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.

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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

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and assayed turbimetrically, gravimetrically and by viable counts. The results of the turbidimetric experiments are shown in table 1. At the concentrations employed DS did not inhibit the growth of S. aureus. Gravimetric and viable count experiments yielded similar results.

DS and production of alpha toxin. Staphylococci produce a variety of toxins and enzymes which have been associated with their pathogenicity. The alpha toxin is endowed with lethal, dermonecrotic and hemolytic action. The action of DS on the synthesis of alpha toxin was assessed. Stage four alpha toxin was prepared and assayed by the method of Bernheimer and Schwartz[8]. Table 2 indicates that DS at the subinhibitory level of 1.75 μg/ml diminished the synthesis of alpha toxin. The specificity and identity of alpha toxin is further substantiated by alpha toxin neutralization studies. The inferiority of the toxin produced in the presence of DS was indicated by the finding that hemolysis of rabbit erythrocytes by alpha toxin from control cultures occurred in the presence of a 1:16 dilution of specific antibody, while a dilution of 1:128 of the same antibody was required to allow lysis of the erythrocytes by alpha toxin prepared from cultures containing 1.75 μg/ml DS.

Table 1. Growth of S. aureus in brain heart infusion broth

Diethylstilbestrol μg/ml	Klett reading at various hours 0	2.5	3.5	4.5	6	10
0	0	40	93	219	335	440
1.5	0	44	90	216	365	445
1.75	0	40	91	218	360	460
2.0	0	35	91	206	355	440

Table 2. Effect of DS on the synthesis of various toxins or products of S. aureus

Parameter assessed	Alpha toxin synthesized at 9–10 h Control	1.75 μg/ml DS
Mg dry wt cells/ml of medium	1.5	1.6
μg stage 4 purified toxin/ml of medium	19	11
Reciprocal of hemolytic titer/mg of toxin	1280	160
Dermonecrosis in cm^2/μg of toxin	1.4	0.0
Parameter assessed	**Coagulase produced at 10 h Control**	**1.75 μg/ml DS**
Units of coagulase/ml protein	48,761	14,628
Mg dry wt cells/ml of medium (BHI)	1.17	1.20
Parameter assessed	**DNA-ase formed at 9–10 h Control**	**1.75 μg/ml DS**
μg of partially purified DNA-ase/ml of medium	47	40
Relative viscocity of DNA at 15 min incubation with 1.8 μg DNA-ase	50	66
Mg dry wt of cells/ml of medium	2.1	2.1
Parameter assessed	**Penicillinase synthesized at 9 h Control**	**1.75 μg/ml DS**
Units of penicillinase per liter of culture supernatant	540	200
Mg dry wt of cells/ml	3.07	3.14

Effect of DS on the synthesis of purified coagulase. Correlation between the pathogenicity of certain strains of staphylococcus and their ability to produce coagulase has incited interest in this macromolecule. Purified coagulase was prepared and assayed by the method of Tager[9]. It can be seen in table 2 that coagulase produced in the absence of DS contained 48,761 clotting units and only 14,628 units per mg of purified protein in the presence of 1.75 μg/ml DS. The titer of coagulase prepared in the absence of DS and exposed subsequently to 2 μg/ml DS was in every case equal to that of the control suggesting that once coagulase had been formed addition of DS could not influence the activity of the molecule. A similar situation was encountered with alpha toxin. The scanning isoelectric pattern of both preparations assayed by the method described by Catsimpoolas[10–12] indicated no differences in the electrophoretic mobility, composition, or the isoelectric point of the 2 coagulase preparations. The isoelectric patterns of both coagulases showed 2 major and at least 5 minor components.

DS and synthesis of DNA-ase. Deoxyribonucleases play a prominent role in cellular metabolism. In addition, there is correlation between DNA-ase activity and positive coagulase reaction which appears to be useful for identifying the species of S. aureus. DNA-ase precipitated with saturated ammonium sulfate[13] from culture supernatants of S. aureus Georgio strain, grown for 9–10 h in brain heart infusion broth with 1.75 μg/ml DS was less abundant and not as active[14] as the corresponding molecule isolated from control cultures (table 2).

The action of DS on penicillinase. An effort was also made to determine if DS could influence the synthesis and/or activity of penicillinase[15,16]. The results of these studies presented in table 2 indicate that under the experimental conditions DS at the subinhibitory concentration of 1.75 μg/ml had a retardive action on the formation of penicillinase.

It is generally agreed that it is usually the quality and quantity of a given toxin, which is of germane importance in the outcome of an infectious process. The data recorded in this paper demonstrate that both the elaboration as well as the activity of certain extracellular products of staphylococci, which may be contributing to the pathogenicity of staphylococci, are adversely influenced by the presence of subinhibitory levels of DS during their formation; and suggest that the reported[6] host beneficial actions of DS and other gonadal hormones might be partially related to the retardive action of hormones on the production of certain toxins, or enzymes of S. aureus. Furthermore, it would appear that staphylococci provide useful experimental models in assaying the as yet poorly understood biochemical actions of the now widely employed contraceptive hormones.

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