ATCh and PT was negligible, being 0.0016 optical density per minute at 412 nm for ATCh and 0.0066 optical density per minute at 412 nm for PT.

Hence PT can be easily utilized for (i) histochemical detection of cholinesterase in intact tissues, (ii) electrophoretic detection of PT-hydrolyzing enzymes, (iii) colorimetric detection and quantitation of cholinesterase, and (iv) as a reagent to compare with the use of aliphatic choline analogs. The advantages of handling and synthesizing PT as compared to ATCh and the similarity of results obtained make it likely that PT will find wide applications in teaching and research programs with vertebrate and invertebrate tissues.

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Trisomy-3,4 and Triploidy (3A-ZZZW) in Chick Embryos: Autosomal and Sex Chromosomal Nondisjunction in Meiosis

Abstract. *The first known cases of trisomy-3, trisomy-4, and triploidy (3A-ZZZW) in chickens are reported. The trisomic embryos were near death at 4 days of incubation; the triploid embryo appeared normal and probably could have developed further. Although the triploid (3A-ZZZW) was found after sampling more than 2000 embryos, the trisomic-3 and trisomic-4 embryos were discovered in the first sampling of a line of chickens selected for low egg production. These studies suggest that the sex chromosomes (Z and W), as well as the autosomes, can undergo nondisjunction in meiosis of chickens.*

Testing the hypothesis that most chromosomal abnormalities result in early embryonic death, I began the cytological screening of early embryos derived from several strains of chickens (*1*, *2*). Since August 1968, 59 embryos with chromosome abnormalities (35 haploids, 16 triploids, 8 trisomics) have been identified out of a total of 2768 embryos (2.1 percent aberration rate). Of the embryos classified as "early deads," 7.6 percent had chromosome aberrations. In this report the first known cases of trisomy-3, trisomy-4, and triploidy (3A-ZZZW) are described.

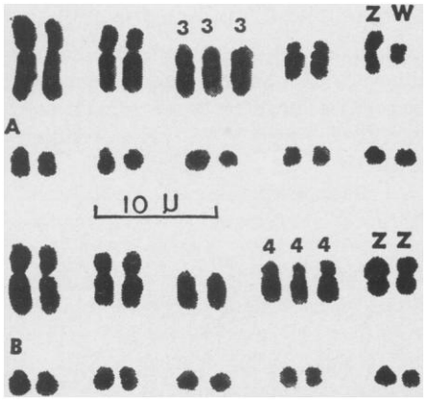

Fig. 1. Karyotypes of the ten largest chromosome pairs from trisomic-3 (A) and trisomic-4 (B) embryos.

Trisomy-3 (of the third largest chromosome) was observed in a 4-day embryo classified as "dead" on the basis of candling. The embryo was small (about the size of a 3-day embryo), and the extraembryonic vascular network was poorly developed. Trisomy-4 (of the fourth largest chromosome) was observed in a second 4-day "dead" embryo. These trisomic embryos were found in the first sampling of a line of chickens selected for low egg production (*3*). Karyotype analysis of 150 metaphases revealed the trisomy-3 and trisomy-4, as well as female (ZW) and male (ZZ) sex chromosome complements, respectively (Fig. 1).

Triploidy (3A-ZZZW) was observed in 72 metaphases from an anatomically normal 4-day embryo. This triploid, derived from a strain cross, was discovered after sampling of over 2000 embryos. It is likely that this 3A-ZZZW embryo would have developed further, possibly to term.

Complete karyotypes were constructed for 15 triploid metaphase cells. In each of these karyotypes a small, unpaired metacentric (or sometimes submetacentric) chromosome, in the size class No. 7 to 8, was present (Fig. 2). Additional data led to the identification of this unpaired metacentric as a W chromosome. In each of the karyotypes the metacentric was clearly larger than chromosome No. 9 (a submetacentric pair) and had a more medially located centromere (Fig. 2). Measurements were made of arm lengths for the Z and W chromosomes of the triploid; arm ratios (ratio of long arm length to short arm length) and sex chromosome ratios (ratio of W chromosome length to Z chromosome length) were calculated (Table 1). These data agree well with unpublished data from my laboratory, as well as with previous reports (*4*, *5*). Further karyological considerations (particularly pairing of microchromosomes) led me to conclude that the unpaired metacentric was not the result of fusion of two telocentric microchromosomes.

Trisomy for chromosomes 1, 2, 3, 4, and 5 has now been reported for chickens (*2*, *6*, *7*). Meiotic nondisjunction in the male or female parent is one probable mechanism to account for the trisomic cases. The effects of several genetic, physiological, and environmental variables on the production of trisomy may now be investigated.

In a previous report it was demonstrated that each of chromosome pairs 1, 2, and 5(Z) did not organize the nucleolus (*2*). In trisomy-3 and trisomy-

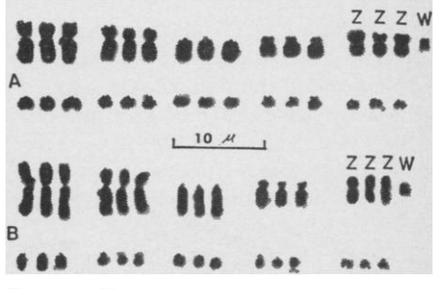

Fig. 2. Karyotypes of the ten largest chromosome triplets from two different cells (A and B) of a triploid (3A-ZZZW) embryo.

Table 1. W chromosome arm ratios (long arm length to short arm length) and sex chromosome ratios (W/Z) expressed as means with standard deviations for ZZZW triploidy and ZW diploidy.

Case	W chromosome arm ratio	Sex chromosome ratio (W/Z)	Reference
Triploid (ZZZW)	1.19 ± 0.09	0.46 ± 0.06	Present study
Diploid (ZW)	1.08 ± 0.13	0.42 ± 0.06	*6*
Diploid (ZW)	1.10		*4*
Diploid (ZW)		0.48	*5*

-4, diploid nucleolar distributions were also found. Thus, chromosomes 1, 2, 3, 4, and 5(Z) can be eliminated as the sole nucleolar-organizing chromosomes. This lends additional strength to the argument that a group of microchromosomes organizes the nucleolus (*8*).

Mechanisms for the origin of the 3A-ZZZW triploid were considered. Nondisjunction of the sex chromosomes in meiosis of the female could produce an A-ZW egg nucleus. If fertilized by a diploid (2A-ZZ) spermatozoon, or two haploid (A-Z) spermatozoa, a 3A-ZZZW zygote would result. The ZZZW sex chromosomal complement reported here is analogous to the XXXY modified Klinefelter's syndrome in man (*9*).

Note added in proof: After this paper was submitted, a case of double trisomy (trisomy-2,5Z) was found. The occurrence of trisomy-5Z lends further support to the hypothesis of meiotic nondisjunction of sex chromosomes. Further, simultaneous nondisjunction (in the male or female) of an autosome and a sex chromosome is suggested.

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8 July 1970

Rapid Axonal Transport of Sulfated Mucopolysaccharide Proteins

Abstract. *When sulfur-35–labeled sodium sulfate is injected intraocularly in the goldfish, labeled sulfated mucopolysaccharides rapidly appear in the contralateral optic tectum of the brain, demonstrating the axonal flow of sulfated mucopolysaccharides. The transport rate is the same as that observed for proteins labeled after intraocular injection of tritiated proline. Treatment of the sulfur-35–labeled material with precipitants and enzymes reveals the presence of substances with properties similar to those of heparan sulfate (the major component) and chondroitin sulfate. Dermatan sulfate was not detected.*

Recent evidence has shown that chondroitin-4-sulfate and heparan sulfate are produced by rat glial cells in tissue culture (*1*). While histochemical (*2*) and autoradiographic (*3*) studies have suggested the presence of sulfated mucopolysaccharides (SM) in neurons as well and roles for SM have been postulated in nerve function (*2*), little is known about their origin and distribution.

In view of the possible functional significance of SM in neurons, it was of interest to determine whether they are among the rapidly transported axonal proteins. The optic tract of the goldfish provides a suitable system for approaching this question (*4, 5*). The eye is a convenient site for reproducible introduction of labeled precursors; the ganglion cells of the retina terminate in the contralateral optic tectum, which is easily removed; and the ipsilateral tectum can be used as an internal control for systemic labeling of the brain arising from precursor that escaped from the eye.

Twenty goldfish (*Carassius auratus*, 6 to 7 cm in length and maintained at 20°C) were injected in the right eye with 40 μc (5 μl) of carrier-free $Na_2{}^{35}SO_4$ and killed 12 hours later. Left and right optic tectal hemispheres were collected separately with an equal amount of unlabeled tectum and fractionated (Table 1). There was no significant difference between the left and right optic tectal hemispheres in radioactivity present in either chloroform–methanol or methanol–water fractions, an indication that there had been no transport of free or lipid-bound labeled sulfate. The large difference in the protease digests from left and right tectal hemispheres was suggestive of rapid transport of labeled SM proteins. This view was substantiated by the fact that about half of the radioactivity in the digest had the solubility characteristics of SM—that is, precipitability in cetylpyridinium bromide (CPB) and ethanol and solubility in 10 percent trichloroacetic acid (TCA). In other experiments, a comparable distribution of radioactivity was seen. The yield of hexosamine in the isolated SM from tectum and whole brain was 50 μg/g (wet weight). This is similar to the values found previously in mammalian brain (*6*). The ratio of hexosamine to uronic acid was 1.1, a value expected for SM.

Further evidence for the SM nature of the material precipitable in CPB was obtained by electrophoresis (Fig. 1). The fastest moving band migrated with standard chondroitin-6-sulfate and contained 29 percent of the recovered radioactivity. The middle band migrated somewhat slower than standard heparan sulfate and contained 68 percent of the radioactivity. The slowest band migrated with hyaluronic acid and accounted for less than 5 percent of the radioactivity. The distribution of radioactivity in the two sulfate bands corresponded approximately with the intensities of staining, suggesting that the specific activities are similar.

The bands were further characterized by treatment with hyaluronidase, which is known to degrade hyaluronic acid and chondroitin sulfate but not heparan sulfate. Fraction 8 (25 μl) was hydrolyzed with 5 μl of a solution (1 mg/ml) of bovine testicular hyaluronidase (Worthington, 3600 unit/mg) in a mixture of 1*M* sodium acetate (*p*H 5.2) and 1.5*M* NaCl at 37°C for 4 hours. Only the center band stained after electrophoresis; it retained more than 95 percent of its original radioactivity. Paper electrophoresis of the hyaluronidase digest at high voltage (80 volt/cm in pyridine acetate, *p*H 4.3, for 20 minutes) yielded three radioactive regions. One remained at