Effects of fasting and refeeding on morphological and biochemical properties of the porcine lens. Y. Lee,* R. G. Kaufman,** and C. deVenecia.***

Severe starvation caused minor morphologic changes in porcine eye lenses, fine punctate opacities in cortex, a few water vacuoles, and enlarged prominent Y sutures. However, these abnormalities disappeared upon rehabilitation. The sedimentation analyses of porcine lens soluble proteins exhibited three components with values for S' x 10^3 of 18.2, 9.0, and 2.7 S. The relative distribution of these components were 21, 36, and 41 per cent for 18.2, 9.0, and 2.7 S components, respectively. The sedimentation patterns and the distribution of three protein components were not affected by severe starvation.

In the lens, older cells become displaced centrally making it unique to the rest of the animal body since old cells are retained and not desquamated. The continuous growth of lens fibers with a concomitant increase in absolute quantity of lens protein at a diminishing rate throughout life is apparently not affected by dietary stress. This unique feature of the lens has made it possible to use the lens as an indicator of age in various animals disregarding the state of nutrition.

The purpose of this study was to investigate the effect of severe starvation and subsequent rehabilitation on the morphologic changes and the distribution of soluble crystallin proteins in the porcine lens.

Materials. Two trials were conducted. In trial 1, nine Hampshire littermate pigs averaging 106 kilograms live weight (7 months old) were divided into two groups. Group 1 consisted of five pigs that were provided water only for 37 days, after which time two pigs of this group were killed and the other three were refed for 32 days to regain their original body weight at which time they were killed. Group 2 consisted of four pigs that were fed ad libitum, two of which were killed after 21 days of fasting. By 42 days of fasting, the water fissures gradually disappeared in most lenses of fasted pigs. On refeeding, the water fissures disappeared and the lenses essentially appeared to be normal. At 57 days of fasting (trial 1) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 2) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 1) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 2) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal.

Results and discussion. Morphologic study. In trial 1, some of the fasted pigs developed small punctate opacities in the anterior lens cortex after 21 days of fasting. By 42 days of fasting, some lenses exhibited anterior or posterior cortical water fissures in addition to a few punctate round opacities. One fasted pig developed small water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 1) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 2) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 1) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal. At 57 days of fasting (trial 2) the anterior Y suture became prominent and engorged. Several water vacuoles adjacent to the inferior arm of the anterior suture. Another fasted pig developed an anterior subcapsular water cleft. Except for these minor changes, all the lenses essentially appeared to be normal.
Fig. 1. A and B, sedimentation patterns for soluble porcine lens proteins of control (upper) and fasted-refed pigs (bottom). C and D, sedimentation patterns for soluble porcine lens proteins of control (upper) and fasted pigs (bottom). Protein concentration was 9.5 mg per milliliter. Photographs were taken at 17 minutes (A and C) and 33 minutes (B and D) after top speed (59,780 r.p.m.) was reached. Sedimentation is from right to left.

and the Y sutures became normal, although some fine opaque dots still remained. In trial 2, similar changes as noted in trial 1 were observed for both "young" and "old" age groups. The lenses of fasted pigs were essentially normal except for a few dotted opacities, small vacuoles, and slightly widened and prominent sutures. Upon refeeding, the vacuoles disappeared and essentially no abnormalities except a few small opaque spots were observed. The only ocular abnormality noted in one pig starved for 101 days (not in the Methods section above) was three to four fine vacuoles in the posterior cortex.

Sedimentation analyses of lens protein. There was no significant difference between the treated group and its corresponding control in fresh lens weight and total lens protein. The sedimentation patterns of soluble lens proteins are illustrated in Fig. 1. The sedimentation coefficients of three components, I, II, and III were 18.2 S, 9.0 S, and 2.7 S, respectively. These values were similar to those reported for bovine lens.7 There was no difference in the sedimentation patterns between breeds, or between full-fed and fasted or fasted-refed pigs.

The relative proportion of three major protein components was determined. Regardless of nutritional stress, age, or breed, approximately 21 per cent of the total soluble protein was accounted for by component I, 38 per cent by component II, and 41 per cent by component III. Cobb, Price, and Koenig8 reported slightly different values; 14, 41, and 45 per cent for components I, II, and III, respectively. These distribution values for porcine lens were appreciably different from those of bovine lens reported by Cobb and Koenig.7

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Valinomycin is a cyclic antibiotic known to selectively mediate K⁺ transport in mitochondria, red blood cells, and artificial membranes. Analogous agents, both microbiologic and synthetic in origin, share with valinomycin the characteristic of forming complexes with alkali metal ions and facilitating their transmembrane movement. These cation conductors have been referred to as ionophores. The implications of these observations may have relevance to carrier-mediated ion transport in the lens. The elucidation of the mechanism of cation selectivity by valinomycin and other related compounds may provide important insight into the mechanisms by which naturally occurring ionic carrier systems function.

In the experiments reported below, valinomycin was used to further characterize specific K⁺-Rb⁺ transport carrier mechanisms in cultured rabbit lenses.

Valinomycin in concentrations of 10⁻⁴ M and 10⁻⁵ M significantly increased the ⁸⁶Rb efflux from rabbit lenses (Fig. 1). The mean ± S.D. ⁸⁶Rb efflux (per cent of total in lens) at four hours incubation (n=18 lenses) was 14.60 ± 2.58, 19.48 ± 4.07, and 16.60 ± 2.73 for controls and 10⁻⁵ M ouabain was 3.95 ± 0.52 x 10⁻⁵, 4.22 ± 0.30 x 10⁻⁵, and 4.56 ± 0.79 x 10⁻⁵ for lenses exposed to valinomycin in concentrations of 10⁻⁴ M, 10⁻⁵ M, and 10⁻⁶ M, respectively. Thus, valinomycin, in concentrations of 10⁻⁴ M increased ⁸⁶Rb lens efflux by 67.9 per cent, at 10⁻⁵ M by 61.9 per cent, and at 10⁻⁶ M had no effect. The ⁸⁶Rb uptake studies demonstrated that valinomycin in concentrations of 10⁻⁴ M, 10⁻⁵ M, and 10⁻⁶ M significantly increased ⁸⁶Rb transport compared to control lenses (Fig. 2). The mean ± S.D. ⁸⁶Rb uptake of lenses incubated for four hours (counts per minute per lens; n=18 lenses) in Tyrode’s media with 10⁻⁴ M ouabain was 3.95 ± 0.52 x 10⁴, 4.22 ± 0.30 x 10⁴, and 3.97 ± 0.14 x 10⁴ for controls and 6.19 ± 0.62 x 10⁴, 4.49 ± 0.64 x 10⁴, and 4.56 ± 0.79 x 10⁴ for lenses incubated in the same media with the addition of valinomycin in concentrations of 10⁻⁴ M, 10⁻⁵ M, and 10⁻⁶ M, respectively. Thus, valinomycin in concentrations of 10⁻⁴ M increased ⁸⁶Rb lens uptake by 56.7 per cent while concentrations of 10⁻⁵ M and 10⁻⁶ M had no effect. The