great deal, thereby suppressing water intake. In warm weather water at warm ambient temperatures cools the tongue very little. As a result, tongue cooling during drinking adjusts water intake to meet water needs.

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Pregnancy Suppression by an Antiserum to the Sperm Specific Lactate Dehydrogenase

Abstract. *An antiserum induced against the isozyme of lactate dehydrogenase (LDH-X) that is unique to spermatozoa reduced significantly the number of pregnancies in mice treated at varying times after they had mated. This effect of the antiserum occurred both prior to and following implantation. The fecundity of treated animals appeared to be normal in subsequent matings.*

The sperm specific isozyme of lactate dehydrogenase (LDH-X) is the tetrameric product of a gene that is active only during the primary spermatocyte stage of spermatogenesis. A homogeneous crystalline preparation of this isozyme has been obtained from mouse testes and used to induce antibody formation in rabbits (*1*). Purity of the antigen preparation was established by disc-gel electrophoresis, analytical ultracentrifugation, and immunochemical analysis. Thus, we have available a potentially useful means of developing information on the relation between a specific, and, more importantly, a well-defined component of the sperm and the process of reproduction. Of particular interest is the area of immunoreproduction. There have been many attempts to influence fertility and to identify immunological causes of infertility by sensitization of both males and females against sperm and testes extracts (*2*). Results obtained from such studies have been variable and interpretations have been conjectural, in part, certainly, because of the complexity of the antigenic material. In this report we present evidence that a specific antiserum to a chemically defined antigen—that is, LDH-X—can reduce fertility in females.

Purification of LDH-X and induction in rabbits of antibody specific to it have been described (*1*). In our work, the complement in the antiserum was inactivated by heating at 56°C for 30 minutes before injection. For most of the experiments 6- to 8-week-old female mice were used from a random-bred Swiss Webster strain maintained in our laboratory during the past several years. In each experimental trial the same proven fertile males were used to fertilize females receiving antiserum (AS) or control serum (CS). Females were placed in a breeding cage during proestrus, and mating was evidenced by the presence of copulation plugs. Injections were made subcutaneously with 0.1 ml of serum. Each female received one injection per day during days 1 to 4, 4 to 7, 7 to 10, or 10 to 13 after coitus. Pregnancy was determined either by examining the uterus or allowing parturition to occur. All animals were caged under a 12-hour daily artificial light regimen and maintained on a diet of Purina chow with free access to water.

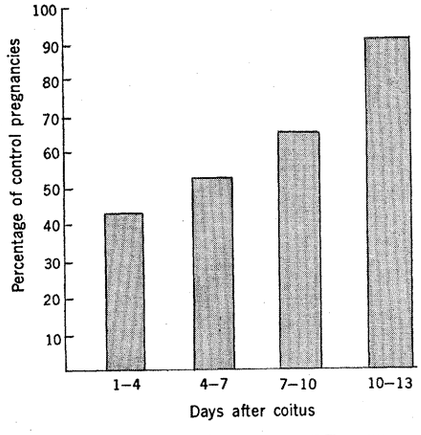

Fig. 1. Effect of antiserum to LDH-X on pregnancy rate in mice. The numbers of animals in each treatment group were as follows: days 1 to 4, 67 CS and 81 AS; days 4 to 7, 72 CS and 72 AS; days 7 to 10, 97 CS and 96 AS; and days 10 to 13, 10 CS and 10 AS.

That postcoital treatment of mice with antiserum depresses the percentage of pregnancies is shown by the data in Fig. 1. The effect of the antiserum appears to be most pronounced when injected on days 1 to 4, and nearly as effective in terminating pregnancy when used on days 4 to 7 after mating. The phenomenon appears to be all or none in a given animal, since AS-treated mice which remained pregnant delivered normal litters, comparable in size to the controls. The antiserum also suppressed pregnancy when injected on days 7 to 10 and apparently was relatively ineffective after day 10 following coitus (Fig. 1).

There are several important controls in the experimental design which are not apparent from the data in Fig. 1 and which should be noted here. Antiserums were obtained from eight rabbits immunized with the same antigen preparation. At least two different antiserum preparations were used for each of the treatment periods during these studies, and control serum was obtained from the same rabbits before their immunization. In all of the test matings, proven fertile males and females were used. There were 67 percent successful pregnancies recorded in the total of 246 control animals. In one treatment group of nine animals (day 1 to 4 after coitus), not included in Table 1, serum absorbed with antigen was injected, and 78 percent of these animals remained pregnant. This result confirms the conclusion that pregnancy suppression was due specifically to the LDH-X antibody.

Injection schedules were established in the experimental protocol to cover the pregnancy stages of oviducal passage of fertilized ova (days 1 to 4 after coitus), implantation (days 4 to 7 after coitus), and postimplantational development. From the results of these treat-

ments it is reasonably certain that antiserum to LDH-X acts primarily by interfering with implantation or causing abortion (or both) after implantation occurs. Whether the antiserum affects the fertilized ovum directly, for example, by causing embryonic failure, or whether it induces the creation of a hostile endometrial environment (or both) remains an open question.

We also ascertained that the antiserum action was temporary, at least after the 1- to 4-day regimen of treatment. Seven mice of an AS group were mated 21 days after their last injection, and all of them delivered normal litters. In addition, the serum injections had no obvious deleterious effects on the mice in terms of weight loss or feeding behavior, nor were there any gross pathological lesions detectable from postmortem examination of viscera.

There is no detectable LDH-X in the female, nor is there any associated with the female reproductive system. We have been unable to detect by immunological tests nonspecific antigens in extracts of various portions of the female reproductive tract which react with the antiserum. Nevertheless, precedents for this phenomenon exist. As was noted above, there is a long history of efforts to control fertility of females immunologically. Of the more recent work, McLaren (*3*) and Bell (*4*) reported fertility reduction in female mice immunized with homologous spermatozoa or testis homogenates. Similarly, infertility has been induced immunologically in cattle (*5*), guinea pigs (*6*), and rabbits (*7*). Bell and McLaren (*8*) found that a subcellular fraction of spermatozoa, the 1200*g* supernatant of disrupted cells, depressed fertility in female mice. Their work indicated that whole spermatozoa were not required for antigenicity and that reduced fecundity was due to immunological impairment of the fertilization process.

Passive immunization causing fertility depression in female mice was reported by Sadri *et al.* (*9*). In this study antiserum induced in rabbits against a saline extract of mouse testes completely inhibited pregnancy in females injected on days 1 to 4 or 6 to 8 after mating, presumably by interfering with implantation. Treatment of two-cell embryos from the rabbit with bovine antiserum against rabbit semen did not reduce their survival rate (*10*). Such antiserum treatment did, however, have a detrimental effect on blastocyst survival (*11*).

Apparently, this is the first report of the effect of an antibody to a specific enzyme on reproductive processes. The data clearly illustrate that this antibody to LDH-X can disrupt pregnancy. Most intriguing is the fact that the enzyme is found only in the male reproductive system while the antiserum to it can act in the female. How this occurs is of course a compelling question of immediate significance to an understanding of the normal reproductive process. Also, the prospect of exploiting this finding in a fertility control program does exist.

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Nucleotide Sequence of a Lysine Transfer Ribonucleic Acid from Bakers' Yeast

Abstract. *The nucleotide sequence of one of the two major lysine transfer RNA's from bakers' yeast has been determined. Its structure is compared to that of a lysine tRNA from a haploid yeast. A total of 21 nucleotides differ in the two molecules. Only the T-ψ-C-G (thymidine-pseudouridine-cytidine-guanosine) loop and its supporting stem are identical.*

The nucleotide sequence of a major $tRNA^{Lys}$ (*1*) from bakers' yeast has been worked out. Its sequence is shown in Fig. 1A in the cloverleaf arrangement. The $tRNA^{Lys}$ was purified from bakers' yeast tRNA (*2*) by countercurrent distribution in an ammonium sulfate system (*3*). The faster moving $tRNA^{Lys}$ was further purified by reverse-phase chromatography with tricaprylmethylammonium chloride (*4*).

The fragments produced by ribonuclease T1 and pancreatic ribonuclease are shown in Fig. 2. Their nucleotide sequences were determined by the specificity of the nuclease used, digestion of pancreatic ribonuclease fragments with ribonuclease T1, digestion of ribonuclease T1 fragments with pancreatic ribonuclease, partial hydrolysis with snake venom phosphodiesterase (*5*), and polynucleotide phosphorylase (*6*). The large fragments isolated after incomplete digestion with pancreatic ribonuclease and ribonuclease T1 in the absence of Mg^{2+} are shown in Fig. 2. These large fragments were analyzed by complete digestion with ribonuclease T1 or pancreatic ribonuclease.

Seventeen of the 76 nucleotides in the $tRNA^{Lys}$ are modified; only the rat liver $tRNA^{Ser}$ (*7*) and the torula yeast $tRNA^{Tyr}$ (*8*) are as highly modified. The $tRNA^{Lys}$ has five pseudouridine residues, including a 5′-terminal pψp. Although Gray and Lane (*9*) found that 4 percent of mixed bakers' yeast tRNA contained pψp, this is the first time that pψp has been found in a purified tRNA.

The anticodon is S-U-U or Z-U-U. The structure of S has been determined by Baczynskyj *et al.* (*10*) to be 2-thio-5-carboxymethyluridine methyl ester. The alternate nucleotide (Z) is probably a derivative or a degradation product of S, since Z and S were both converted to 5-carboxymethyluridine by desulfurization with cyanogen bromide (*11*). Z is probably an oxidation product of S, as treatment with iodine converted S into a product whose spectral properties were very similar to those of Z. The dinucleotide ZpUp does not have a sufficient number of negative charges for Z to be a symmetrical disulfide. It could be a mixed disulfide formed with an unknown sul-