reflection of a type of altered cell immunity that may result from prolonged natural exposure to staphylococci. Alternately, or in addition, the changes in the cell may merely reflect a physiological change due to aging which might be associated with the availability of certain nutrient factors for the parasite. The ability of DNS + DNT to modify cell properties, so that the latter resemble those from newborn animals, certainly suggests the possible involvement of non-specific alterations. The present data do not permit a distinction between these two possibilities just cited or the possible involvement of still other factors. The data, however, show that alterations in cellular properties rather than alteration in humoral factors are of greatest significance to the intracellular fate of virulent staphylococci. It is possible that the differences in the intracellular fate of staphylococci in monocytes from newborn and adult rabbits relate to certain problems of human infections with staphylococci which, although they occur in all age groups, are more frequent in children and adolescents than in adults(7).

In previous studies(4) enhancing effects of enzymatic digests of DNA on growth of virulent staphylococci *in vitro* and in abcesses were observed. It appears noteworthy that components of such digests, namely the nucleotides and nucleosides, now also have been implicated in the intracellular fate of staphylococci. This strengthens the previously expressed thought that by antagonizing the effects of DNA breakdown products *in vivo* one may achieve beneficial therapeutic effects.

Summary. By studying the fate of staphylococci in monocytes maintained *in vitro* it was established that intracellular survival time of virulent staphylococci is longer in monocytes from newborn rabbits than in monocytes from adult rabbits. Supplementation of the bacterial growth medium and cell maintenance medium with a mixture of deoxynucleosides, deoxynucleotides and $MgSO_4$ resulted in a lengthening of the survival of intracellular staphylococci in phagocytes from adult rabbits. Alterations of survival time were not obtained when the DNA precursors were added to cultures containing cells from newborn animals. Intracellular multiplication of the cocci did not occur under any of the conditions studied. Factors that may be responsible for the differential intracellular survival times of the pathogen, their possible relationship to therapeutic problems and to infections in man have been discussed.

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An Anti-Androgen Assay Using the Castrated Mouse.* (27818)

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Anti-androgenic compounds may be assayed in the androgen stimulated castrated rat using the end-points of prostate and seminal vesicle weights. This test however lacks sensitivity(1). The use of the testosterone stimulated castrated immature mouse, reported in this paper, provides this increase in sensitivity with no loss in convenience and reproducibility.

Methods. Swiss albino mice were castrated at 21 to 23 days of age. On the day of operation and for a total of 7 consecutive

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TABLE I. Anti-Androgenic Activity of Various Steroids in a Castrated Mouse Assay.

Material injected					Tissue ratio ± S.E.	
Designation	Total dose, mg	Testosterone inj, mg	No. of mice	Mean body wt, g	Prostate	Seminal vesicles
0	0	0	10	16	.08 ± .006	.08 ± .004
0	0	2	9	18	.35 ± .03	1.10 ± .15
A-norprogesterone	100	2	9	16	.34 ± .04	.64 ± .11
Progesterone	100	2	9	16	.29 ± .03	.40 ± .05
Ro 2-7239	100	2	6	16	.24 ± .02	.56 ± .03

days the testosterone dissolved in 0.1 ml of sesame oil or an aqueous suspending medium† was injected subcutaneously. The test material dissolved either in 0.2 ml of sesame oil or the aqueous suspending medium was injected once daily for 7 days also starting on the day of operation. Twenty-four hours after the last injections the animals were autopsied and the body, prostate, and seminal vesicles weights determined. The results are expressed as tissue ratios defined as milligrams of tissue per gram of body weight.

Results. A total dose of 2 mg of testosterone injected in an oil solution was inhibited by 100 mg doses of 3 different compounds (Table I). The solvent injected control group had prostate and seminal vesicle ratios of 0.08 ± 0.006 and 0.08 ± 0.004 respectively. Testosterone at a total dose of 2 mg did not produce a significant difference in body weight, but did produce 4-fold increases in the prostate ratio and a 12-fold increase in the seminal vesicles ratio. One hundred mg of A-norprogesterone did not decrease the prostatic ratio significantly. However, the ratio of the seminal vesicles decreased from 1.10 ± 0.15 to 0.64 ± 0.11. Progesterone caused a similar but somewhat more intense response at the same dose level. Ro 2-7239 (2 - acetyl-7-oxo-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene) at the total dose of 100 mg inhibited the action of testosterone on the prostate and seminal vesicle ratios.

Table II deals with a series of experiments on the influence of progesterone on the action of testosterone when both compounds were administered in sesame oil solution. In these experiments 0.4 and 0.8 mg of testosterone were injected and the dose of progesterone ranged from 10 to 40 mg. No significant anti-androgenic effect was elicited on the prostate at the 10, 20 or 40 mg dose levels of progesterone in Experiment A (Fig. 2). The 10 and 20 mg dose of progesterone was ineffective as an anti-androgen on the seminal vesicles but the total dose of 40 mg produced a highly significant inhibition of seminal vesicle ratio from 0.62 ± 0.057 to 0.46 ± 0.049. In Experiment B (Table II) the 0.4 ml of testosterone injected over the 7-day period caused a significant increase in both prostate and seminal vesicle ratios. The prostate ratio was increased almost 100%, while the seminal vesicle ratio was increased something less than 4-fold. In this experiment as little as 10 mg per day total dose of progesterone significantly decreased the seminal vesicle ratio from 0.93 ± 0.122 to 0.77 ± 0.064, while the prostatic ratio remained unchanged. At the 20 mg total dose in Experiment B no change in prostate ratio was found, but again the seminal vesicle ratio was decreased. At 40 mg, however, a significant decrease in both prostatic and seminal ratios were observed. Experiment C was essentially similar to Experiment A, and Experiment D was similar to Experiment B.

Discussion and conclusions. The fact that progesterone is an effective anti-androgen in the castrated mouse stimulated with testosterone confirms previous studies in the testosterone stimulated castrated rat and chick. The use of the castrated mouse permitted the demonstration of the anti-androgenic activity of progesterone at the 40 mg dose level in 4 trials out of 4 attempts with respect to the seminal vesicles and 3 trials out of 4 attempts on the prostate. Some positive anti-androgenic effects were observed at the 20 mg dose

† This medium consists of sodium chloride (0.9%), polysorbate 80 (0.4%), carboxymethyl cellulose (0.5%), and benzyl alcohol (0.9%).

TABLE II. Anti-Androgenic Activity of Various Steroids in a Castrated Mouse Assay.

Exp	Material inj	Total dose, mg	Testosterone inj, mg	No. of mice	Mean body wt, g	Tissue ratio ± S.E. Prostate	Seminal vesicles
A	0	0	0	8	18	.07 ± .008	.13 ± .014
	0	0	.4	10	19	.21 ± .019	.62 ± .057
	Progesterone	10	.4	10	18	.22 ± .025	.70 ± .077
		20	.4	10	14	.30 ± .052	.73 ± .069
		40	.4	7	17	.20 ± .018	.46 ± .049
B	0	0	0	10	18	.19 ± .032	.24 ± .025
	0	0	.4	7	17	.33 ± .047	.93 ± .122
	Progesterone	10	.4	10	20	.34 ± .027	.77 ± .064
		20	.4	8	17	.38 ± .041	.73 ± .066
		40	.4	10	22	.19 ± .018	.37 ± .026
C	0	0	0	8	18	.07 ± .008	.13 ± .014
	0	0	.8	8	17	.23 ± .025	.85 ± .068
	Progesterone	10	.8	9	18	.26 ± .017	.81 ± .038
		20	.8	9	17	.24 ± .017	.72 ± .035
		40	.8	8	18	.18 ± .012	.47 ± .080
D	0	0	0	10	18	.19 ± .032	.24 ± .025
	0	0	.8	9	20	.36 ± .047	.90 ± .088
	Progesterone	10	.8	5	19	.34 ± .060	.65 ± .092
		20	.8	10	22	.33 ± .038	.71 ± .081
		40	.8	10	19	.26 ± .023	.46 ± .047

level of progesterone on the seminal vesicles. In the rat 50 mg of this compound were required for seminal vesicle effects while 250 mg doses were required for prostate effects (2).

The testosterone stimulated mouse also showed a more intense response to the anti-androgen progesterone than had been observed in the treated rat. Forty mg of progesterone produced a mean inhibition of 61% on the seminal vesicle ratio in the 4 experiments (A,B,C,D – Table II) while previous studies indicated that 250 mg of progesterone were required to produce a 52% inhibition response in the testosterone stimulated castrated rat(2).

The mechanism of anti-androgenic action awaits elucidation.

Summary. An anti-androgen assay, using the testosterone stimulated castrated mouse is described. This method, which employs the end-points of weight of the seminal vesicle and the prostate, is reasonably reproducible, convenient, and possesses a better sensitivity than is obtained by using the castrated rat.

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