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DNA in the Core of the Calf Lens (37926)

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(Introduced by E. S. Higgins)
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The lens is a unique organ, as it is composed entirely of a single cell type in its various differentiated forms. Because it is made up of an embryologically pure line of cells, it provides an excellent system for the study of the mechanisms of cellular differentiation. The entire vertebrate lens is enclosed by an outer collagenous capsule, below which is a single layer of cuboidal epithelial cells completely covering the anterior surface. The epithelial cells in the equatorial region undergo cellular differentiation to form the fiber cells which comprise the bulk of the lens. The newer fiber cells constitute a cortex region, while those found in the center of the lens date back to the embryonic growth period and comprise the nuclear region of the lens.

One of the terminal events in the differentiation of bovine lens fiber cells is the pycnosis and degeneration of the nuclei followed by their disappearance. These cells, however, continue to synthesize protein, even in the core of the bovine lens (1). No DNA can be detected in the core by histological or fluorometric methods (1). One could rationalize the simultaneous absence of DNA and continued protein synthesis by the existence of stable messenger RNA (2). However, low levels of RNA synthesis have been detected in these cells (3).

Similarly, no DNA can be detected in the chick lens core by autoradiographic (4) or microspectrophotometric methods (5). However, DNA was shown to be present by an elegant autoradiographic experiment utilizing calf thymus DNA polymerase (6). It was postulated that these pieces of DNA were large enough to code for proteins, yet too small to be detected by more traditional methods.

We were intrigued by the possibility that, like the chick lens, DNA was indeed present in the bovine lens core, but had escaped detection. In this communication, we present evidence that DNA is present in the core of the bovine lens.

Materials and Methods. Calf lenses were obtained from George H. Meyer and Sons, Inc., Meat Packers, Richmond, VA. The calves were approximately 3 months old. [^{3}H]Thymidine (6 Ci/mmole) was obtained from Schwarz/Mann. Chromatography, followed by radioactive scanning revealed no radioactive impurities. Deoxyribonuclease (DNase), RNase-free, was obtained from Worthington Biochemical. Fifty lenses were incubated for 2 hr at 37° in 125 ml of Hanks solution containing 1000 units of penicillin, 1000 μg of streptomycin, and 0.2 ml of [^{3}H]thymidine (0.2 mCi). At the conclusion of the experiment, the medium was filtered through Millipore filters, which were dried, and the radioactivity determined. No radioactivity above background was detected. Hence, it was concluded that no significant microbial growth occurred during the course of the incubation. The lenses were washed in cold Hanks solution (4–6°) and held in cold Hanks solution prior to the next step. The lenses were

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separated into three preparations, according to Papaconstantinou (3). This method affords a reasonable separation (3). An incision was carefully made in the lens capsule, and the capsule was removed with the aid of small forceps. Most of the epithelial cells adhere to the capsule. The capsule was then rinsed several times in Hanks solution to remove adhering cortex cells. This is the epithelial layer. The remainder of the lens was stirred in homogenization buffer (0.1 *M* Tris, 0.1 *M* KCl, 0.015 *M* $MgCl_2$) containing 1% sodium dodecyl sulfate (SDS). The buffer was decanted about 15 sec after the addition of the decapsulated lens, to allow any adhering epithelial cells to slough off. This was done for each lens. After an additional 5 min, the buffer was decanted and retained. This is the cortex layer. The remaining portion of the lenses were pooled in buffer. Alternate stirring and decantation was performed several times over the next 30 min. The remaining portion of the lens, about 20% by weight, constitutes the nuclear layer or core of the lens. The epithelial and cortex layers were homogenized with a Kontes ground glass homogenizer. The nuclear layer was homogenized in a Virtis omni-mixer. All of the homogenates were centrifuged in a Sorvall type SS-34 rotor at 16,000 rpm for 30 min at 4° using a Sorval Superspeed RC2-B. The supernatant was retained for determination of radioactivity, and 0.1 ml was applied to filter paper discs (Whatman 3MM). Some of the discs were treated with 5% trichloroacetic acid (TCA) for 10 min, rinsed with TCA (2 times), and then washed in 95% ethanol (3 times) and ether (3 times). The discs were dried and counted in toluene–fluors (2,5-diphenyloazole-1,4-bis[2-(5-phenyloxazolyl)]benzene) in a liquid scintillation counter (Nuclear-Chicago, Mark I) at 35% efficiency for tritium. Control experiments revealed no loss of DNA from the discs. Rat liver DNA (mol wt 2.5×10^6, sp act 400 cpm/μg) remained bound throughout the procedure.

The approximate sedimentation coefficient, S, was determined from a 5–20% sucrose gradient with a 4S RNA as a marker (7). The marker RNA was centrifuged in a separate tube in the same rotor as each sample.

Supernatant (0.5 ml) from homogenized lens fractions previously described was layered onto a 5–20% sucrose gradient and centrifuged in a Spinco SW-39 rotor at 20,000 rpm for 17 hr at 4° with an L3-50 ultracentrifuge. The tubes were punctured from the bottom with a device designed by Salo (8), and 12-drop fractions were collected on Whatman 3MM discs. The radioactivity was determined as previously described. After counting, the discs were rinsed with toluene, dried, and treated with pancreatic deoxyribonuclease (DNase), 10 μg/ml for 30 min at 37°. Control experiments revealed that previous counting followed by toluene washes had no effect on the DNase sensitivity of DNA bound to the discs. The reaction was stopped by placing the discs in cold 5% TCA for 10 min. The discs were rinsed with cold TCA (2 times), then 95% ethanol (3 times), then ether (2 times). The procedure itself does not remove DNA from the discs. Discs containing radioactive DNA, but not treated with DNase, lost less than 2% of their radioactivity. In addition, the DNase was shown to be ribonuclease free, as rRNA with a specific activity of 15,700 cpm/μg (9) was unaffected by treatment with this DNase.

Results. All attempts to detect DNA in the core by the diphenylamine method (10) failed. We also prepared extracts from the cores of 200 calf lenses, employed a usual DNA purification procedure (9), and again failed. It is probable that the large amounts of protein in the lenses would trap small amounts of DNA during conventional procedures designed to achieve a partition between DNA and protein. It should be stressed that our use of SDS in the extraction buffer not only aids in a clean separation of the three layers of the lens, lysis of the cells, and removal of much of the protein, but also would be expected to dissociate most protein-DNA complexes (11).

The incorporation of [^{3}H]thymidine into macromolecules of the various layers of the calf lens is presented in Table I. There is considerable transport of thymidine even

TABLE I. Incorporation of [^{3}H]Thymidine Into Calf Lens.[a]

Layer	Untreated (cpm/lens)	TCA-Treated (cpm/lens)
Epithelial	4,559	551
Cortex	44,677	5,673
Nuclear (core)	34,125	12,338

[a] The procedure is described in "Materials and Methods."

into the core of the lens (nucleus fraction), as indicated in the second column which represents total radioactivity per lens. The estimated amount of incorporation of [^{3}H]-thymidine into macromolecules is given in the third column. There is less total incorporation in the epithelial layer, because this is the smallest cell population, comprising a layer under the capsule which is one cell thick. There is extensive incorporation into the cortex layer. This is the layer underneath the epithelial cells which is comprised of fiber cells that have differentiated from the epithelial cells. Most surprising is the extensive incorporation into the nucleus layer. This part of the lens is composed of fiber cells that were formed during the embryonic life of the organism and have been without a nucleus for several months. These experiments were repeated two additional times, with essentially similar results.

In an attempt to further certify that [^{3}H]-thymidine was indeed incorporated into macromolecules as revealed by Table I, 0.5 ml of the supernatant from each fraction was sedimented through 5–20% sucrose gradients. The results are presented in Fig. 1. The S values (Svedberg constants) obtained for each fraction indicate that [^{3}H]thymidine was incorporated into DNase-sensitive molecules which possess a large molecular weight. These results were repeated two additional times (with different lens preparations) with similar results.

Discussion. The data presented in this paper are consistent with the interpretation that DNA occurs in the core of the calf lens. It is difficult, however, to believe that DNA synthesis occurs in the nuclear, or core layer, as it is comprised of cells devoid

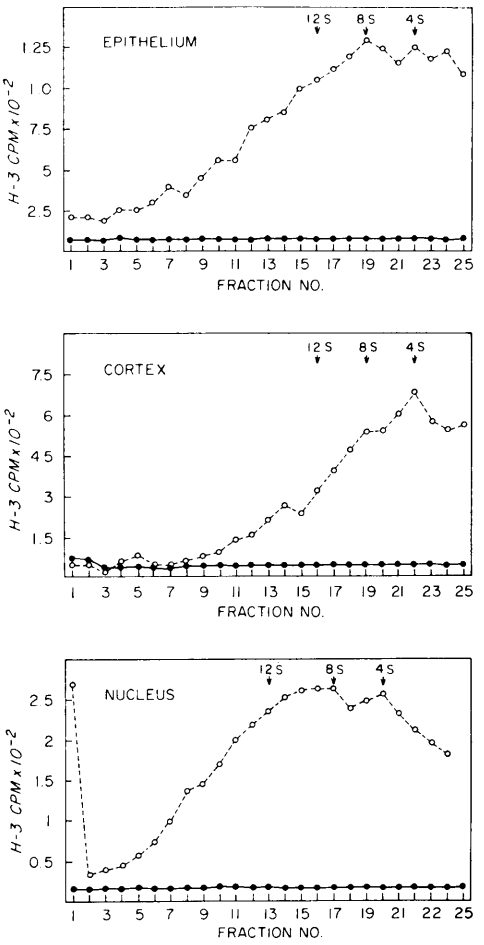

FIG. 1. Sedimentation of DNA isolated from calf lens. The conditions are given in "Materials and Methods." (○—○) no DNase treatment, (●—●) DNase treatment.

of a nucleus. It is to be expected that our procedure would remove any contaminating epithelial or cortex layer cells. The occurrence of DNA synthesis in the cortex layer could perhaps be accounted for if one assumed that some of the cells present have just finished the differentiation process, but have not yet lost their nuclei, and that others still retain DNA, but this assumption is difficult to apply to the nuclear layer. The most probable explanation is that these cells have retained small pieces of DNA which can be utilized as templates, probably by a repair enzyme (or enzymes) other than a true, replicative, DNA polymerase.

Summary. A study of DNA in the bovine lens has indicated its presence in all layers of the lens. Lenses were obtained from freshly slaughtered calves and cultured in a medium containing [^{3}H]thymidine. The lenses were then separated into three cellular layers: epithelial, cortex and nuclear. Measurements of radioactivity of fractions from sucrose gradients of each preparation provided evidence for the presence of DNA. The radioactivity was sensitive to DNase. Apparently, pieces of DNA have been retained even in the core of the lens and these pieces can be utilized as templates by a repair enzyme(s).

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