flashed adapting background. Substitution of a steady long wavelength adapting field did not appear to significantly alter the results. The rat increment threshold curves still showed rod saturation. The flashed background, however, simplifies the interpretation by excluding the possibility of attributing the effect to the exhaustion of rhodopsin in the rods.

The next experiment, aimed at identifying the pigments responsible for dark- and light-adapted rat ERG's, involved determining the flash energies needed to produce criterion responses at several different wavelengths. Spectral sensitivity measurements were made on the dark-adapted rat retina. These measurements are plotted in Fig. 3 where the solid curve is for a Dartnall nomogram pigment peaking at 500 nm. These determinations show that the dark-adapted retina has the sensitivity of ordinary rods. Next, the retina was light adapted with a flashed background adjusted to be 1 log unit above the lowest level of background which produces saturation. Light adaptation produces a second spectral sensitivity curve showing increased long wavelength sensitivity without a significant shift in the peak.

Many years ago Granit (*13*) measured the spectral sensitivity of single units in the light-adapted rat retina. His photopic spectral sensitivity curves had two maximums around 500 nm and 600 nm. This led to the hypothesis that cones filled with visual purple and cones with a long wavelength sensitive pigment were connected to a common channel which mediated the light-adapted responses. These notions are consistent with the findings reported here.

There is suggestive physiological evidence in the literature for two mechanisms contributing to flicker fusion of the rat ERG (*2, 3*). Previous experiments have shown a clear break in the curve relating flicker fusion frequency and light intensity. To examine the relationship between the findings reported here and the flicker fusion studies, a sectored disk driven by a variable-speed motor interrupted the light from the flashed background. The speed of disk rotation was varied to produce a 20-μv flicker response. The flicker fusion frequency increased only slightly (Fig. 2) over the first 3-log increase in stimulus intensity. At about −4 log mean intensity the flicker fusion frequency rapidly increased. The break in the flicker fusion frequency curve occurred at about the same intensity as the shift in spectral sensitivity.

In summary these findings suggest that there are at least two receptor mechanisms in the rat retina: a classical rod, and another mechanism differing from a rod in absolute threshold, spectral sensitivity, and speed of response. The presence of more than one receptor mechanism explains the discrepancy between the limited response range found for the human rod system and the great range of light adaptation exhibited by the rat retina.

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Calcium Carbonate Concretions: Cyclic Occurrence in the Hamster Vagina

Abstract. *Three crystalline forms of calcium carbonate were identified in washings of the hamster vagina. Spherical concretions of vaterite and hexagonal concretions of calcite predominate on days 3 and 4 of the 4-day estrous cycle. Dumbbell-like concretions of aragonite predominate during pregnancy and pseudopregnancy. Each polymorph is associated with an acid-insoluble matrix. Concretions disappear after ovariectomy and reappear during daily injections of estrogen and progesterone.*

It is common practice to follow the 4-day estrous cycle of the golden hamster (*Mesocricetus auratus*) by examining vaginal washings or smears daily for their cellular content. A massive discharge of nucleated epithelial cells suspended in mucin occurs every fourth day (*1*). We designate this as day 1 of the cycle. Under our laboratory conditions, ovulation occurs about 2:00 a.m. of day 1 and estrus begins about 9 hours earlier (*2*). Cellular changes characterizing each day of the hamster cycle have been described in detail (*3*), but the occurrence of noncellular calcareous concretions in the vagina has not been reported.

We observe these concretions in vaginal washings, obtained each morning with a dropper and tap water and examined while still wet under a microscope (× 100), on days 3 and 4 of the estrous cycle and during pregnancy and pseudopregnancy. They are absent on days 1 and 2 of the estrous cycle, during lactation, during acyclic periods due to

Table 1. Analysis by atomic absorption spectrophotometry of vaginal concretions collected on days 3 and 4 of the 4-day estrous cycle (E) and on days 5 to 15 of pregnancy and pseudopregnancy (P).

Sample source	Hamsters (No.)	Collections (No.)	Concretions					
			Micrograms	Micrograms per day*	Ca (%)	Mg (%)	Na (%)	K (%)
E	68	558	31.5	56	33.7	1.18	0.34	
E	34	218	11.8	54	33.9	1.24	.37	0.036
P	36	351	28.1	80	34.6	0.54	.27	
P	22	196	14.2	72	34.4	.53	.31	.028

* Micrograms of concretions produced per hamster per day.

ovarian regression, and from the eighth day of age, when the vagina opens, to 1 or 2 days before puberty (the first vaginal discharge), which occurs on about the 28th day of age. They disappear after ovariectomy and reappear during daily subcutaneous injections of both 17β-estradiol (0.1 or 25 μg) and progesterone (1.25 mg), neither hormone alone being effective. As little as 100 pg of estradiol in 10 μl of oil placed in the vagina of ovariectomized hamsters injected with progesterone (1.25 mg daily) produced concretions within 24 hours. This sensitive animal system may be useful for the bioassay of estrogen or progesterone, or both. The number, form, and occurrence of concretions are unaffected by supplementing the standard diet of Purina Laboratory Chow (0.85 percent Ca) with oral doses of calcium lactate or acetate (0.56 g of Ca per kilogram of body weight daily) or by replacing the standard diet with Corn Chex (Ralston Purina Co.), a low-calcium (<0.01 percent), vitamin-enriched cereal. Concretions are not found in uterine washings, feces, or urine; nor is urine, which might conceivably enter the vagina, the source of calcium (*4*). It therefore appears that the concretions form from substances secreted by the vagina under the influence of estrogen and progesterone from the ovaries.

Concretions occur in three basic forms, each with characteristic variations. Spherical (Fig. 1A) and hexagonal (Fig. 1B) forms greatly predominate over dumbbell-like forms (Fig. 1C) on days 3 and 4 of the estrous cycle. If mating occurs on day 4, as evidenced by the presence of sperm in the vaginal discharge of the next morning (day 1 of pregnancy), enormous numbers of small spherical forms appear on day 3 of pregnancy. This phenomenon also occurs on day 3 of spontaneous or mating-induced pseudopregnancy. On day 4 of pregnancy or pseudopregnancy, enormous numbers of dumbbell-like forms usually appear. These predominate from day 5 to the day of birth (day 16) or to the end of pseudopregnancy (usually day 9).

Occasionally in an individual, either spherical or hexagonal forms greatly predominate on day 3 or 4 of the estrous cycle. On such occasions we collected almost pure samples of each. A third sample of almost pure dumbbell-like forms was collected on day 5 of pregnancy. Concretions were separated from cells and debris by making use of the greater density of concretions (*5*). Each sample was photographed while still wet through a light microscope at $\times$ 100 (Fig. 1). Analysis (*6*) of x-ray diffraction photographs of the three samples (Fig. 2) indicated that the spherical, hexagonal, and dumbbell-like forms contain vaterite, calcite, and aragonite, respectively. The spotty appearance of the calcite diffraction lines and the uniform intensity of the vaterite and aragonite lines indicate that the calcite sample contains much larger crystals. This suggests that the hexagonal forms (Fig. 1B) are single crystals and the spherical (Fig. 1A) and dumbbell-like forms (Fig. 1C) are aggregates of crystallites. When vaterite is prepared synthetically, growth frequently occurs in the form of spherulites composed of radial arrays of small crystals (*7*). Such arrays could account for the uniform intensity of diffraction lines observed for spherical concentrations.

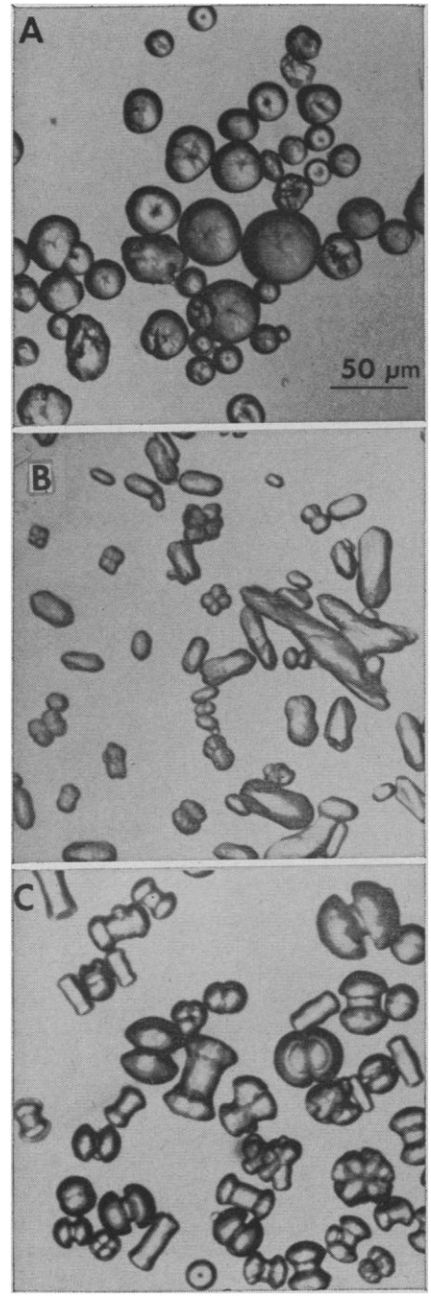

Fig. 1. Spherical (A), hexagonal (B), and dumbbell-like (C) concretions of calcium carbonate normally occurring in the hamster vagina.

Four additional samples, two of which were mixtures of vaterite and calcite forms from cyclic hamsters and two predominantly aragonite forms from pregnant hamsters, were analyzed for calcium, magnesium, sodium, and potassium content (Table 1). Only traces of sodium and potassium were detected. The magnesium contents of the vaterite-calcite mixtures were more than twice those of the aragonite samples. Note in Fig. 2 that x-ray diffraction lines for vaterite and calcite, but not aragonite, forms are displaced to slightly higher angles when compared with their respective standards. These shifts to higher angles indicate a displacement of *d*-spacings to lower values and are probably a result of lattice substitution of the smaller Mg^{2+} ion for Ca^{2+} ions. All four samples contained about 34 percent calcium, which is less than the calcium content of $CaCO_3$ (40 percent). This sets an upper limit of about 85 percent for the $CaCO_3$ content of concretions; $MgCO_3$ could account for an additional 4 percent. At least part of the remaining 11 percent was noncarbonate material. This was indicated in the first step of the analysis when 6*N* HCl was added to dissolve the samples. After vigorous effervescence, a residue remained. It dissolved when heated and remained in solution after cooling.

When concretions were observed under a microscope ($\times$ 100) as 6*N* HCl was added, all forms immediately effervesced and amorphous particles, much smaller than the original concretions, remained. However, when 0.01 to 1*N* HCl or 0.01 to 6*N* acetic acid was added, the rate of effervescence was reduced and concretions dissolved slowly, revealing a matrix that retained the shape of the original concretion. Vaterite forms dissolved first, then aragonite, and then calcite. Matrices are insoluble in water, dilute acid and base, methanol, and chloroform. Their identity is unknown.

The principal forms of $CaCO_3$ found in nature are calcite and aragonite (*8*, *9*), one or both constituting, for example, coral reefs, foraminiferous deposits, molluscan shells, otoliths, and algal cases (coccoliths). Vaterite has been found in a calc-silicate rock in

Northern Ireland (*10*); in sedimentary rock in Israel (*11*); in spicules of a nudibranch (*12*); in coccoliths of algae grown in a nitrogen-deficient medium (*13*); in regenerated parts of molluscan shells (*12, 13*); in mantle tissue of a freshwater snail (*14*); in urinary, biliary, and pancreatic calculi (*15*); in aberrant otoliths of codfish (*16*); and in normal otoliths of the sturgeon, bichir, bow-fin, and gar-pike (*9*). The latter (*9*) and present findings appear to be the only reports of the normal occurrence of vaterite in vertebrates.

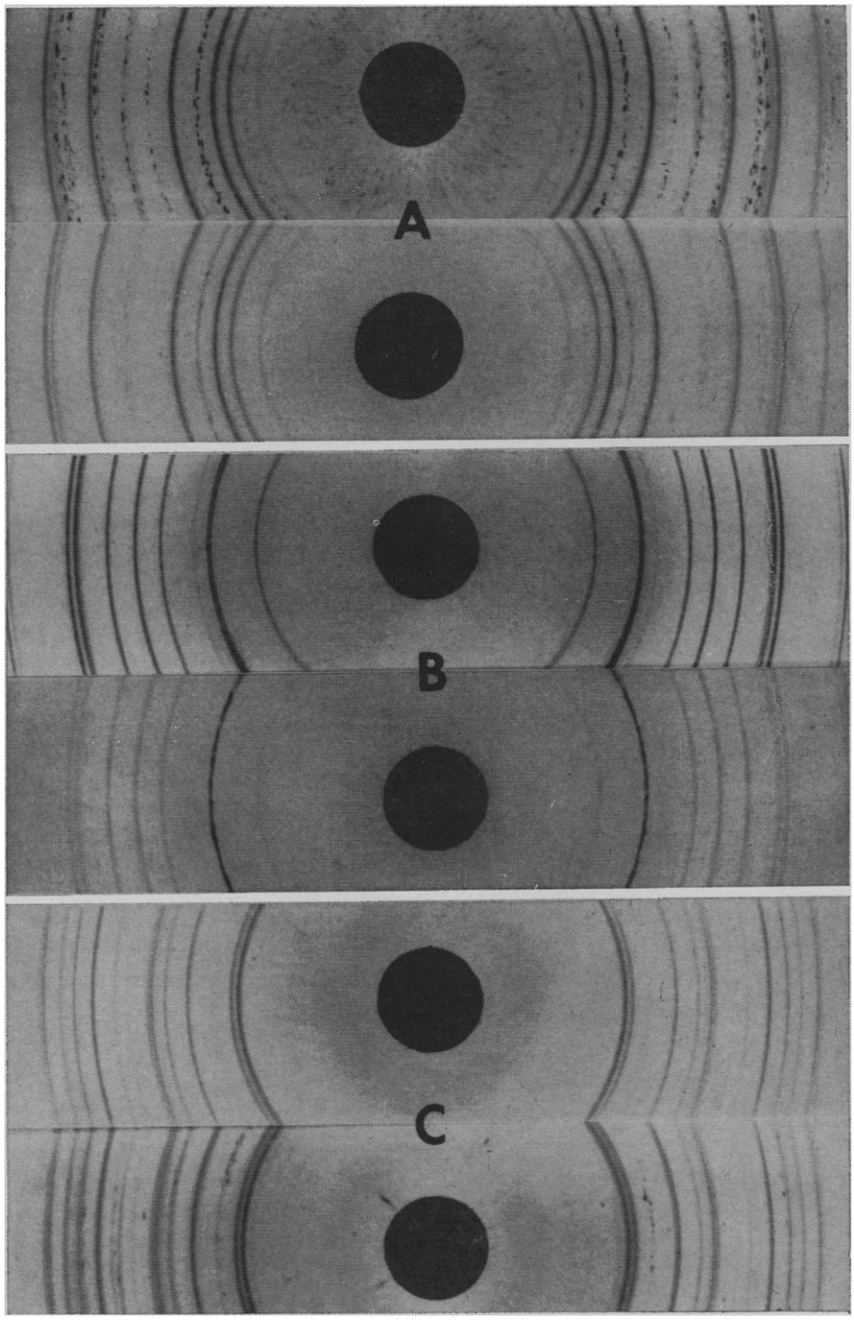

Fig. 2. X-ray diffraction photographs of calcium carbonate concretions from the hamster vagina. (A) Upper pattern, synthetically prepared mixture of vaterite and calcite; lower pattern, sample of predominantly spherical concretions (Fig. 1A). The uniformly intense lines are from vaterite and the spotty lines are from calcite. The dominance of the uniformly intense lines in the lower pattern indicates that the sample consists mainly of vaterite. No aragonite lines are evident. (B) Upper pattern: synthetic calcite; lower pattern: sample of predominantly hexagonal concretions (Fig. 1B). The matching of these two patterns establishes the hexagonal forms as calcite. No vaterite or aragonite lines are evident. (C) Upper pattern, geological aragonite [from Aragon, Spain (Smithsonian Institute Collection No. 16900)]; lower pattern, sample of predominantly dumbbell-like concretions (Fig. 1C). The dominance of the aragonite lines in the lower pattern establishes this polymorph as the major phase in this sample. The spotty lines are from calcite. No vaterite lines are evident.

The rarity of vaterite is probably due to its instability and tendency to recrystallize to calcite and aragonite. The stabilizing factor in the hamster vagina may be the chemical makeup of the matrix associated with vaterite in spherical concretions. The three forms of concretions might result from chemically different matrices, each specifying the polymorph precipitated. Shell formation in mollusks is thought to proceed in this way. Insertion of decalcified matrix from an aragonitic species between the shell and mantle of a calcitic species resulted in deposition of aragonite (*13, 17*).

Morita and Chang (*18*) recently reported that the presence of Ca^{2+} was necessary to maintain sperm motility, under in vitro conditions, in the hamster but not in the rat, guinea pig, or rabbit. We have not found calcareous concretions in vaginal washings obtained daily from rats. While calcium phosphate concretions were obtained from the vagina of mice made permanently acyclic by neonatal injections of estrogen, they were not found in the vagina of normal cyclic mice (*19*). The presence of calcium carbonate concretions in the hamster vagina during the evening of day 4, when mating occurs, supports the view that calcium may be needed for sperm motility in this species.

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5. Vaginal washings were placed on a microscope slide. As the slide was rocked, cells became suspended and were removed by tilting the slide long enough for the cell suspension, but not concretions, to fall off. This procedure was repeated at least five times with additional water.
6. X-ray diffraction photographs of the three

samples were taken in a Philips Debye-Scherrer type powder camera (114.59 mm diameter) with the use of nickel-filtered copper radiation and exposure times of 2 to 4 hours. Samples were prepared for examination by mounting them in thin-walled glass capillaries (0.3 mm in diameter). The identity of the diffraction lines in the recorded patterns was established in two ways: (i) by visual comparison with the x-ray diffraction patterns of standard materials recorded under identical conditions; (ii) by comparing interplanar spacing, *d*, and the intensities, *I*, of the observed lines against published standards (Powder Diffraction File, American Society for Testing and Materials, Philadelphia, Pa.). For this latter comparison, the angular position of each diffraction line was measured to a precision of 0.05° 2θ and then converted to the corresponding *d*-value through use of appropriate tables [H. E. Swanson, *Tables for Conversion of X-ray Diffraction Angles to Interplanar Spacing*, National Bureau of Standards Applied Mathematics Series, vol. 10 (U.S. Government Printing Office, Washington, D.C., 1950). The relative intensities were estimated visually.

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Informational DNA Synthesis Distinguished from That of Nuclear DNA by Inhibitors of DNA Synthesis

Abstract. *With inhibitors of DNA synthesis used in the presence of ethidium bromide, it has been possible to distinguish between synthesis of informational DNA (I-DNA) and that of nuclear DNA. Hydroxyurea depresses I-DNA synthesis preferentially but does not affect DNA transport between cell compartments. 5-Fluorodeoxyuridine and cytosine arabinoside reduce synthesis of I-DNA to a much lesser degree than that of nuclear DNA.*

The discovery in chick embryos of a class of DNA's, informational DNA (I-DNA), which, it was suggested, represented copies of nuclear genes recovered in the cytoplasm was reported a little over a year ago (*1*). In response to the original paper Fromson and Nemer, on the basis of experiments with sea urchins, said that the finding was artifactual and represented contamination of the cytoplasm with nuclear DNA, which occurred during cell homogenization and fractionation (*2*). The Fromson and Nemer report has been cited uncritically (*3*).

If synthetic processes in developing sea urchin cells are like those in embryonic chick or mouse cells, Fromson and Nemer would have seen virtually *no* I-DNA synthesis during the 3-hour labeling period employed, especially since their experiments were carried out at 18°C, at which temperature rates of biochemical reactions must be substantially lower than those at 37°C. After labeling mouse or chick cells for 3 hours at 37°C, no 16*S* or larger-sized free I-DNA–containing particles (I-somes) can be observed in cytoplasmic extracts. It is therefore unlikely that Fromson and Nemer would have seen I-DNA in their cells, if it was being synthesized.

Nevertheless I have tried to settle the problem of whether the DNA in question arises in the nucleus and belongs there but not in the cytoplasm.

Control experiments which support the reality of I-DNA and describe its functional association with other particles under conditions of relatively high ionic strength, in the cytoplasm, have since been performed (*4–6*). However, to add to the weight of evidence needed to strengthen a heterodox view, we have continued to look for ways to distinguish between nuclear DNA and nonmitochondrial cytoplasmic DNA (I-DNA) of probable nuclear origin.

A new set of experiments has revealed fresh distinctions. By means of inhibitors of DNA synthesis, it has been possible to view separately the synthesis of I-DNA from that of nuclear DNA. It appears that I-DNA is much more sensitive to the inhibitory action of hydroxyurea (HU) than is nuclear DNA. The reverse is true when DNA synthesis is inhibited with 5-fluorodeoxyuridine (FUdR). A third inhibitor, cytosine arabinoside (CA), like FUdR, appears to favor the synthesis of cytoplasmic nonmitochondrial DNA but only of low molecular weight. Cytosine arabinoside blocks the formation of 16*S* and larger DNA-containing particles seen in extracts from control cells.

In general, for all of the experiments reported here, chick breast muscle from 11-day embryos was first incubated with an inhibitor of chromosomal DNA synthesis. [^{3}H]Thymidine was then added and the incubation was continued. Ethidium bromide (EB), which blocks mitochondrial DNA synthesis at a concentration of 1.0 μg/ml (*7*), was present throughout the incubation period. Cells of the tissue pieces were dissociated with trysin, and the washed cells were opened without homogenization (*8*) by means of Triton X-100. Nuclei were separated from the cytoplasm by centrifuging the extract at 10,000 rev/min in a Sorvall centrifuge at 4°C.

Hydroxyurea inhibits the synthesis of I-DNA; it affects the synthesis of nuclear DNA to a much lesser degree. In cells incubated for 5 hours with the inhibitor, the amount of labeled I-DNA that is recovered in the cytoplasm is 18.7 percent of that in the control cells, whereas the amount of labeled nuclear DNA recovered is 67.5 percent of that in the control cells, a 3.5-fold difference (Fig. 1A and Table 1).

In a similar experiment FUdR acts in an opposite sense (Fig. 1B and Table 1) when cells are incubated with the inhibitor continuously. During a 5-hour period of incubation, synthesis of nuclear DNA is reduced to about 9 percent (Table 1) of that in the control cells, while that of I-DNA is reduced to 44 percent of the control

Table 1. Incorporation of radioactivity (count/min) into nuclear DNA. Nuclei from cells used in the experiment described in the legend to Fig. 1 were dissolved overnight in 2 ml of SDS buffer (0.1*M* NaCl, 10^{-3}*M* EDTA, 0.5 percent sodium dodecyl sulfate, 0.01 tris; *p*H 7.4) made 1.0 percent with deoxycholate. Before a portion was taken for determination of radioactivity associated with DNA, the volume was raised to 3 ml with distilled water.

	HU	FUdR	CA
Control	3.74×10^6	2.79×10^6	1.73×10^6
Experimental	2.53×10^6	0.23×10^6	0.02×10^6