sources that the infant thyroid, in spite of its much smaller size, is more sensitive to radiation-induced carcinogenesis than the adult thyroid.

[7] Campbell, J. E., G. K. Murphy, A. S. Goldin, H. B. Robinson, C. P. Straub, F. J. Weber and K. H. Lewis, *Am. J. Public Health*, **49**, 225 (1959).

[8] "Strontium Program—Quarterly Summary Report," *Health and Safety Laboratory Document HASL-55*, U.S. Atomic Energy Commission, New York Operations Office (1959).

[9] The evaluation of the constant in this equation is based on physical and biological data for iodine-131, which have been summarized, for example, in "Recommendations of the International Commission on Radiological Protection," *Brit. J. Radiol.*, Supplement No. 6 (1952). It is assumed that the effective half-life of iodine-131 is 7.7 days and that the effective beta energy is 0.19 Mev.

[10] A recent survey of 312 children under the age of six in suburban Long Island by H. H. Neumann, *Arch. Pediat.*, **74**, 456(1957), gave a mean value of 840 ml of fluid cow's milk consumed per day with the lower third of the group averaging 647 cc per day and the upper third averaging 1,005 cc per day.

[11] Since evaporated milk will tend to be several months old before it is consumed, its iodine-131 activity should be virtually nil.

[12] Boyd, E., in *Handbook of Biological Data*, ed. by W. S. Specter (Philadelphia: W. B. Saunders Co., 1956).

[13] Dunning, G. M., *Nucleonics*, **14**, No. 2, 38 (1956).

[14] See, for example, R. L. Gunther and H. B. Jones, *U.S. Atomic Energy Commission Document UCRL-2689 and addendum* (1954).

[15] Oddie, T. H., *Brit. J. Radiol.*, **24**, 333 (1951).

[16] Middlesworth, L. Van, *Nucleonics*, **12**, No. 9, 56 (1954) and *Science*, **123**, 982 (1956); Comar, C. L., B. F. Trum, U. S. G. Kuhn III, R. H. Wasserman, M. M. Nold, and J. C. Schooley, *Science*, **126**, 16 (1957).

[17] Spiers, F. W., *Brit. J. Radiol.*, **29**, 409 (1956).

[18] It should be noted that with the cessation of nuclear weapons testing in November, 1958, human thyroid levels of radioiodine should decline exponentially and by January, 1959, there should no longer have been any appreciable contamination of milk with radioiodine from past weapons tests. The level of radioiodine in cow's milk may well be the most important index of short-term environmental contamination with fission products whether from weapons tests or other sources [see discussion in *Safety Aspects of Nuclear Reactors*, ed. C. R. McCullough (New York: D. Van Nostrand Co., 1957), 28].

THE ELIMINATION OF DNA FROM SOMA CELLS

BY THEOPHILUS S. PAINTER

THE UNIVERSITY OF TEXAS

Read before the Academy, April 29, 1959

The current widespread interest in the role of DNA in protein synthesis, on the parts of cytologists and biochemists alike, brings into question anew a very old enigma of cytology, which is, the reason for the elimination of parts of or whole chromosomes from somatic cells or nuclei of embryos so that in some animals the chromosome complex of germ cells is markedly different from that of the soma cell of the same individual. In the fungus-feeding fly, *Oligarces paradoxus*, for example, germ cells show 66 chromosomes while soma cells contain only 10 such elements (Reitberger[1]).

The loss of parts of chromosomes from soma cells was first observed by Boveri[2] in the cleaving eggs of *Ascaris megalocephala*, and sporadically through the years other examples of such aberrant behavior have been recorded. The elimination

of whole chromosomes from soma cells occurs regularly in species of Diptera belonging to the families Cecidomyidae, Chironomidae, and Sciaridae.

The very intriguing question, which has aroused much speculation since Boveri's original observations is: What useful purpose is served by the elimination of DNA from soma cells during early embryonic development? The fact that species in which this regularly occurs are spread all over the earth suggests that this anamolous behavior must have an adaptive value else it would not have become established.

Many different suggestions have been made to account for DNA elimination from soma cells but none has been free from objection nor subject to experimental verification (for a recent review see Goldschmidt[3]). It is clear, however, that the persisting somatic chromosomes (hereafter designated S-chromosomes) carry all the genetic factors needed for the normal development of adult males and females, from which it may be inferred that the eliminated chromosomes (E-chromosomes) have no immediate genetic function.

Today, in the light of a better understanding of the functions of DNA and especially the new discoveries in nuclear chemistry, it becomes possible to correlate seemingly unrelated facts, and there emerges a plausible and very convincing explanation which not only allows us to understand the meaning of DNA elimination but sheds some light, perhaps, on the role of heterochromatin in the economy of cells. The writer believes that the evidence to be reviewed below justifies the conclusion that the excess DNA carried in germ cells of certain flies and in at least one genus of plants, plays an essential role in building up, in the cytoplasm of oocytes or the seed, part of the proteins and nucleic acids (both DNA and RNA) required for the development of embryos. In short, it is an ovarian adaptation to meet a specific need. The subsequent elimination of an excess of DNA from soma cells is highly advantageous to the organism because it frees the soma cells from the necessity of synthesizing anew, or reassembling, nucleotides which are not required for somatic development. In this way, the supply of nucleic acid precursors previously stored in the cytoplasm of the female gamete is conserved and the more rapid development of the embryo is made possible.

Biologists have always understood, of course, that in oviparous animals the mature egg must carry within itself all the food reserves needed for the development of the embryo to the point when it can get additional food from its environment. And before the turn of the present century cytologists had recognized and described two different cellular mechanisms by which essential foodstuffs are synthesized and stored in the egg's cytoplasm. At one extreme the synthesis of proteins, nucleic acids and other materials is carried out by specialized nutritive cells—nurse cells—and subsequently this material is transferred to the egg's cytoplasm in several ways. In such cases the egg nucleus remains small until late in oocyte development and appears to play a minor role in syntheses. At the other extreme, in the absence of typical nurse-cells, the egg's nucleus itself becomes enormously enlarged to a germinal vesicle and is extremely active in synthesizing proteins and nucleic acids. In some animals the oocytes show both nurse-cell and germinal vesicles. Of especial interest are species of mosquitoes in which the adult female must feed upon blood before oocyte development is complete, for it has been shown that a mixture of essential amino acids may be substituted for a blood meal. Obviously, the problem of getting enough raw materials from which to synthesize egg cyto-

plasmic constituents, especially proteins, is a critical one for all organisms. Examples of these two synthetic mechanisms will be helpful in considering the main questions before us.

In the ovary of the fruit fly, *Drosophila melanogaster*, an early step in oocyte development is the formation of the egg chamber containing 15 nurse-cells and one oocyte. The nurse-cells, which are modified oogonia, now undergo a series of endomitotic division cycles during each of which the chromosome number is doubled so that the largest nurse-cells may carry from 512 to 1024 haploid sets of chromosomes (Painter and Reindrop[4]). There is a corresponding increase in cytoplasmic volume after each endomitotic division cycle. Nucleoli are extremely numerous and voluminous in nurse-cells and since, as Vincent[5] has shown, nucleoli are composed mainly of proteins in a highly dehydrated state, it is obvious that nurse-cell nuclei synthesize large amounts of nuclear protein. Eventually, the contents of all nurse-cells are incorporated into the cytoplasm of the oocyte, sometimes by a breakdown of the cell wall with direct absorption but more usually by indirect absorption. As a result of the absorption process the cytoplasm of the mature egg contains a large amount of protein synthesized in the nuclei of nurse-cells as well as in the cytoplasm of the oocyte and in addition the degradation products (probably depolymerized) of many thousands of chromosomes.

In the ovary of the frog or toad the story is quite different as to details, but in the end the same situation obtains as in the insect egg. Germ cells pass through the early stages of meiosis up to the diplotene stage without a very great increase in nuclear or cytoplasmic volume. But from this point on, for a period of months there is a consistent increase in nuclear and cytoplasmic volume until the nucleus reaches a diameter of about 150 microns and the whole egg is a millimeter or more in size. During this long period very significant changes occur within the nucleus. In the leptotene stage Painter and Taylor[6] reported that in addition to the thread-like chromosomes, and quite separate from them, many hundreds of Feulgen-positive granules lie just within the nuclear wall forming a sort of cap of DNA particles on the side of the nucleus opposite that toward which the zygotene threads are oriented. In early pachytene small nucleoli, rich in RNA, arise in direct contact with one or more of the independent DNA granules. Such nucleoli with the DNA and RNA components persist for a time and then begin to disappear. First, the DNA granule fades, then the RNA and finally the proteins of the nucleolus seem to melt away. Concomitant with nucleolar disappearance, a gradient of RNA builds up in the cytoplasm which is densest nearest the nuclear wall. After the DNA granules visible in the leptotene stage are used up, and all through the months of the germinal vesicle growth, new crops of nucleoli appear. These are rich in RNA and apparently arise initially in close association with heterochromatic regions of the diplotene chromosomes, and often (always?) a DNA granule is attached to the small nucleolus. We seem to be dealing with a dynamic process during which the chromosomes shed nucleolar material and this passes to the nuclear wall and disappears.

From the above sequence of events Painter and Taylor concluded that, in ways not then understood, the nucleolar material (consisting mainly of RNA and concentrated proteins) passed through the nuclear wall into the cytoplasm of the oocyte. Recently, very striking support for this inference has come from two different

sources. Hoff-Jorgensen,[7] using a bioassay method, has shown that the cytoplasm of the frog's egg contains over 4,500 times as many nucleotides (measured as thymidine) as the haploid nucleus of a sperm, from which it is evident that the DNA granules, attached to nucleoli, pass out into the cytoplasm. Recently, some excellent electron photographs have been published of the germinal vesicle of the frog's egg, both by Kemp[8] and Wischnitzer,[9] and these clearly show that the disappearance of nucleoli, as seen by the light microscope, is due to a shredding of the nucleolar material into granules about 150 A in diameter. That such granules pass through the extremely abundant pores in the wall of the germinal vesicle is strongly supported by Kemp's figures.

From the evidence presented it is clear that in the growth of oocytes large quantities of DNA are required for the synthesis of nuclear proteins, whether the actual site of synthesis be in small, separate factories (nurse-cells) or in one large center, the germinal vesicle. Viewed in this light it seems extremely probable that in those families of Diptera, which carry high numbers of chromosomes in their germ cells, as compared to the soma complexes of the same individual, the extra E-chromosomes are used to synthesize nuclear proteins and thus correspond functionally to the DNA granules of the frog's egg. The entire behavior of the E-chromosomes both in meiosis and in early cleavage is consistent with this interpretation.

In the Cecidomyidae, the family of gall-flies which has been most extensively studied cytologically by White,[10] the behavior of the E-chromosomes during meiosis is quite different in males and in females. During sperm formation all the E-chromosomes (with exception of one transitional species) are eliminated during the first division so that mature sperm carry only a haploid number of S-chromosomes. During the meiosis of females both a haploid number of S-chromosomes and representatives of all the E-chromosomes are found in the female gamete. After fertilization the zygote carries a diploid number of S-chromosomes and representatives of all the E-chromosomes derived from the mother. During early cleavage stages, usually the third, the E-chromosomes are eliminated from the spindles of potential somatic nuclei but are retained in the most posterior nucleus, which becomes the germ cell.

According to White adult females do not require feeding prior to laying mature eggs. Incidentally, in the Cecidomyidae, to judge from published figures, both nurse cells and a germinal vesicle function.

In the dog-roses of Europe a somewhat similar situation exists as in the gall-flies. In *Rosa canina* and related species, there are 35 chromosomes in both male and female germ cells, consisting of 7 pairs of homologous chromosomes (corresponding to S-chromosomes) and 21 univalent elements (E-chromosomes). During the meiosis of male germ cells all the univalent E-chromosomes are lost so that functional pollen carries only a haploid set of S-chromosomes. In female germ cells the mature gamete carries a haploid number of S-homologues and a full complement of univalent E-chromosomes. The later development of the so-called embryo-sac is quite complicated but one cell functions as a female gamete and is fertilized and the rest of the cells, including triploid nuclei become a part of a cytological mechanism for synthesizing the food material of the seed. Thus in plants the fundamental requirement of providing food for the development of the

embryo is delayed until after fertilization but, as in animals, much DNA is required for synthetic processes.

It goes without saying that the elimination of parts of chromosomes in early cleavage from potential soma cells may be explained in the same way as the elimination of whole chromosomes.

The recent work of Allfrey and Mirsky[11] greatly contributes to the understanding of reserve food mechanisms found in the oocytes of animals. These authors have shown three important facts: (1) that DNA is essential for the synthesis of proteins in the nucleus; (2) that as DNA is removed from thymocyte nuclei, by the use of DNase, in measure, the ability of nuclei to synthesize protein falls off; (3) that in a nucleus depleted of its DNA, the ability to synthesize proteins can be partially restored by adding to the medium of such nuclei a polyanion which is not related structurally to nucleic acids. These authors point out that the role of DNA in protein synthesis may be due, in part, to the configuration of the DNA molecule.

Applying the above chemical information to the nurse-cell and germinal vesicle mechanisms, it may be said that the ability of a nucleus to synthesize proteins depends on the amount of DNA it contains, or on the amount of a special kind of DNA. In nurse-cells the repeated reduplication of chromosome sets by endomitosis greatly increases the "nuclear horsepower" of each nurse-cell so that the ability to synthesize proteins of the largest of such cells in the ovary of the fruit fly may be from 256 to 512 times greater than that of a diploid nucleus. Similarly, the initial presence of extra chromosomal granules in the young germinal vesicle of the frog greatly increases the protein synthesizing power of this cell component.

It appears quite clear, from the evidence reviewed above, that in the growth of oocytes, much more DNA is needed for the synthesis of nuclear protein than can be supplied by the diploid or, after synapsis, the tetraploid nucleus. One way of meeting this need is for the germ cells to carry an excess of DNA—in the form of the E-chromosomes—but there are serious disadvantages of having such extra DNA in all cells of the organism where it is not needed. First of all, in male gametes, whether sperm or pollen, lightness of weight or small size would presumably have an adaptive value. In soma cells, were the E-chromosomes retained in all nuclei during embryonic development, the replication of DNA structures not needed for development would impose a heavy drain on the supply of nucleotides or other nucleic acid precursors stored earlier in the cytoplasm of the oocyte. From this the advantages of eliminating unnecessary material are very clear.

The question may now be raised, What becomes of the discarded chromatin, i.e., mainly DNA? The rapid disappearance of whole or parts of chromosomes from cytoplasm is undoubtedly due to the presence of DNase in the cytoplasm, but it appears to the writer that nucleic acid precursors are much too valuable a material to be discarded. In this relation the insect egg is admirably adapted for the conservation of discarded DNA because cell walls are not formed in the embryo until some 2000 nuclei are present, so all such nuclei would have free access to deoxyribotides or -sides. This would greatly hasten development of the soma cells, as I pointed out some years ago (Painter[12]).

The elimination of DNA from soma cells raises many questions, chief of which is the origin of the extra DNA found in germ cells. Since in *Rosa canina* the 35 chromosomes present in germ cells is known to be a pentaploid complex ($n = 7$

chromosomes) several writers have assumed that in animals the high chromosome number of germ cells is a derivative of an originally polyploid condition. At the moment this appears as the most likely explanation.

A second, and related question, is raised by the fact that the chromatin thrown out of the spindle, during the chromatin diminution in *Ascaris*, has come to be regarded as being made up of nongene bearing chromatin, i.e., is heterochromatin. Are the 55 extra chromosomes in the germ cells of *Oligarces*, for example, made up wholly of heterochromatin? No answer can be given to this question but since the original findings of Muller and Painter[13] in 1932, it has been evident that not all the DNA along the chromosomes is gene-bearing and now the striking experiments of Mirsky and Allfrey suggest a way in which DNA may lack the specificity of the gene-bearing areas and still contribute to the synthesis of nuclear protein.

[1] Reitberger, A. *Chromosoma*, **1**, 391 (1940).
[2] Boveri, Th., *Anat. Anz.*, **2**, (1887).
[3] Goldschmidt, R., *Theoretical Genetics* (Berkeley: Univ. of Calif. Press, 1955.).
[4] Painter, T. S., and E. Reindrop, *Chromosoma*, **1**, 276 (1939).
[5] Vincent, W. S., *Intern. Rev. Cytol*, **4**, 269 (1955).
[6] Painter, T. S., and A. N. Taylor, these PROCEEDINGS, **28**, 311 (1942).
[7] Hoff-Jorgensen, E., *Recent Developments in Cell Physiology* (London: Butterworth, 1954).
[8] Kemp, E. N., J. *Biophysic. and Biochem. Cytol.*, **2**, 281 (1956).
[9] Wischnitzer, S., *J. Ultrastr. Res.*, **1**, 201 (1958).
[10] White, M. J. D., Univ. of Texas Publ., No. 5007 (1950).
[11] Allfrey, V. G., and A. E. Mirsky, *Science*, **128**, 1142 (1958).
[12] Painter, T. S., *Trans. Conn. Acad. Arts & Sciences*, **36**, 443 (1945).
[13] Muller, H. J., and T. S. Painter, *Zeitsch. Ind. Abst. Verer.*, **50**, 129 (1932).