amount of precipitation versus the concentration of the reactants. Incubation was at room temperature (21 °C) with measurements taken at 4, 8, 24, 48 and 72 h.

The quantitative precipitin test in gel[9] was also performed since it provided the same information as the tube test, was somewhat less cumbersome and was amenable to large scale testing. Diffusion plates were made by pouring 12 ml of 1.5% agarose in deionized water into the bottom of 100 mm diameter petri plates. Parallel wells of 5 mm diameter and 15 mm apart from their centers were cut in the agar. As with the tube precipitin test one class of reactant was held constant during one test and the other diluted in a geometric series. The concentration range used in the tube test was employed in the gel test. The equivalence point was taken as the concentration of reactants which produced the sharpest line of precipitation without Lisegang bands.

Results and discussion. Tube precipitation studies produced the typical bell-shaped curves observed for the quantitative determination of antigen-antibody precipitation. Both pro-zone and post-zone phenomena were seen. The polymyxins, polymyxin B and colistin, yielded the greatest turbidity and precipitation with polyanionic detergents and had the widest equivalence zone of the tested antibiotics. The aminoglycosides, as typified by the kanamycin versus SPS study shown in the table, all produced bell-shaped curves, but with lesser amounts of precipitation than the polymyxins. There was no precipitation with any detergent tested and penicillin G, nor was there precipitation with tween 80 and benzalkonium chloride with any examined antibiotic.

Quantitative checkerboard precipitation tube dilution test: SPS and kanamycin

Kanamycin (mcg/ml)	SPS (mcg/ml)* 50000	25000	12500	6250	3125	1560	780
10000	−	+	+	+	+	+	+
5000	−	+	+	+	+	±	−
2500	−	−	+	+	+	−	−
1250	−	−	−	+	−	−	−
625	−	−	−	−	−	−	−
312	−	−	−	−	−	−	−
156	−	−	−	−	−	−	−

* The molecular weight of SPS is not known.

In gel (figure) the aminoglycoside and polymyxin class antibiotics reacted with the polyanionic detergents in the classical manner. In each, the equivalence point was determined as that area where the precipitation line was the sharpest and where precipitation bands switched position from antibiotic to detergent excess. The typical Lisegang bands seen in gel are representative of the prozone and postzone phenomena one observes during tube testing. In gel, the point of first precipitation is also the equivalence point. As a result the test can be accomplished within 4 h since first precipitation appears at this time. Consequently it is possible to rapidly determine the amount of antibiotic in solution utilizing a constant concentration of polyanionic detergent. The concentration of detergent needed to elucidate the equivalence point for any given antibiotic sample varies by the amount of antibiotic in solution. Several varied detergent concentrations can be set-up at the same time on different gel plates.

Based on classical precipitin testing by tube dilution and gel diffusion it would appear that the interaction of polyanionic detergents with polymyxin and aminoglycoside class antibiotics fulfills the requirements of a quantitative reaction. Accordingly, one may utilize either technique for measuring levels of these antibiotic classes and for the preparation of standards. Because the reactions can be read within 4 h these procedures should be useful in those instances where rapid measurements are needed.

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Growth-modulating serum tripeptide is glycyl-histidyl-lysine[1]

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19 November 1976

Summary. The structure of a growth-modulating tripeptide from human serum and plasma has been determined to be H-glycyl-histidyl-lysine-OH.

We have previously reported the isolation of a small peptide from human serum which, in concentrations ranging from 20 to 200 ng/ml, promoted the growth of hepatoma cells in HTC and prolonged the survival of nonreplicating rat liver cells in low-serum (1%) monolayer culture[2,3]. Amino acid analyses of the purified active fraction suggested that the native factor was a tripeptide composed of glycine, histidine and lysine[2].

Among synthetic analogs tested, glycyl-histidyl-lysine (GHL) had bioactivities which were comparable to those of the serum factor in the hepatoma and normal liver cell systems[4]. Recently, GHL in nmole concentrations was shown to stimulate growth and differentiation of cortical neurons in vitro and to inhibit growth of glial elements[5]. Suppression of growth in fibroblast cultures by GHL at higher concentrations has also been reported[6]. At neutral pH ranges GHL has chelation properties toward several transition metals (Cu^{+2}, Zn^{+2}, Co^{+2}, Fe^{+3}) and seems to function synergistically with the metals to increase the adhesion of hepatoma cells (HTC_4) growing in low-serum (0.5–1.0%) to the monolayer support[7].

We present data herein that confirm that the native serum factor is a tripeptide with the structure: glycyl-histidyl-lysine. The serum tripeptide was isolated by methods detailed elsewhere[8]. In brief, the serum proteins are removed by heat coagulation and centrifugation. The supernatant which contains the tripeptide is passed through a molecular filter with a nominal cutoff at 500 daltons (Amicon, UM-05, Lexington, Mass.) then lyophilized. After reconstitution in 1% acetic acid, the frac-

tion is further purified on a Sephadex G-10 column (Sigma Chem. Co., St. Louis, Mo.). Final purification of the active fraction from the G-10 column is achieved using a high pressure silica gel (5 μm) column[8].

When the purified tripeptide was mixed with an equimolar amount of synthetic glycyl-histidyl-lysine and chromatographed on thin-layer silica gel plates (Polygram Sil G, 0.25 mm, Macherey-Nageland Co., Duren, Federal Republic of Germany) only one resultant spot was found (solvent: $CHCl_3$/MeOH/17% NH_4OH: 2/2/1 by volume). When the same experiment was done with synthetic glycyl-lysyl-histidine, the native factor and the synthetic could be narrowly separated.

The sequence was elucidated by manual Edman degradations and C-terminal analysis using carboxypeptidase B. Purified native GHL (40 nmoles) was subjected to manual Edman degradations[9] and the resulting PTH amino acids were identified and quantitated by gas[10] and thin layer chromatography[11]. After the first cycle of the Edman degradation, 19.6 nmoles of glycine was obtained and after the second cycle 5 nmoles of histidine were obtained as the only detectable PTH amino acids at each step. To more firmly establish the C-terminal sequence of GHL, the native peptide was digested with carboxypeptidase B for periods of 15 sec to 2 h. The time dependent liberation of lysine and histidine from native peptide is presented in the table. These results are consistent with a C-terminal dipeptide sequence of His-Lys and conclusively demonstrates the structure of this growth stimulating peptide to be H-Gly-His-Lys-OH.

Release of free amino acids from the COOH-terminus of native GHL

	15 sec	60 sec	10 min	2 h
Lysine	1.00*	1.00	1.00	1.00
Histidine	0.69	0.73	0.79	0.96

* Molar ratio of amino acids, taking lysine as 1.00 in each time period.

1 Acknowledgment. This work was supported by grants from the U.S. Public Health Service (HD-03148 and P17AM 18520) and from the National Cancer Institute (NO-1-CB53868).

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Effects of diethylstilbestrol on the production of various extracellular products of Staphylococcus aureus[1]

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Summary. The synthetic estrogen diethylstilbestrol at a subinhibitory level of 1.75 μg/ml diminished the production of staphylococcal alpha toxin, coagulase, deoxyribonuclease and penicillinase. Thus, the reported host beneficial effects of diethylstilbestrol may be partially related to its retardive action of certain toxins, or enzymes of S. aureus.

The biochemical action of hormones on mammalian[2–5] and microbial cells[6] have been adequately reviewed, shown to influence the metabolism of these cells and suggest that they may be of importance in a host parasite relationship.

Diethylstilbestrol (DS) is used as replacement therapy in estrogen deficiency, in carcinoma and is now also employed in a daily dose of two 25 mg pills for postcoital contraception. Investigations in this laboratory indicated that injection of gonadal hormones to rabbits and mice enhanced the resistance of these animals to induced staphylococcal infections[6,7]. The present investigations suggest that the reported host beneficial actions of DS and other gonadal hormones might be partially related to its interference with the production of various toxins or products of S. aureus.

Assay of the subinhibitory levels of DS. As a prelude to the studies related to the action of DS on the synthesis of staphylococcal products the subinhibitory doses of DS were determined. The system contained 1000 ml of the appropriate culture medium in a 2500 ml Erlenmeyer flask, DS at a final concentration of 0–2 μg/ml, and an inoculum of approximately 1×10^9 colony forming units of S. aureus. Flasks were incubated on a rotary shaker at 37 °C, portions were removed at varying time intervals

1 Acknowledgments. This work was supported by United States Public Health Service, grant AI 06618, a grant in aid from Loyola University, United States Public Health Service, grant GRSG PRO 5368, and grants from Eli Lilly, Upjohn, Syntex, and G. D. Searle drug companies.

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