and rotation axes are coincident, then we have here at least a theoretical basis for a determination of the direction of the projected rotation axis on the plane of the sky.

* NOTE: The work leading up to the writing of this paper was done at the Harvard College Observatory during the spring of 1950.

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HORMONAL INFLUENCE ON NUCLEAR SYNTHESIS. I. ESTROGEN AND UTERINE GLAND NUCLEI

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In a recent publication[1] Salvatore has pointed out that injection of estrogen into ovariectomized rats results in a doubling of nuclear volume in uterine gland cells. Progesterone does not produce this effect. Consequently the author concluded that his observations and other evidence "clearly show(s) the genic nature of rhythmical growth" and that "estrogen acts on the genes by inducing their reproduction."

Inasmuch as these conclusions were based solely on karyometric analysis, it seemed desirable to test Salvatore's hypothesis of genic duplication by comparing the amounts of some nuclear components in uterine gland nuclei before and after doubling of nuclear volume. Recently developed photometric techniques[2, 3] make it possible to measure amounts of protein as well as desoxyribonucleic acid (DNA) in single nuclei. Furthermore it has been shown repeatedly that the latter substance constitutes a reliable measure of the state of ploidy of a nucleus—a definite amount of DNA being associated with a haploid set of chromosomes.[4–7]

For the purpose of this experiment several 4-month-old Long-Evans S-1 rats were ovariectomized; 6 weeks later, some of these were injected intramuscularly with 250 I. U. of estrone in sesame oil, others with 0.5 mg. of progesterone, daily for 4 days. Vaginal smears were taken at the time of sacrifice, 6 hours after the last injection, to determine the efficacy of castration and of hormone treatment. Uterine segments were fixed in acetic alcohol. Paraffin sections of the uterus from one castrate control, one estrogen-treated and one progesterone-treated animal were mounted together on slides and treated with the Feulgen reagent for determination of DNA and the Millon reagent for determination of tyrosine and trypto-

phane. The amount of colored substance in single nuclei was determined from its specific light absorption measured with a sensitive phototube through a microscope. Techniques and methods of calculation are discussed in a previous publication.[8] The results of the DNA measurements are given in table 1.

The volume determinations incidental to these measurements confirm the observation of Salvatore, namely, that estrogen treatment caused a doubling of nuclear volumes in the uterine gland cells. Progesterone alone caused no such increase. The effect of simultaneous administration of estrogen and progesterone is still to be determined. The basic nuclear volume in the present material is slightly different from that reported by Salvatore (77.1 μ^3 instead of 88.1 μ^3). This may be due to a difference in fixation and/or to differences in the strain of rats used and is not considered to be important. The Feulgen measurements clearly indicate that the nuclei in all three animals contain essentially the same amount of DNA.

TABLE 1

AMOUNTS OF DNA (ARBITRARY UNITS) IN INDIVIDUAL NUCLEI OF UTERINE GLANDS OF THE RAT

ANIMAL	NUMBER OF NUCLEI MEASURED	NUCLEAR VOLUME IN μ^3	AMOUNT OF DNA
Castrate control	20	77.1 ± 1.0	2.41 ± 0.09
Estrogen-treated	20	157.5 ± 1.6	2.36 ± 0.08
Progesterone-treated	12	81.6 ± 1.5	2.15 ± 0.08
Tetraploid liver nuclei of a normal animal	10	[a]	5.04 ± 0.15

[a] Nuclear volume omitted, since there is often no consistent relationship among values obtained from nuclei of different tissues.[4]

Furthermore, this amount is that of a typical diploid nucleus, as is demonstrated by comparison with values obtained from tetraploid liver nuclei of a control rat measured on the same slide. Thus, injection of estrogen has not resulted in doubling of DNA content in uterine gland nuclei, and polyploidy, as would be indicated by an increased amount of DNA, evidently has not been induced by the hormone.

Doubling of nuclear volume, however, is a feature which normally accompanies polyploidy within a tissue. In mouse liver, for example, three size classes and three corresponding classes of DNA content in a ratio of 1:2:4 occur.[4, 6, 7] It has been argued[9] that the increase in nuclear volume in such a case is probably not due to the increase of DNA alone but rather to increased protein content, inasmuch as such nuclei contain anywhere from 8 to 15 times more protein than DNA.[10] This argument is supported by observations on a number of cases, where changes in nuclear volume are not accompanied by changes in DNA content but by parallel changes in

total protein content.[8, 9, 11] Table 2 indicates that this is also true in the present case.

The extinctions due to the Millon reaction are nearly the same in sections of equal thickness of small and large nuclei; this indicates that the protein concentrations in nuclei of castrate control and estrogen-treated animals are very similar. The total amounts of protein in these nuclei are accordingly correlated with the nuclear volumes, and it appears that estrogen treatment has induced an approximate doubling of the protein content in uterine gland nuclei.

This particular case of nuclear protein synthesis independent of DNA synthesis is similar to that reported by Schrader and Leuchtenberger.[9] In the testis of the hemipteran insect *Arvelius albopunctatus*, spermatocyte nuclei in different lobes of the testis stand in a volume relation of 1:2:8 but contain the same amount of DNA. The same concentration of protein was found in the medium- and large-sized nuclei. The authors then draw attention to the amazing parallelism between nuclear size and protein content and indicate their opinion that a rhythmic growth of extrachromosomal proteins is involved. The functional significance of this

TABLE 2

PROTEIN CONCENTRATIONS IN UTERINE GLAND NUCLEI AS INDICATED BY THE EXTINCTIONS (OPTICAL DENSITIES) DUE TO THE MILLON REACTION OVER 4 μ PATH LENGTH

ANIMAL	E_{TEST} (12 MEASUREMENTS)	E_{BLANK} (10 MEASUREMENTS)	$E_{CORRECTED}$ (TEST − BLANK)
Castrate control	0.157 ± 0.010	0.037 ± 0.002	0.120 ± 0.010
Estrogen-treated	0.147 ± 0.006	0.042 ± 0.003	0.105 ± 0.007

phenomenon in *Arvelius* is obscure, since both types of spermatocytes eventually give rise to apparently normal sperm cells. In the estrogen-treated rat, on the other hand, the phenomenon of rhythmic protein synthesis may be related to the function of uterine gland cells which are prepared for secretory activity by estrogen.

Protein measurements as employed herein do not permit distinction between chromosomal and extra-chromosomal proteins of the nucleus, but further attempts to specify the chemical nature of these proteins are being undertaken in order to obtain evidence bearing on the concepts of chromosome structure proposed by Mirsky and Ris.[12] Exact duplication of one or all nuclear protein components, if confirmed by more refined methods, may indicate that a multiplication of some basic units is involved. Our investigations are being extended to include the study of other instances of hormonal alteration of nuclear and cytoplasmic constituents with special reference to the anabolic activities of the nucleus.

Summary.—Our work confirms that injection of estrogen into ovariectomized rats results in a doubling of nuclear volume in uterine gland cells. Photometric analysis shows that the increase in nuclear volume is paralleled

by an increase in protein content, while the amount of desoxyribonucleic acid remains unchanged.

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THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN

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During the past fifteen years we have been attacking the problem of the structure of proteins in several ways. One of these ways is the complete and accurate determination of the crystal structure of amino acids, peptides, and other simple substances related to proteins, in order that information about interatomic distances, bond angles, and other configurational parameters might be obtained that would permit the reliable prediction of reasonable configurations for the polypeptide chain. We have now used this information to construct two reasonable hydrogen-bonded helical configurations for the polypeptide chain; we think that it is likely that these configurations constitute an important part of the structure of both fibrous and globular proteins, as well as of synthetic polypeptides. A letter announcing their discovery was published last year.[1]

The problem that we have set ourselves is that of finding all hydrogen-bonded structures for a single polypeptide chain, in which the residues are