and vaginal cornification following concomitant administration with exogenous estrogen and is also reflected by modification of vaginal cytology during continued administration to cycling female rats. Copulation during extended administration of up to 2.5 mg kg/day of U-11555A, and normal development of ensuing pregnancies if treatment is stopped just prior to breeding, suggests ovarian activity and psychic responses are adequate to support normal reproductive functions. On a mg/kg basis, doses of U-11555A comparable to the minimal effective antifertility dose in rats do not exhibit significant progestational, antiprogestational, antigonadotropic, androgenic, or estrogenic activities.

Summary. U-11555A, a derivative of 2,3-diphenylindene, is an orally active antifertility agent in laboratory mammals. In rats, its efficacy is limited to the period of tubal transport of the zygote. Single oral doses, administered any time within 4 days of breeding, effectively inhibit pregnancy. After the zygote has reached the uterus U-11555A is ineffective as a pregnancy inhibitor. At effective antifertility dosages, U-11555A does not exhibit significant progestational, antiprogestational, antigonadotropic, androgenic, or estrogenic activities. U-11555A inhibits the uterine and vaginal response to endogenous and exogenous estrogens.

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Effects of Enzymatic Digests of DNA on Staphylococci.* (27139)

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Enzymatic digests of DNA have been shown to contain a factor that is capable of promoting the establishment of virulent mutants in initially avirulent cultures of a number of bacterial species(1-6). The active factor has been shown to be non-identical with known breakdown products of DNA, such as purines, pyrimidines, nucleosides, and nucleotides, and is obtained following exposure of DNA to DNAase regardless of the source of the nucleic acid. In the case of pneumococci the selective population change from non-smooth to smooth was found to be attributable to a selective stimulation of DNA synthesis and with it an increase in the rate of multiplication of smooth virulent types; non-smooth avirulent types did not respond to the DNA digest(4,5). The present report will discuss recent attempts to study influences of the DNA digest factor on population changes and growth of staphylococci of different virulence.

Materials and methods. Coagulase-positive and coagulase-negative strains of known relationship were employed. Most of these originated from cultures kindly furnished by Dr. John E. Blair, who had obtained them as primary isolates from human sources. DNA from calf thymus isolated by the procedure of Kay *et al.*(7), was used routinely, but DNA from bacterial sources also was employed. Pancreatic DNAase (Worthington) was used and the mixtures of DNA and

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DNAase in diluent containing 0.03 M $MgSO_4$ plus 0.85% NaCl were allowed to incubate at room temperature for one hour prior to use. They were sterilized by filtration through millipore membranes and stored at 4°C. Growth and population changes were tested in brain-heart infusion broth or in a defined medium(8) which had been inoculated with 10^5 to 10^6 organisms. Inocula either consisted principally of one cell type, *i.e.*, either coagulase-positive or -negative, or were prepared as mixtures of approximately 99% coagulase-negative plus 1% coagulase-positive cells. Appropriate tests showed that differential clumping was not responsible for the results to be described, *i.e.*, the size of each plating unit remained constant under all conditions used. This was determined by direct microscopic observations on the number of cells per aggregate, and by comparative viable counts following dispersion of aggregates by sonication (Raytheon Sonic Oscillator, model 101) sufficient to yield maximum increases in counts.

Results. Both in brain-heart infusion broth and in the defined medium(8), the coagulase-positive type was found to become the dominant cell type following 24 to 48 hours of incubation of initially predominantly coagulase-negative populations. In the early tests the addition of DNA digest resulted in an extremely variable stimulation of the rate with which the coagulase-positive variants established themselves. At the same time, however, it was noted that addition of filtrates from aged coagulase-positive cultures yielded a consistent stimulation of the rate with which coagulase-positive cell types became predominant in aging cultures. Since it is known, particularly from the studies of Burns and Holtman(9), that the vast majority of coagulase-positive staphylococci will also produce a nuclease, and since it is also known that staphylococci lyse fairly rapidly in aging closed culture systems, it was suspected that the staphylococci might produce their own DNA digest factor in aging cultures. To test this assumption, chelating agents (citric acid or versene) were added to cultures inoculated with either coagulase-positive or coagulase-negative organisms. The

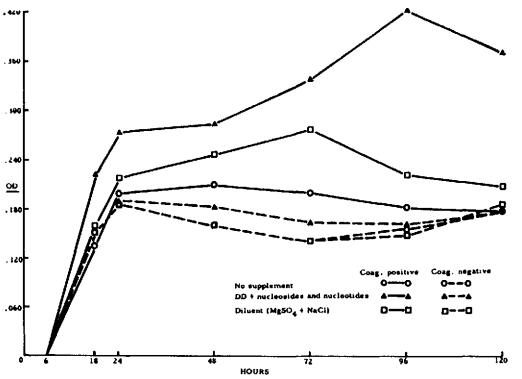

FIG. 1. Effects of DNA digest supplemented with deoxynucleosides and deoxynucleotides (325 μg of each/ml) on growth (optical density) of coagulase-positive and -negative staphylococci in chelated BHI medium. OD determined at 640-700 mμ in a Klett-Summerson Photoelectric Colorimeter. Extent of growth during initial 24 hr can be attributed to "carry-over" effects from prior growth in non-chelated media.

presence of the chelating agent inhibited the multiplication of both types; however, reduced growth did occur. When one ml of DNA digest (DD) produced from 400 μg DNA and 100 μg of DNAase was added to 4 ml of culture, a stimulation of the growth of coagulase-positive cells occurred, whereas the coagulase-negative cells responded little or not at all (Fig. 1). This selective stimulation of the growth of the coagulase-positive type in presence of DD was confirmed by a large series of replicate experiments, involving both viable counts and turbidity readings, and was also reflected in the striking differences in population change that occurred in DD-supplemented chelated cultures compared to unsupplemented chelated cultures (Fig. 2). As in the case of brucellae and pneumococci, further supplementation of the digest-containing cultures with mixtures of deoxyribonucleosides (DNS) and deoxyribonucleotides (DNT) tended to enhance the effectiveness of the digest factor, but this enhancement was not obtained in all tests. DNS and DNT had slight effects in the absence of DD, but as in the case of purines, pyrimidines, DNA alone, or DNAase alone, these supplementations failed to duplicate the effects of DD (Table I). The diluent containing $MgSO_4$ did produce a fair stimulation of the establishment of coagulase-positive variants in ini-

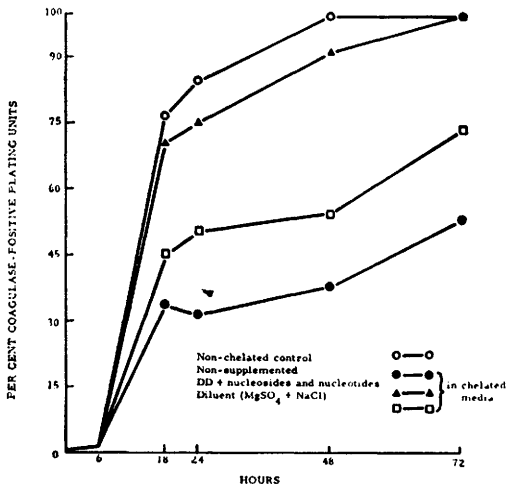

FIG. 2. Effects of DNA digest supplemented with deoxyribonucleosides and deoxyribonucleotides on establishment of coagulase-positive types in initially 99% coagulase-negative plus 1% coagulase-positive cultures in chelated BHI medium. Proportion of coagulase-positive organisms was estimated by determining per cent of pigmented colonies on nutrient agar plates inoculated with samples from the liquid culture.

tially predominantly coagulase-negative cultures but this may be attributed to a partial negation of the chelation. Ashed DNA plus DNAase had no effects on chelated cultures, and tests with DNA from *B. subtilis* and from pneumococci as source of DD yielded the same results as were obtained with calf thymus DNA.

Studies with pneumococci and Brucella had revealed that 2-acetyl amino-5-nitrothiazole is a potent inhibitor of the effects of DNA digest(10). Addition of this compound to the digest-containing cultures of staphylococci now has been found to produce similar inhibitions.

The stimulatory effects of the DNA digest factor on the growth of coagulase-positive staphylococci also expresesd itself under certain *in vivo* conditions. Thus, when mice were inoculated subcutaneously with approximately 5×10^8 coagulase-positive organisms suspended in 100 μg of DNA plus 25 μg of DNAase (0.2 ml) a striking increase of the gross pathology resulted. Fig. 3 shows the appearance of mice sacrificed 14 days after subcutaneous challenge with staphylococci which were injected either with or without DD. Suspending the organisms in DNAase alone, DNA alone, or a mixture of purines and pyrimidines (50 γ of each) failed to produce such effects. Preliminary tests in which intracerebral administration of the pathogen was used have indicated similar enhancing effects of the DNA digest, resulting in more rapid and greater mortality of the animals that had received DD intracerebrally with the infecting organisms. DNA digest has failed to exert any detectable modification on systemic infections initiated by IP or IV challenge. Attempts have been made to antagonize the *in vivo* effects by agents capable

TABLE I. Effects of DD and of Known Components of DNA on Population Changes and Growth of Staphylococci in BHI. Inoculum: 99% BVII coagulase-negative plus 1% BVII coagulase-positive cells; inoculum size: 5×10^5.

Culture conditions	12 hr		24 hr		48 hr	
	OD	% +	OD	% +	OD	% +
Non-chelated BHI controls	.077	60	.276	66	.262	74
In chelated media:						
BHI controls[a]	.0	<1	.077	3	.107	10
DD added[b]	.040	28	.190	63	.271	66
Purines, pyrimidines added[c]	.006	2	.064	7	.079	10
DNS* added[d]	.007	3	.059	11	.071	14
DNT† added[d]	.010	3	.095	14	.111	18
DNS-DNT added[e]	.017	4	.083	20	.110	23
DNAase added	.012	7	.079	21	.106	19
DNA added	.015	7	.082	16	.089	21
Diluent for DD ($MgSO_4$ + NaCl) added	.009	10	.099	30	.134	36

* Deoxynucleosides. † Deoxynucleotides.

[a] 0.5 mg/ml citric acid. [b] DNA 80 γ/ml + DNAase 20 γ/ml. [c] 80 γ/ml of each.
[d] 100 γ/ml of each. [e] 50 γ/ml of each.

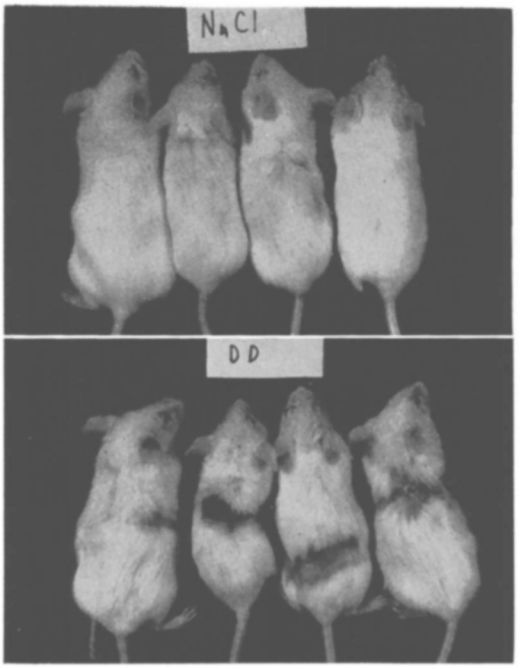

FIG. 3. Mice inj. with coagulase-positive organisms, with and without DD, 14 days following subcut. infection.

of interfering with DNA digests effects *in vitro*. So far, neither chelating agents nor nitrothiazole, administered locally, have altered the course of subcutaneous staphylococcal infections in mice.

Discussion. Our finding that growth of coagulase-positive staphylococci, *in vitro* and *in vivo*, can be stimulated by a factor present in DNA digests raises some interesting questions regarding the significance of such effects in the normal course of staphylococcal infections. It is conceivable that the tendency of virulent staphylococci to prosper in abcesses may be associated with the organism's dependence on cell breakdown factors that accumulate and persist for relatively long periods in such environments. Our findings that staphylococci are not merely dependent on production of the stimulating DNA digest factor by cells of the host but also appear capable of producing their own digest factor in closed environments *in vitro*, would appear to explain the rather unusual direction of population changes of these organisms *in vitro*. It will be recalled that staphylococci tend to undergo changes *in vitro* from avirulent to predominantly virulent populations, whereas members of other pathogenic bacterial species tend to undergo *in vitro* population changes in the opposite direction(11). Finally, it can be hoped that the demonstrated relationship between growth of virulent staphylococci and DNA digest factor can be used for the recognition of inhibitors capable of interfering with *in vivo* propagation of this pathogen. This might be accomplished by preventing the action of the DNA digest factor or by preventing its production and accumulation. A potential therapeutic approach based on this phenomenon could be expected to affect staphylococci without regard to their antibiotic resistance since recent tests have shown that penicillin-resistant and penicillin-sensitive coagulase-positive types respond equally to the stimulating effects of the DNA digest factor.

Summary. The multiplication of coagulase-positive staphylococci was found to be stimulated by a factor present in enzymatic digests of DNA, which previously had been shown to stimulate DNA synthesis and multiplication rates of other gram-positive bacteria, regardless of the source of DNA used. Coagulase-negative variants do not respond and the presence of the DNA digest factor therefore enhances population changes favoring rapid establishment of coagulase-positive organisms in initially predominantly coagulase-negative populations. On the basis of studies with chelated media it was concluded that, by virtue of cell lysis and nuclease production, a DNA digest factor can accumulate naturally in non-chelated, aging staphylococcal cultures containing at least a few coagulase-positive cells. An enhancement by DNA digest of localized lesions that develop following subcutaneous injection of staphylococci into mice also was noted. The implications of these findings for understanding the unusual direction of population changes of staphylococci *in vitro* as well as problems of pathogenesis and therapy have been discussed.

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Interstitial Fluid Pressure in Intestinal Submucosa of Dogs in Endotoxin Shock.* (27140)

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The loss of fluids from vessels has been shown to be associated with changes in lymph flow and interstitial fluid pressure(1,2). Aust *et al.*(3) found a significant sequestration of plasma albumin labeled with I^{131} in the intestine of the dog 5-7 minutes following administration of a lethal dose of *E. coli* endotoxin. Ballin and Meyer(4) have shown an increase in lymph flow rate from the small intestine after endotoxin and more recently Alican and Hardy(5) observed an increase in the thoracic lymph flow early after endotoxin, which was primarily attributed to an increase in hepatic lymph flow. This was followed by a decline and a secondary rise which was chiefly accounted for by an increase in the intestinal lymph flow. It therefore seemed desirable to measure tissue pressure in the intestinal submucosa during endotoxin shock. In addition, since portal hypertension occurs during the initial stages of endotoxin shock (6), tissue pressures were measured during a 10-minute period of obstructive elevation of the portal venous pressure in order that comparisons might be made.

Method. Adult dogs were fasted 18-24 hours, then anesthetized by intravenous administration of sodium pentobarbital. The procedure for measuring tissue pressure was modified after that of McMaster(1,2) and Hinshaw *et al.*(7). A #24 or 27 gauge needle filled with saline was inserted into the intestinal submucosa, connected to a Statham strain gauge *via* a polyethylene catheter and the pressure was recorded on a Sanborn recorder. Pressure within the system was elevated 3 to 10 cm of saline for a short period (0.5 to 1 sec.) and the pressure decay slope was followed until a plateau occurred which was accepted as the tissue pressure (P_{tissue}) in mm of Hg when referred to a zero catheter at atmospheric pressure. Successive values agreed within 0.5 mm of Hg. Pressure in the portal vein (P_{pv}) was also recorded by cannulating the splenic vein following splenectomy.

Tissue and portal pressures were measured in 12 dogs, prior and subsequent to intravenous administration of *E. coli*† endotoxin (1 mg/kg) and in another 12 dogs, before, during, and after obstructive elevation of the portal pressure. Portal pressure was mechanically increased by utilizing a screw-clamp fitted with an extension or by using a long ligature of umbilical tape placed about the vein and passed through a plastic tube which could be depressed against the vessel.

Results. The average tissue pressure in the intestinal submucosa at various times following administration of endotoxin and obstructive elevation of the portal pressure, plus and minus the standard error of the

* Supported by grants from U.S.P.H.S.

† *E. coli* endotoxin was obtained from Difco Labs.