TABLE IV. Histoplasmin-Latex and Histoplasmin-Collodion Agglutination Reactions with Moderately Positive Human Sera.

Specimen No.	Latex Serum dilution 1:5	1:10	1:20	1:40	1:80	1:160	C*	Collodion Serum dilution 1:5	1:10	1:20	1:40	1:80	1:160	C*
31	3	2	2	1	—	—	—	4	4	2	1	—	—	—
32	4	3	2	1	—	—	—	4	4	3	2	1	—	—
33	3	3	2	1	—	—	—	4	4	3	3	2	1	—
34	4	3	2	1	—	—	—	4	3	2	1	—	—	—
35	4	3	3	2	1	—	—	4	4	3	2	1	—	—
36	3	3	2	1	—	—	—	4	3	2	1	—	—	—
37	4	4	2	1	—	—	—	4	4	3	2	1	—	—
38	3	3	2	1	—	—	—	4	4	3	2	1	—	—
39	4	4	4	2	1	—	—	4	3	2	1	—	—	—
40	4	4	4	3	1	—	—	4	3	3	2	1	—	—
41	4	3	2	1	±	—	—	4	3	2	1	—	—	—
42	4	3	2	2	1	—	—	4	4	3	2	1	—	—

* Control—Serum 1:5 and particles without histoplasmin.

Summary. This laboratory has now had 10 years experience with the collodion agglutination test for histoplasmosis(3-9). We use it routinely in screening for active histoplasmosis. The test itself is simple to perform and read. However, smaller laboratories or institutions with lesser demand for serologic studies for histoplasmosis find the preparation of collodion particles too time-consuming for their purposes. Commercially-prepared latex particles show similar results as collodion particles when both are sensitized with histoplasmin. It is suggested from these studies that, as in the collodion agglutination test, a 4+ reaction at 1:5 dilution or higher is compatible with active histoplasmosis. In 4 instances 3+ latex agglutinations were observed in sera showing 4+ collodion tests. Thus, at the present time, 3+ agglutinations with latex are placed in the "suspicious" category. As with collodion agglutinations, 1-2+ reactions are considered negative.

Studies are in progress to evaluate further the use of histoplasmin-latex agglutinations in serologic diagnosis of histoplasmosis.

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Effect of Certain Amino Acids and Peptides on Hyaluronidase Production by *Staphylococcus aureus*.* (23852)

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Rogers(1) devised a semisynthetic medium for production of hyaluronidase by a number of bacteria, including *Staphylococcus aureus*. This medium consisted of acid hydrolyzed

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casein, cystine, tryptophan, thiamin, nicotinic acid, and the necessary salts to keep the medium well-buffered, since hyaluronidase synthesis ceases when pH of culture falls below 6.0. Although *S. aureus* has been cultivated in chemically defined media(2,3), synthesis of hyaluronidase in a completely synthetic medium has not been described. During investigation on synergistic infections produced by concerted action of anaerobic streptococci and *S. aureus* injected into the rabbit skin, it was observed that hyaluronidase containing cell-free filtrates of an *S. aureus* brain heart infusion culture were capable of enhancing anaerobic streptococcal infections(4). To identify and characterize further the factors in this staphylococcal filtrate responsible for this enhancement phenomenon, a synthetic medium was devised for growth and hyaluronidase production by this strain of *S. aureus*. Certain amino acids appear essential for enzyme synthesis but not for growth and certain dipeptides stimulate both growth and hyaluronidase production.

Materials and methods. The strain of *S. aureus*, designated as SAB2, was originally isolated from a human blood culture. This hemolytic, coagulase positive organism was maintained on sheep-blood agar by weekly transfer. Brain heart infusion broth (Difco) and heart infusion broth (Difco) served as reference nonsynthetic media. Good growth of this organism was obtained in a synthetic medium of pH 7.5 containing per 100 ml of medium: L-arginine hydrochloride, 40 mg; L-cystine, 40 mg; DL-valine, 40 mg; L-proline, 20 mg; DL-leucine, 20 mg; DL-phenylalanine, 40 mg; glycine, 20 mg; L-histidine hydrochloride, 40 mg; L-aspartic acid, 20 mg; L-lysine hydrochloride, 40 mg; L-glutamic acid, 10 mg; DL-tryptophan, 40 mg; L-tyrosine, 20 mg; nicotinic acid, 2 μg; thiamin hydrochloride, 2 μg; glucose, 1 g; $MgSO_4 \cdot 7H_2O$, 1 mg; $MnSO_4 \cdot H_2O$, 1 mg; $FeSO_4 \cdot 7H_2O$, 1 mg; K_2HPO_4, 500 mg; KH_2PO_4, 500 mg; and NaCl, 500 mg. This medium was sterilized by filtration through a Selas 02 candle. Solutions of glycyl-L-tryptophan and glycyl-L-tyrosine were sterilized separately by filtration and added to medium to give a concentration of 70 mg/100 ml of medium. In experiments that follow "complete synthetic medium" refers to the chemically defined medium outlined above minus the 2 dipeptides. The adequacy of media for growth and hyaluronidase production was determined by inoculation of 50 ml Erlenmeyer flasks containing 25 ml of media with 0.05 ml of a suspension containing 3 x 10^9 twice-washed cells/ml as determined by a McFarland nephelometer. All cultures were incubated on a rotary shaker at 37°C. The inocula were collected from a 24-hour culture in heart infusion broth. Bacterial growth was estimated on a Klett-Summerson photoelectric colorimeter using red filter No. 66. An uninoculated tube of medium was used to zero the instrument. Hyaluronidase concentrations of culture supernates were determined by the turbidimetric method(5) using potassium hyaluronate isolated from human umbilical cord(4) as substrate.

TABLE I. Effect on Growth and Hyaluronidase Production of Omitting Amino Acids from a Synthetic Medium for *Staphylococcus aureus*.

Amino acids omitted	Growth*	Hyaluronidase (TRU/ml)
None	100	20
Lysine, arginine, cystine	37	0
Glycine, valine, leucine	4	0
Phenylalanine, tyrosine, tryptophan	89	0
Aspartic acid, glutamic acid, histidine, proline	7	0
Phenylalanine	112	16
Tyrosine	108	0
Tryptophan	82	0
Control: Heart infusion broth	233	40

* Expressed as % of turbidity of 24-hr culture in complete synthetic medium. 100% approximates colorimeter reading of 240.

Results. *Staphylococcus aureus*, strain SAB2, synthesized 40-50 turbidity reducing units (TRU) of hyaluronidase/ml of culture when grown 24 hours in either brain heart infusion or heart infusion medium. Although only one-half this amount of enzyme was produced in the complete synthetic medium (16-20 TRU), there was a similar reduction in bacterial growth (Table I).

By omission of amino acids in groups, as recommended by Lederberg(6), it was found (Table I) that certain groups appeared essential for optimal growth with the exception of

TABLE II. Effect of Peptides and Varying Concentrations of Amino Acids on Growth and Hyaluronidase Production by *Staphylococcus aureus*.

Medium	Growth*	Hyaluronidase (TRU/ml)
Complete synthetic, 1× full complement of amino acids	100	16
Complete synthetic + glycyl-L-tyrosine and glycyl-L-tryptophan	198	32
Complete synthetic, 2× tyrosine, tryptophan and glycine	126	16
Complete synthetic, 2× full complement of amino acids	148	20

* Expressed as % of turbidity of 24-hr culture in complete synthetic medium.

phenylalanine, tyrosine, and tryptophan. The simultaneous elimination of these amino acids, however, abolished hyaluronidase production. In the absence of each of these 3 amino acids singly, growth was comparable in all cases to that in the complete synthetic medium, but enzyme production could not be detected in the absence of tyrosine or tryptophan. Omission of phenylalanine alone, however, had no effect on hyaluronidase production by this organism. Although a moderate amount of growth took place in the medium lacking lysine, arginine, and cystine, hyaluronidase could not be detected in this culture. It seems quite possible that small quantities of hyaluronidase were present (<0.5TRU) but that the method of assay is not sensitive enough to determine these lower concentrations with certainty.

Omission of single amino acids demonstrated that glycine was indispensable for growth of this organism and that valine and leucine, although not completely essential for growth, were highly stimulatory. With the knowledge that glycine was essential for growth, additions to the synthetic medium of dipeptides containing glycine and the 2 amino acids essential for hyaluronidase production were tested for their possible stimulatory effect on growth and hyaluronidase production. The effect of the addition of these peptides to the synthetic medium on growth and hyaluronidase production was compared with the response observed in the complete synthetic medium, the complete synthetic medium containing twice the usual concentrations of the amino acids glycine, tyrosine, and tryptophan, and the complete synthetic medium containing twice the usual concentrations of all amino acids (Table II). Although growth was stimulated in most cases by increase in free amino acids, the greatest enhancement of growth and hyaluronidase production took place in the synthetic medium supplemented with the dipeptides. A further experiment demonstrated that addition of either peptide alone resulted in the same stimulation of growth and enzyme production.

By sampling a continuous culture at various intervals of time, it was shown (Table III) that hyaluronidase synthesis and growth have a considerable lag period in the synthetic medium as compared with brain heart infusion. In the synthetic medium, bacterial turbidity was first detected at about 3 hours and it appears that enzyme production did not begin until the 7th hour and then increased as growth developed. Both growth and hyaluronidase production were detected in supernates from the nonsynthetic medium after a shorter lag period (3 hours) and both increased considerably in the next 24 hours. It seems reasonable to assume that hyaluronidase production reaches a limiting peak between 12 and 24 hours since samples taken after 24 hours failed to detect an increase in hyaluronidase in either medium.

Discussion. Lower production of hyaluroni-

TABLE III. Hyaluronidase Production at Various Time Intervals during a Continuous Culture Experiment.

	Complete synthetic + peptides*		Brain heart infusion*	
Time, hr	Growth†	Hyaluronidase, TRU/ml	Growth†	Hyaluronidase, TRU/ml
1	0	0	0	0
2	8	0	18	0
3	12	0	128	4
4	12	0	204	32
5	—	—	264	32
6	28	0	300	48
7	32	0.5	345	—
11	135	8	415	48
12	200	20	428	48
24	345	38	545	48

* 250 ml Erlenmeyer flask containing 100 ml media incubated on rotary shaker at 37°C.
† Colorimeter reading.

dase by *S. aureus* in a chemically defined medium than in nonsynthetic media demonstrated the necessity for additional nutritional factors for optimal production of this enzyme. Rogers(1) has reported similar results. The same situation has been reported for production of other enzymes such as lecithinase by *Clostridium perfringens*(7). Tyrosine, phenylalanine, and tryptophan were dispensable for optimal growth of *S. aureus*, strain SAB2, in the synthetic medium used. This result agrees with previous work demonstrating that these 3 amino acids are dispensable for growth of a number of strains of staphylococci(3). However, this organism required tryptophan and tyrosine for synthesis of hyaluronidase. Glycine was completely essential for growth. Furthermore, an increase in growth and hyaluronidase production was noted when the synthetic medium was supplemented with either glycyl-L-tryptophan or glycyl-L-tyrosine. This may indicate that the peptides enhance growth and enzyme production by supplying the essential amino acids in a more utilizable form(8).

Summary. *Staphylococcus aureus*, strain SAB2, synthesized hyaluronidase in a chemically defined medium only in the presence of tyrosine and tryptophan, even though these amino acids were not required for optimal growth. Glycine was indispensable for growth of this organism. Supplementation of the synthetic medium with glycyl-L-tyrosine or glycyl-L-tryptophan enhanced both growth and hyaluronidase production.

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Liver Ketogenesis: Role of Glucose-Cyclo-Acetoacetate on Ketogenesis From Fatty Acids and Pyruvate. (23853)

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After having shown(1-7) that acetoacetate plays an important role in development of experimental diabetes, we noticed that compounds formed by condensing acetoacetate with glucose are not only non-diabetogenic but act as an important agent in prevention of various kinds of experimental diabetes induced by alloxan, dehydroascorbic acid and acetoacetate(8-12). Because unlike other diabetogenic substances, acetoacetate is a normal metabolite, it is reasonable to assume that gradual accumulation of acetoacetate is responsible for progressive development of clinical diabetes. Such accumulation may be caused either by excessive hepatic ketogenesis (formation of acetoacetate) or by decreased extra hepatic utilization. But it has been demonstrated by Chaikoff *et al.*(13) that tissues of diabetic animals can oxidize ketone bodies as readily as those of normal animals. Raper and Smith(14) are also of the opinion that ketosis must be due, mainly, to this overproduction of ketone bodies by liver rather than to its underutilization by extra-hepatic tissues. Jowett and Quastel(15) found that liver slices form 10 to 40 times more ketone bodies than other organs and Edson(16) has shown that when butyric and crotonic acids

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