biotics, and if so, whether such treatment would significantly reduce the mortality.

Korol(10) obtained equivocal results in one experiment in which mice poisoned with nitrogen mustard were treated with streptomycin, but the treatment was not continued beyond the 4th day. In one unpublished experiment Korol(11) found a slight reduction in mortality in mice treated for 12 days with 7000 μg of streptomycin a day.

While the development of bacteremia may hasten the death of animals poisoned with nitrogen mustard, it seems doubtful that its importance as the cause of death is as great as it appears to be after moderate doses of total body x-radiation.

Summary. 1. Nine to 10 weeks old female mice, averaging 27 g in weight were injected intraperitoneally with 0.2 mg (approximately 7 mg per kilo = LD_{90}) of nitrogen mustard (Methyl bis (β-chloroethyl) hydrochloride). Cultures of heart's blood and spleen of mice sacrificed for that purpose were made at daily intervals. 2. Bacteremia was found to be present in over $\frac{1}{3}$ of the mice on the 5th, 6th and 7th days. A number of additional mice on these days (6-17%) showed positive cultures of spleen only. The species of bacteria recovered from blood and/or spleen were all members of the normal intestinal flora, with the exception of *Corynebacterium pseudotuberculosis murium*. 3. Generalized infection of enteric origin seems to play a less important role as a cause of death in mice poisoned with nitrogen mustard than in mice subjected to moderate doses of total body x-radiation.

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Growth Stimulation of *Lactobacillus gayoni* by N-D-Glucosylglycine.* (20046)

DEXTER ROGERS, TSOO E. KING, AND VERNON H. CHELDELIN.
(Introduced by R. Wulzen.)
From the Department of Chemistry and the Science Research Institute, Oregon State College, Corvallis.

Early studies on the nutrition of *Lactobacillus gayoni*(1) provided evidence for a "gayoni factor", inasmuch as this organism could not be cultured on existing synthetic media unless supplemented by liver or yeast fractions. Subsequent experiments(2,3) showed that yeast nucleotides and unusually high levels of pteroylglutamic acid were required in addition to the active principle of liver or yeast. Recently, it has been found possible to replace in part the nutrients in liver or yeast by glutamine or asparagine, although maximum growth could not be obtained(4).

The growth of *L. gayoni* also has been found to be dependent upon heating the complete

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TABLE I. *L. gayoni* Response to Asparagine and Yeast Extract as Influenced by Heating the Culture Medium and by Supplements of Ascorbic Acid.

Test materials	Filter-sterilized medium*		Heat-sterilized medium*	
	Control	Ascorbic acid (4 mg/10 ml)	Control	Ascorbic acid (4 mg/10 ml)
	Growth response (O.D.)†			
None	.08	.10	.12	.16
Asparagine (10 mg/10 ml)	.19	.23	.73	.89
Yeast extr. (50 mg/10 ml)	.79	1.02	1.17	1.24

* Culture medium minus asparagine and ascorbic acid. Heat sterilization—10 min. at 120°C 50 mg of yeast extr. = ca. .5 mg of asparagine. *L. gayoni* asparagine requirement = .1–.3 mg per 10 ml.

† O.D. (optical density) = 2-log % transmission. Each value is average of 2 determinations. Time—12 hr at 37°C.

culture medium. Heating the components of the medium separately and mixing after cooling or filter sterilizing the medium did not provide for any stimulatory factor. This factor could also be supplied by high levels of a yeast extract.

Procedure. The test organism was strain 45 of *L. gayoni* 8289 which was maintained according to previously described procedures (2,3). The culture medium of Cheldelin and Nygaard(2) was employed with supplements of ammonia-hydrolyzed yeast nucleic acid† (Schwarz) (1 mg/10 ml), L-asparagine • H_2O (1 mg/10 ml), ascorbic acid (4 mg/10 ml), xanthine (0.1 mg/10 ml) and pyridoxal • HCl (10 γ/10 ml). The previously listed concentrations of salts A and Tween 80 were increased 3-fold. Such a medium was complete enough to allow for maximum growth within 20 hours of incubation, even when unheated. However, for a maximal growth rate during 10 to 14 hours of incubation, a large supplement of yeast extract (Difco) (ca. 50 mg/10 ml) was required. When the components of the culture medium were heated separately or in various combinations, each component was adjusted individually to pH 6.8 before heating. When heat sterilization was not applied, the medium and supplements were filter sterilized using either sintered glass or Seitz filters. N-D-*glucosylglycine* ethyl ester was prepared by condensing glucose with glycine ethyl ester according to the procedure of Wolfrom *et al.*(5) (m.p. 106.5-107.5°C;‡ literature m.p. 108°C). Henceforth in this report, this ester will be referred to as GG. GG did not give a positive ninhydrin test under conditions where a positive test was obtained from free glycine. Neither was a positive reaction obtained by the aniline acid phthalate test for reducing sugars(6). Upon heating an aqueous solution of GG, positive tests were obtained for the free amino acid and reducing sugar. By *saponification* of GG with alcoholic KOH, the potassium salt was obtained in quantitative yield. This salt was isolated as a hygroscopic white powder which contained bound amino and reducing groups. When warmed with ninhydrin, the solution "browned" before giving a positive test for the free amino acid. The *alkaline treatment* of GG or glucose consisted of autoclaving 120 mg of material in 50 ml of one per cent potassium phosphate buffer (pH 9.5) for 10 minutes at 120°C. After cooling, the solution was adjusted to pH 6.8 and diluted to 60 ml. In certain experiments as noted, a few of the supplements were omitted from the basal medium and added as test materials.

Results. When the test organism was cultivated in an unheated medium, it did not grow as rapidly as it did when the medium had been heated (Table I). Although ascorbic acid provided some stimulation, especially in the more complete media, the effect of heating the medium was considerably greater.

In experiments which are not being reported here in detail, it was found that omitting the glucose or acid-hydrolyzed casein from the medium during heating reduced the heat activation effect. When only glucose and the

† Employed as a source of the yeast nucleotides.

‡ Uncorrected (Fisher-John block).

TABLE II. *L. gayoni* Response to the Substance Formed by Heating Together Various Components of the Culture Medium.

Components of medium heated together*	Growth response (O.D.)†
0 (all filter-sterilized)	.23
G + S	.27
G + S + casein hydrolysate	.51
glycine	.64
D,L-α-alanine	.12
β-alanine	.42
D,L-valine	.41
D,L-norvaline	.41
D,L-leucine	.34
D,L-isoleucine	.39
D,L-serine	.25
D,L-threonine	.36
L-cystine	.18
D,L-methionine	.22
L-lysine • HCl	.41
L-histidine • HCl	.39
L-arginine • HCl	.39
D,L-aspartic acid	.36
L-glutamic acid	.42
L-tyrosine	.35
D,L-phenylalanine	.30
D,L-tryptophane	.38
L-proline	.29
L-hydroxyproline	.25

* G = glucose (100 mg); S = salts A (.15 ml). All amino acids and casein hydrolysate were tested at 4 mg (except cystine which was tested at 2 mg). Total volume = .4 ml. Heating time—10 min. at 120°C.

† O.D. (optical density) = 2-log % transmission. Time—12 hr at 37°C.

TABLE III. *L. gayoni* Response to Graded Levels of N-D-Glucosylglycine, Ethyl Ester and Potassium Salt.

Test materials (mg/10 ml)		Growth response (O.D.)*
0		.32
Ethyl ester	.01	.35
	.1	.42
	1	.67
	10	.62
	100	.12
K salt	1	.37
	10	.50
	100	.05

* O.D. (optical density) = 2-log % transmission. Each value is average of 3 determinations. Time—13 hr at 37°C.

acid-hydrolyzed casein (previously adjusted to pH 6.8) were heated together, only slight amounts of activity were formed; the presence of salts A (potassium phosphates, pH 6.8) was required in order to obtain the full effect of heat activation. Heating glucose with salts A also resulted in only incomplete activation.

When the amino acids of the acid-hydrolyzed casein were treated individually with glucose and salts A, glycine was found to be the amino acid most effective for activation (Table II). Many of the other amino acids were slightly effective and some were inhibitory. In a subsequent experiment, only α-alanine was found to be inhibitory when heated with glucose, salts A and glycine.

Solutions of glucose, salts A and amino acids were observed to "brown" on heating and the possibility that activation was related to the Maillard reaction(7) was explored. Several intermediates of the "browning" reaction involving glucose and glycine were tested, among them N-D-glycosylglycine, as the ethyl ester and the potassium salt. These materials were found to be active when tested in the unheated medium (Table III), but the potassium salt was only about one per cent as active as the ester. When these materials were heated with the culture medium, the broth containing the potassium salt "browned" more than the broth containing the ester. Perhaps, a free carboxyl group favored destruction of activity.

The GG response curve deviated from that of yeast extract (Fig. 1). With increasing concentrations of GG, the response increased slowly at first, then progressively more rapidly than the yeast extract response. Ultimately, a maximum was reached and higher concentrations of GG inhibited growth. Neglecting these higher levels, where inhibition was evident, the GG curve was reminiscent of curves obtained with deficient media. Several limiting factors appeared to have been increasing simultaneously, or in other words the activity of GG may have represented multiple effects. When GG was autoclaved under alkaline conditions, an overall decrease in activity was observed. However, the response curve then paralleled the yeast extract curve and no inhibitions were observed at the high supplement levels. Subsequent tests have shown that although some activity was always destroyed by heating, prolonged heating (60 minutes instead of the 10 minutes employed here) did not reduce the activity further. These data were interpreted as indicating a

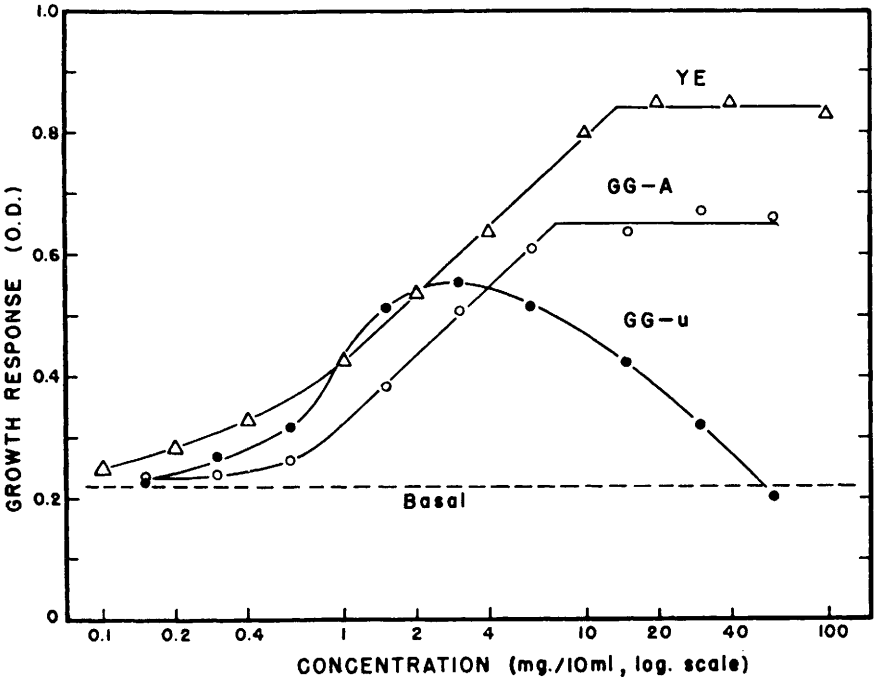

FIG. 1. *L. gayoni* response to graded levels of N-D-glucosylglycine ethyl ester, unheated (GG-U) and autoclaved at pH 9.5 (GG-A), and yeast extract (YE). Time—13.5 hr at 37°. Each point is average of 4 determinations.

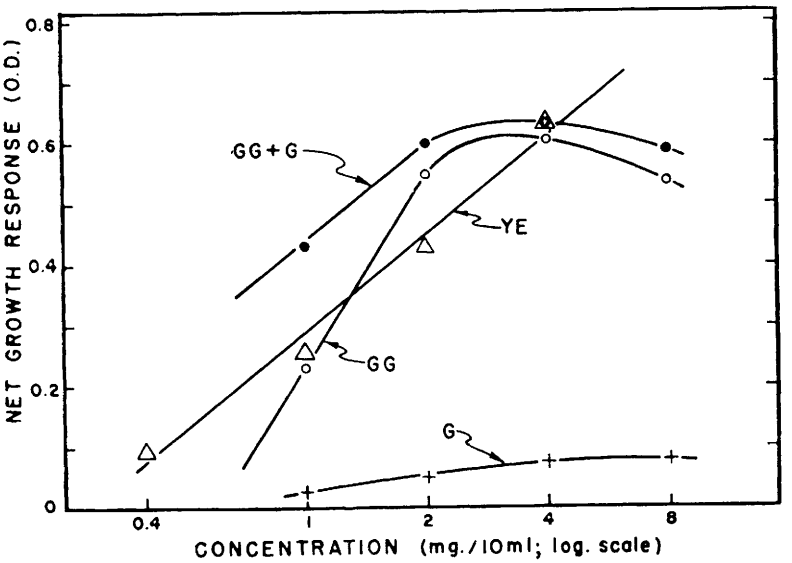

FIG. 2. *L. gayoni* response to graded levels of yeast extract (YE), N-D-glucosylglycine ethyl ester (GG) and glucose (G) autoclaved at pH 9.5. (GG + G) = graded levels of GG with constant amounts of G added to basal medium (4 mg/10 ml). Time—13 hr at 37°. Each point is average of two determinations.

conversion of GG into another, heat stable form, perhaps even more active. The initial loss of activity was apparently due to destruction of GG before it could be converted into the other form, and the total activity was regarded as a net effect of several competing

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)

VERNON H. CHELDELIN, JENS G. HAUGE,† AND TSOO E. KING.
(Introduced by R. Wulzen.)
From the Department of Chemistry and Science Research Institute, Oregon State College, Corvallis.

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† Fellow of the Royal Norwegian Council for Scientific and Industrial Research.