CHROMOSOME STRUCTURE IN THE SALIVARY GLANDS OF SCIARA[1]

BELLING, in a series of papers,[2] strongly supported the hypothesis that chromioles in chromosomes represent gene loci, but was unable in his material (lilies) to make the necessary genetic tests to identify individual chromioles with individual genes. Recently attention has again been directed to this subject by the finding of Heitz and Bauer,[3] of Painter[4] and of King and Beams[5] that the deeply staining cross bands or disks, long known to be visible in the salivary gland "spireme" of Diptera, represent a definite architecture and may, like the chromioles, be identified individually in different chromosomes. Painter has shown further that in *Drosophila* these bear a definite relation of some kind to gene loci, and Koltzoff[6] has suggested that the small granules visible in the "disks" or rings probably represent the genes or the spaces between the genes. According to press reports[7] and personal communication, an essentially similar interpretation has been developed by Bridges. On the view of Bridges and Koltzoff each of the large salivary gland chromosomes is compound and represents a bundle of slender gene strings which have been derived by repeated division of an original gene string (or pair of strings) and each disk or ring is a rosette, made up of the genes derived from one original gene (or pair). Threadlike lines have been described connecting the granules in successive bands or disks as required by the hypothesis (Koltzoff).

In this interpretation no reference is made to the claim of earlier investigators, such as Alverdes[8] and Kaufmann,[9] that the disks or rings in the chromosomes are present in pairs. If such a paired condition exists it requires interpretation. With a view toward throwing light on this and other features, as well as of identifying individual chromosomes in our material, we have made careful observations on the salivary gland chromosomes in *Sciara*. Although the study is by no means complete, certain features have been observed which seem to warrant brief note at this time. In material fixed and stained in the usual iron-aceto-carmine mixture we have obtained distinctly different appearances after different modes of treatment. Dissection in Ringer's solution or tap water gives conditions resembling those previously described. The chromosomes contain bands or disks of various sizes ranging down to very thin lines or rows of granules. Many of these are in pairs.

[1] From the Department of Embryology, Carnegie Institution of Washington, and the Department of Zoology, Johns Hopkins University, Baltimore, Md.

[2] *E.g., Univ. of Calif. Pub. in Bot.*, 14: 307, 1928.

[3] *Zeits. f. Zellf. u. mik. Anat.*, 17: 67, 1933.

[4] SCIENCE, 78: 585, 1933; *Genetics*, 19: 175, 448, 1934.

[5] *Anat. Rec.*, Sup., p. 89, 1933.

[6] SCIENCE, 80: 312, 1934.

[7] *New York Times*, September 17, 1934; *Science News Letter*, September 29, 1934.

After dissection in body fluid or directly in the aceto-carmine mixture, however, the chromosomes often contain a series of what appear to be compressed biscuit-like or wafer-like vesicles. These vesicles likewise range in size down to mere lines or rows of "granules" (Fig. 1, *A*). And like the bands

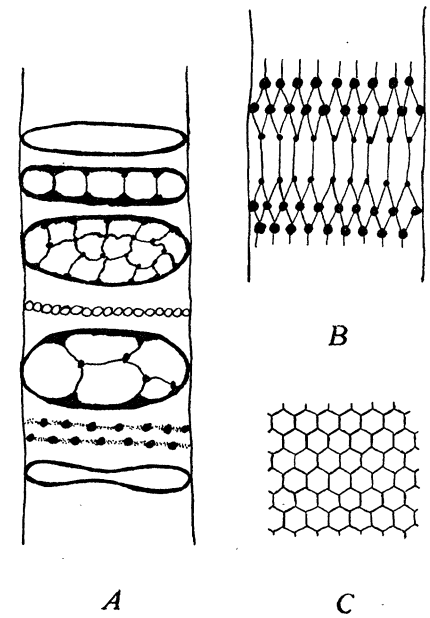

FIG. 1

or disks they exhibit a constancy in organization, corresponding in homologous regions of homologous chromosomes. Under suitable conditions both disks and vesicles may be secured in the same chromosome. In some cases at least it seems clear that a vesicle represents a pair of disks, together with the intervening material, and that the two faces of the vesicle correspond to the two disks.

The segments or vesicles appear in fixed material to be vacuolated or alveolar in structure. Their faces often appear granular, like the disks. The granules, in such cases, appear to bear a definite relation to the "alveolar" structure. The granule, or thickening, lies at the junction of two or more "alveoli," as indicated in the accompanying figure 1, *A*. Thus the number of granules bears a definite relation to the number of alveoli, and each alveolar wall or interface may make a thread-like connection across the vesicle connecting two granules on opposite faces. By focusing, the granules may often be followed along the alveolar junctions, up the faces of the vesicle and across the top, where those from opposite faces meet. Such "granules" are clearly long and slender. We have not found them consistently arranged about the axis of the chromosome like spokes of a wheel.

In material prepared so as to bring out the disk or

[8] *Arch. f. Zellf.*, 9, 168, 1912.

[9] *Amer. Nat.*, 65: 555, 1931.

banded structure many of the "bands" are represented by what appear to be rows of granules across the chromosome, and in some cases faint longitudinal lines appear to connect granules in successive bands, giving the impression of strings of beads, as described by Koltzoff. This is particularly true where a chromosome has been stretched. Careful study of the finer structure in such cases indicates that these lines represent the walls of "alveolar" spaces, and that the structure, in fixed material, is in reality honeycomb-like, as indicated schematically in figure 1, *B*. In the clearest cases we have examined, the lines are not continuous, but forked, following the walls of the (often hexagonal) "alveoli." In some cases the alveoli appear to be elongated in a more or less diagonal direction, suggesting that the chromosome is twisted. In others the honeycomb structure is comparatively uniform and lines may be traced diagonally in both directions (clockwise and counter-clockwise) from a given point as indicated schematically in figure 1, *C*.

In our material each region in the chromosome appears to have a definite type of protoplasmic structure which usually extends through the chromosome transversely at that level. The type may change abruptly in passing from one region to another. At some places the protoplasm appears to be essentially homogeneous, while at others the appearance of fine or coarse alveolation is evident, suggesting that qualitative chemical differences are associated with the morphological differences.

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DEMONSTRATION OF THE EXISTENCE OF TWO FORMS OF VITAMIN D IN FISH LIVER OILS

SINCE the discovery that the vitamin D of irradiated ergosterol is different from that of cod liver oil, the latter form has sometimes been distinguished by such terms as "natural vitamin D" or "fish oil vitamin D." The implication that the vitamin D naturally occurring in different fish oils is always the same qualitatively has been put to test, with the surprising observation that fish oils differ qualitatively as well as quantitatively in their vitamin D content.

To distinguish the two forms in fish oil we employed essentially the same procedure that was used in 1930 to differentiate between cod liver oil and irradiated ergosterol, *i.e.*, the administration to chickens of materials previously assayed with rats. A precision method of assay with chickens, which will be described elsewhere, was developed to measure the response of this species with accuracy comparable to that attained in the critical method with rats.

The first experiment was done with oil extracted from the livers of halibut, *Hippoglossus hippoglossus*. This was assayed with rats, and diluted with maize oil. The dilution was administered to chickens in parallel with cod liver oil of the same potency. Rat unit for rat unit, the halibut liver oil induced slightly less calcification in the chickens than did cod liver oil, but the difference was perhaps no greater than the errors of assay. We were left with the suspicion that a greater difference might be observed in other oils.

Compared with certain liver oils, cod liver oil is a weak source of vitamin D, containing usually about 100 international units per gm. Halibut liver oil contains on the average about 1,200 I. U. per gm, but even this is weak in comparison with several other fish oils that we have examined. One of the more potent liver oils is that of the bluefin tuna, *Thunnus thynnus*, which contains on the average 40,000 I. U. of vitamin D per gm. A pure specimen of this oil was assayed with rats, diluted and administered to chickens as before. Rat unit for rat unit, it was only one sixth as effective as cod liver oil. Similarly, the unsaponifiable fraction of the tuna liver oil was found to be one seventh as effective, rat unit for rat unit, as the unsaponifiable fraction of cod liver oil.

The effectiveness ratio which was thus found to be 1:6 or 1:7 is several times greater than the probable error of the assays. One must therefore conclude that the vitamin D of bluefin tuna liver oil and the vitamin D of cod liver oil are different substances (or different mixtures of substances), one rat unit of the former having only 15 per cent. of the antirachitic effectiveness of one rat unit of the latter *for the chicken*. A detailed account of these experiments will be published elsewhere, together with data on additional liver oils.

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