0.1 M sodium sulfate.

Summary. Data were presented which revealed that the paucity of growth of a mutant strain of yeast at 38°C is not only related to an inability to carry out the biosynthesis of pantothenic acid but also the requirement for an increased osmotic pressure or perhaps simply a high solute concentration. Results were discussed in terms of possible mechanisms by which these environmental conditions might serve to permit growth of the mutant at increased incubation temperatures. Since at lower temperatures the organism is genetically capable of synthesizing pantothenic acid, the role of environment in genetic expression is reemphasized.

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L Phase Variants Related to Antibiotic Inhibition of Cell Wall Biosynthesis.* (28753)

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It is generally accepted that certain antimicrobial agents inhibit cell wall biosynthesis. Biochemical demonstration of this action by penicillin and D-cycloserine has been reported by Strominger(1,2) who has measured the accumulation of cell wall precursors in different bacteria exposed to these antibiotics. Parallel studies have shown that bacitracin, novobiocin, and gentian violet also interfere with cell wall synthesis(1). Jordan(3) has demonstrated the inhibition of cell wall mucopeptide production by vancomycin. Biologic evidence for a similar action can be obtained by observing protoplast formation and/or L phase growth when selected bacteria are exposed to antimicrobial agents under appropriate environmental conditions. If L phase growth occurs, it has been assumed that only cell wall synthesis is interrupted. Penicillin and D-cycloserine are 2 antibiotics which are known to induce L phase growth,

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