liver are not able to remove the specific antibodies from the serum.

Discussion. Homologous testes antisera contain antibodies that localize with the achrosome of the spermatid and the head of spermatozoa. This specific localization occurs in the testes of sensitized as well as unsensitized animals. It would appear, therefore, that the autoantigen constituent of testes extracts resides in this cytological component. This finding confirms by fluorescent technics the suggestion of Freund *et al.*(1) and Katsh(5) that aspermatogenic antigen may be a polysaccharide associated with the achrosome. The results are in agreement also with those of Baum(8) whose pictures indicate localization of fluorescence in the mature sperm but do not permit identification of specific cytological regions. The low grade fluorescence we observe in the albuguinea and blood vessel walls can be expected on the basis of previous experiments. It has been shown that normal serum proteins deposit to a limited extent in the extra-vascular connective tissue structures(9). Further evidence for the specificity of fluorescent localization in the spermatid achrosomes and sperm head is provided by the absorption experiments in which lyophylized guinea pig testes are effective in removing antibodies while liver and kidney extracts are not. The lack of correlation between circulatory levels of antibodies and morphologic damage to the testes is similar to the results reported by other investigators and points to the fact that much remains to be elucidated with respect to the mechanisma of the immunological insult to the testes as well as the chemical identification of antigens involved in the induction of aspermatogenesis.

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Influence of Host Age and DNA Precursors on Intracellular Staphylococci.* (27817)

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Previous investigations on the intracellular fate of virulent staphylococci in monocytes from "normal" adult rabbits(1,2,3) had shown that virulent *S. aureus* organisms survive intracellularly for some time but are eventually destroyed. No intracellular multiplication was observed in these studies, employing monocytes maintained *in vitro,* nor in the studies of others (see 3). If one assumes that staphylococci have a basic potential for intracellular residence and multiplication, then the observations with phagocytes from adult rabbits suggest the possibility that failure of multiplication in cells from the adult host may be due to the host's natural attainment of a considerable degree of resistance as a result of prior natural exposure. If this were true then monocytes from newborn rabbits, which presumably lack signifi-

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cant prior exposure to staphylococci, could be expected to permit longer intracellular survival or possibly even intracellular multiplication.

Another factor that might influence the intracellular fate of staphylococci might be the intracellular availability of certain growth factors. McKee and Braun(4) reported that the enzymatic breakdown products of DNA can enhance the growth of virulent staphylococci *in vitro,* and similar effects have been noted more recently after addition of a mixture of deoxynucleosides (DNS), deoxynucleotides (DNT) and $MgSO_4$(5). It was of interest to determine whether these supplements might also influence the intracellular fate of staphylococci in monocytes from adult and newborn rabbits.

Materials and methods. Staphylococcal strains. S. aureus, 18-Z, described previously (1) was used throughout these experiments. In addition *S. aureus,* BV11+, used in the prior studies *in vitro* by McKee and Braun (4) was employed in some experiments. Organisms of this strain produce bound and soluble coagulase, alpha and delta hemolysins, hyaluronidase and fibrinolysin. Their phage type is 79. They are sensitive to streptomycin, tetracycline, chloramphenicol, erythromycin, novobiocin, and resistant to penicillin and oleandomycin. The intracellular fate of cells of the BV11+ strain was similar to that of the 18-Z strain. The experimental results of this report, therefore, will be limited to data obtained with the 18-Z strain.

Technics of cell culture. Mononuclear phagocytes were collected from rabbits of the New Zealand strain 2 days following intraperitoneal injection of 30 ml mineral oil to adult, or 10 ml of oil to weaned, and 5 ml of oil to newborn rabbits. The peritoneal cavity was washed with Hanks' solution containing heparin at 1:20,000, and the exudate thus collected was centrifuged and the cells resuspended in Hanks' containing 10% autologous serum. Tissue culture chambers and their use have been described in detail(1). Briefly, plastic chambers holding 16 coverslips were used. An overnight culture of staphylococci that had been washed twice with saline was mixed with monocytes in a ratio of 25 cocci per monocyte and 9×10^6 of these bacteria-exposed monocytes were then placed in each plastic chamber. After incubation at 37°C for 30 minutes the chambers were washed, and unless otherwise noted, a medium containing 10% autologous serum and 50 μg streptomycin per ml was added. At regular intervals thereafter, 2 coverslips and a sample of the medium were removed. One coverslip was stained to count the number of monocytes, the other was used for recovery of intracellular staphylococci with the aid of saponin and small glass beads. The recovered cocci were diluted and plated on nutrient agar plates. Simultaneously a sample of medium was plated to estimate the number of any extracellular organisms. All tissue cultures were kept in a 5% CO_2 incubator. In addition to Hanks' solution, McCoy's 5a medium(6) was used in some experiments. Results with this medium were identical to those to be described for Hanks'. In supplemented cultures 100 μg of each DNS and each DNT, plus 12.5 μg of ADP and 0.0016 of $MgSO_4$ were added to each ml of culture.

Experimental results. The intracellular fate of virulent staphylococci in monocytes from 4- to 5-day-old newborn rabbits, their mothers, adult male rabbits and weaned rabbits was compared in a series of experiments. Fig. 1 presents the results of one of several similar experiments. The cocci survived longer in the monocytes from the newborn and weaned rabbits than in corresponding cells from adult animals. However, some weaned rabbits yielded cells that behaved more like those from adult animals than those from newborn animals. There was no evidence for bacterial multiplication within any of these cells.

To determine whether monocytes or serum have the greatest effect on the intracellular fate of staphylococci, several experiments were performed with monocytes from newborn rabbits maintained in sera from either young or old animals. Also, monocytes from adult animals were maintained either in autologous sera or sera from newborn rabbits. Fig. 2, illustrating the results of such an experiment, indicates that the intracellular be-

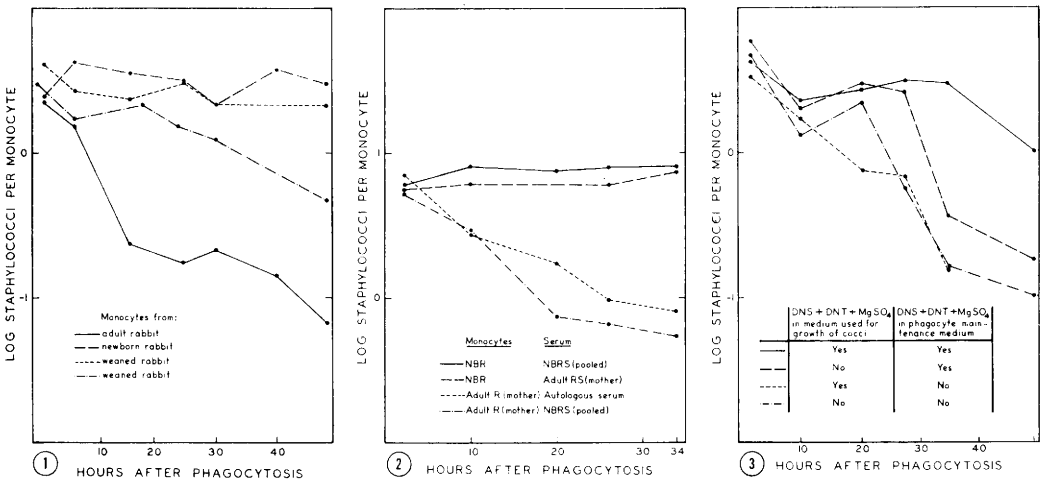

FIG. 1. Intracellular survival of *S. aureus*, 18-Z, within monocytes from newborn, weaned, and adult rabbits. The adult rabbit was the mother of the newborn rabbits.

FIG. 2. Intracellular survival of *S. aureus*, 18-Z, within monocytes from newborn (NBR) and maternal adult rabbits (Adult R) which were suspended in sera from either newborn (NBRS) or adult rabbits (Adult RS).

FIG. 3. Effect of supplementation with a mixture of deoxynucleosides, deoxynucleotides and $MgSO_4$ (DNS, DNT, $MgSO_4$) on intracellular survival of *S. aureus*, 18-Z, in monocytes from an adult rabbit.

havior of the staphylococci is determined principally by the source of the cells and is apparently independent of the source of the serum used.

For tests on the possible influence of a mixture of DNS + DNT + $MgSO_4$, the 18-Z strain of staphylococci was grown in brain heart infusion broth with and without the nucleoside-nucleotide supplement. The bacteria were washed twice with saline and then mixed with monocytes and introduced into unsupplemented tissue culture medium or media supplemented with DNS + DNT + $MgSO_4$. The results (see example in Fig. 3), with monocytes from adult rabbits, showed that intracellular survival was prolonged when bacteria grown in the presence of DNS + DNT + $MgSO_4$ were ingested by monocytes maintained in DNS + DNT + $MgSO_4$-supplemented media. The most pronounced effects occurred when the supplement was added to both the bacterial and the tissue culture medium; when supplementation was restricted to only one of these media it became apparent that supplementation of the tissue culture medium was the most critical factor.

The intracellular fate of staphylococci was not affected by the just described supplement when monocytes from newborn and from certain weaned animals were used. That is, no prolongation of survival of the cocci occurred in those cells in which survival is normally for a prolonged period of time. However, in the case of monocytes from the weaned rabbits that behaved more like adults than newborns, a prolongation of survival was produced by exposure to DNA precursors.

Discussion. These studies have revealed that intracellular survival of virulent staphylococci in mononuclear phagocytes from newborn rabbits is longer than in monocytes from adult rabbits. Also, the studies with the DNS + DNT + $MgSO_4$ supplements have shown that survival of the cocci in monocytes from adult animals can be prolonged by supplementing the cellular environment with DNA precursors; the extent of such prolongation is similar to that occurring in unsupplemented maintenance cultures of monocytes from newborn animals. The differential survival time of virulent staphylococci in phagocytes from animals of different age may, therefore, be attributable to at least one of two factors: The shortened survival of the cocci in cells from adult animals may be a

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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