($p < 0.001$). Complete turning occurred less frequently in unsupplemented rat serum ($p < 0.05$).

Supplementation of the rat serum with glucose greatly improved embryonic development (table 2). There were significant differences ($p < 0.0005$) in protein content and somite number compared with embryos grown in supplemented human serum. As before, the difference in frequency of closure of the anterior neuropore was statistically significant ($p < 0.01$), there being more embryos with an open anterior neuropore in human serum.

B) Comparison of development in female vs male human sera. There were no statistically significant differences between data obtained from embryos cultured in female serum and those in male serum (table 3). The protein content of the embryos was approximately double that of embryos cultured in human serum described in A. Closure of the anterior neuropore again occurred in only about 50% of the embryos.

Discussion. The comparison of development in rat and human sera (section A) showed that human serum did not support development as well as rat serum when both had the same glucose concentration. Growth and differentiation were retarded in human serum and there was an increased frequency of neural tube defects. Only when the rat serum was not supplemented with glucose, and used at the relatively low volume of 0.6 ml/embryo, did development in human serum compare favorably in terms of growth and differentiation. This is in accord with the observations made by Ellington[15] on the effects of rat starvation and the subsequent reduction in serum glucose concentration on the ability of sera to support normal development. Even so, the use of rat serum, though unsupplemented, still resulted in a lower incidence of neural tube defects compared with supplemented human sera.

The present observations on growth are not in accord with those of Gupta and Beck[10] who found that there was no significant difference in protein content between embryos grown in human serum compared with those in rat serum. However, the overall conclusion, that rat embryos cannot be reliably cultured in human serum, is the same. Potential solutions to this problem include dilution of human serum with rat serum[9] or a synthetic culture medium.

The growth (protein content) of embryos cultured in human serum was greater in section B than in section A. The most likely explanation for this is the increase in the amount of serum from 0.6 ml/embryo to the more widely accepted 1 ml/embryo. The results obtained in section B showed that male and female human sera supported development equally well, confirming the preliminary observations of Chatot et al.[8] with human sera and New[16] with rat sera. This indicates that male human volunteers could possibly be used to screen for teratogens thereby avoiding ethical problems which would arise from the use of female human volunteers of reproductive age.

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Lysozyme activity in female genital tissues of normal and genetically lysozyme-deficient rabbits[1]

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Summary. The internal genitalia of female normal rabbits and mutant lysozyme-deficient rabbits, which lack genetically the leukocytic isozyme of lysozyme, were assayed for lysozyme activity. The ovaries, uteri, and vaginas of the lysozyme-deficient rabbits had less than 20% of the lysozyme activity of normals. The oviducts, and in particular the caudal portions of the oviducts, had lysozyme activities up to 71% of the levels in normals. These observations suggest that the lysozymes of oviduct and leukocytes of rabbits are under the control of different genes.

Key words. Rabbit; lysozyme-deficient; genitalia; lysozyme activity; isozymes.

Lysozyme (EC 3.2.1.17) hydrolyzes the β-1,4 glucosidic linkages of N-acetylmuramic acid and N-acetylglucosamine and is a cationic, small, and ubiquitous enzyme[2]. The primary identification of lysozyme is based upon its lysis of the cell walls of the bacterium *Micrococcus luteus (lysodeikticus)*. Bacterial cell walls are composed of alternating N-acetylmuramic acid and N-acetylglucosamine moieties, the substrate of lysozyme[3]. These observations suggest that the role of lysozyme is antibacterial. In spite of extensive investigations, however, the role of lysozyme in mammals has not been established.

Lysozyme from chicken egg white has been extensively studied and has been well characterized. The majority of studies on lysozyme have utilized this source of the enzyme and hypotheses on the role of lysozyme in mammals have often been based on studies with chicken egg white lysozyme. Lysozyme occurs in high concentration in human leukocytes and it has been suggested that it functions as an antibacterial agent in leukocytes. Some studies have utilized human leukocytic lysozyme isolated from the urine of human patients with leukemia[4].

Recent studies with several species indicate that lysozyme exists

in multiple molecular forms or isozymes[5-7]. We have described an inherited condition of rabbits consisting of a deficiency of lysozyme[8-10]. The condition is inherited as an autosomal recessive, and there is a tissue specific deficiency of lysozyme. Our recent studies indicate that there are at least two primary isozymes of lysozyme in rabbits that differ in electrophoretic, chromatographic, and kinetic properties and the lysozyme-deficient rabbits lack completely the isozyme associated with the leukocytic cells and tissues[11,12].

In the present study the lysozyme activity of the tissues of the genital tract of lysozyme-deficient rabbits relative to normal rabbits was measured with the intention of determining the relationship of the lysozyme in the genital tissues to that of the other tissues of rabbits.

Materials and methods. The internal genitalia were collected from five lysozyme-deficient and six normal (with respect to lysozyme activity) adult anestrus female rabbits immediately after overanesthetization with sodium pentobarbital. These tissues were divided into ovaries, oviducts, uterus and vagina and the oviducts were further dissected into preampulla, ampulla, isthmus, and junctura[13]. The right and left of each paired organ of each rabbit were combined. All tissues were frozen immediately after collection and were stored frozen at −20 °C until thawed for assay.

All samples of a given tissue were thawed, prepared and assayed at the same time. Tissues were homogenized by a modification of the previously described technique[8]. To each gram of tissue was added 2.33 ml of homogenizing solution (pH 6.2). This solution consisted of a 1:1 mixture of 0.1% Triton X-100 in 0.067 M sodium phosphate buffer (pH 7.3): 1% acetic acid in 95% ethanol. Ovaries and oviduct segments were homogenized in chilled micro plexiglass tissue homogenizers (Bellco Glass Inc., Vineland, NJ, USA) and the uteri and vaginas were homogenized in chilled microblenders (Eberbach Inc., Ann Arbor, MI, USA). The tissue homogenates were centrifuged at 3000 × g for 10 min at 5 °C, the supernatant, except of any lipid layer, was collected and recentrifuged. The supernatants were assayed for lysozyme in duplicate by the lysoplate method of Osserman and Lawlor[4] as previously described[8,10] except that low-sulphate agarose (Marine Colloids, Rockland, Maine, USA) was used[14]. The enzymatic activity of the supernatants was determined as chicken egg white lysozyme equivalent μg/ml. Protein in the supernatants was measured in duplicate by the method of Lowry et al.[15] with the addition of 0.5% sodium dodecylsulphate in the alkali reagent[16] to prevent possible precipitate formation by the Triton X-100. The lysozyme activity in the supernatants was expressed as mean ± SEM chicken egg white lysozyme equivalent μg/mg protein.

Results and discussion. The lysozyme activity in the internal female genital tissues of normal and lysozyme-deficient rabbits is summarized in the table. The ovaries of the lysozyme-deficient rabbits had no detectable lysozyme activity. The uterus and vagina of lysozyme-deficient rabbits had 19% and 10% of normal lysozyme levels, respectively. The lysozyme activity in the oviduct of the lysozyme-deficient rabbits increased relative to normal rabbits in the more caudal portions and was 71% of normal in the junctura, the most caudal portion.

Lysozyme activity* in various portions of the genital tract of lysozyme-deficient and normal female rabbits

Tissue	Rabbit phenotype Normal (n = 6)	Lysozyme-deficient (n = 5)	%of normal
Ovaries	5.1 ± 1.3	0 ± 0	0
Oviduct			
Preampulla	11.0 ± 3.2	2.8 ± 1.1	25
Ampulla	24.1 ± 8.0	14.3 ± 6.8	59
Isthmus	21.3 ± 5.8	14.1 ± 5.1	66
Junctura	7.0 ± 2.3	5.0 ± 1.1	71
Uterus	10.3 ± 4.3	2.0 ± 1.2	19
Vagina	18.0 ± 5.4	1.8 ± 0.9	10

* Mean ± SEM chicken egg white lysozyme equivalent μg/mg protein.

Previous studies with the lysozyme-deficient rabbits have revealed that some cells and tissues are markedly deficient in lysozyme activity[8-12]. These included bone marrow, spleen, lung, salivary glands, lymph nodes, polymorphonuclear leukocytes, bone, cartilage, serum, tears, macrophages, and kidney. In the present study ovaries, vaginas, and uteri had similar marked deficiencies of lysozyme. The previous studies demonstrated that other tissues of lysozyme-deficient rabbits had levels of lysozyme which were only moderately deficient (less than 50% reduction) or nondeficient[8-12]. These included thymus, appendix, sacculus rotundus, ileum, cecum, distal colon, and rectum. In this study the ampulla, isthmus, and junctura of the oviduct correlated with these tissues.

Rabbits have at least two isozymes of lysozyme controlled by different genes. The lysozyme-deficient rabbits lack one of these isozymes. The lysozyme in those tissues of the lysozyme-deficient rabbits that are not markedly deficient in lysozyme, therefore, are under control of a gene different from that which controls the isozyme that is markedly deficient in the lysozyme-deficient rabbits. This suggests that the isozyme of lysozyme present in the leukocytes of normal rabbits is not the isozyme of lysozyme that is the primary constituent of the caudal portions of the rabbit oviduct.

Studies by Peeters and Vantrappen[7] demonstrating different isozymes of lysozyme in mouse intestine and leukocytes, more recently by Hammer and coworkers[17] demonstrating different isozymes of lysozyme in mouse small intestine and most other tissues, and by Dobson and coworkers[18] demonstrating a unique stomach lysozyme in ruminants, correlate with results of studies with lysozyme-deficient rabbits that indicate the intestinal lysozyme is under the control of a different gene than leukocytic lysozyme[10-12]. The totality of these observations suggests that oviduct lysozyme and leukocytic lysozyme are isozymes and that chicken egg white lysozyme and mammalian leukocytic lysozyme are products of different genes.

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