(possibly α) had higher binding speed (bimolecular constant, 2.9×10^5 liter mole^{-1} min^{-1}) than the resistant counterpart (bimolecular constant, 1.4×10^5 liter mole^{-1} min^{-1}). The constants for the slow-binding components (possibly β) for each strain, on the other hand, scarcely differ from each other, the values being 4.9 and 5.3×10^3 liter mole^{-1} min^{-1} for the susceptible and resistant components, respectively.

It may be premature to state that all these tendencies of dieldrin-resistant strains, to have less binding capacity of nerve components with dieldrin, are causally related to the mode of resistance in these strains, for any insect colonies from different geographical locations can be expected to have a number of biochemical variations. To show that the two phenomena, dieldrin-resistance and binding of dieldrin with nerve components, are related to each other, genetic analyses [such as those employed to correlate low aliesterase activity with organophosphate resistance (*10*)] or reasonable biochemical evidence must be offered. As yet, no evidence indicates that dieldrin forms a charge-transfer complex with the nerve components of the German cockroach; dieldrin is unsuitable for ultraviolet-spectra analysis. The complex, unlike DDT complex, is inextractable with organic solvents: this fact indicates that the binding phenomenon, at least in part, involves a process of complex formation with nerve components other than simple lipids.

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Triploid-Diploid Mosaic Chicken Embryo

Abstract. *Cytological analysis of an underdeveloped chicken embryo at 6 days of incubation revealed a triploid-diploid mosaic condition. Of the 30 metaphases observed, 19 were triploid and 11 diploid. The triploid cells were 3A-ZZZ and diploid cells 2A-ZZ, as determined for the six largest pairs of chromosomes.*

While the analysis of the normal chicken (*Gallus domesticus*) karyotype has advanced considerably, climaxed by Owen's report in 1965 (*1*), little has been reported with respect to chromosome number deviations. Newcomer *et al.* (*2*) demonstrated polyploid cells in the gonad of a sex-reversed female chicken. Ohno *et al.* (*3*) described an adult triploid chicken with a left ovotestis; the chromosome constitution was established to be 3A-ZZ. In a report on testicular chromosomes, Ford and Woollam (*4*) demonstrated a polyploid nucleus. This report of a triploid-diploid mosaic chicken embryo is a result of an effort to determine if the embryonic mortality among embryos from some matings may be attributed to aneuploidy or polyploidy.

Pedigree matings were made in a stock of Single Comb White Leghorn chickens mated with a male from a segregating population involving the Single Comb White Leghorn, Barred Plymouth Rock, and Cornish varieties of chickens. Embryos in a number of eggs from several females grew slowly, and a few appeared to be near death after 5 or 7 days of incubation. Owen's technique (*1*) was modified and employed on four of the very weak 5- to 7-day embryos. Two hours prior to killing, 0.1 ml of 0.05-percent colchicine was injected into the eggs near the developing embryo. The whole embryo was placed in a test tube and ground with a glass rod. The resultant macerated tissue was exposed to distilled water for 15 minutes. The cell suspension was centrifuged at 500 rev/min for 15 minutes, then the supernatant was decanted. Acetic-alcohol (1:3) fixative was added, and the pellet was resuspended slowly; fixation was for 30 minutes. After further centrifugation and decanting, the tissue was suspended in 45-percent acetic acid for 15 minutes. The cell suspension was filtered through cheese cloth to remove large clumps of tissue and other debris before air-dried preparations were made on a hot plate at 37° to 40°C. The slides were stained in aceto-orcein for 30 minutes, washed in 45-percent acetic acid, air-dried again, and mounted in Canada balsam. Observations were made by phase contrast microscopy.

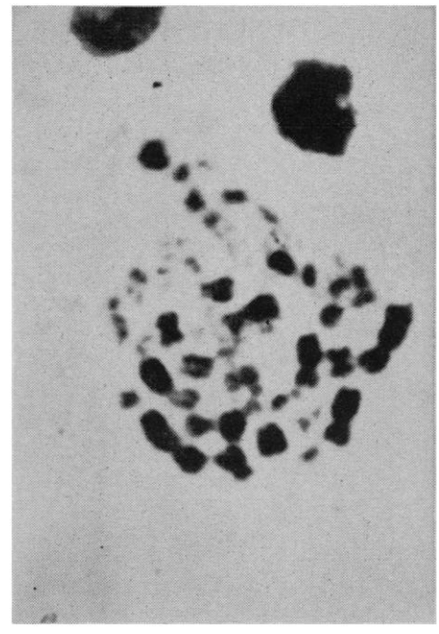

Fig. 1. Colchicinized metaphase from a chicken embryo containing a 3A-ZZZ chromosome constitution.

In one of the four embryos studied, triploid cells were observed. In this abnormal embryo, mitoses were rare, as was expected from the embryo's weak and underdeveloped condition.

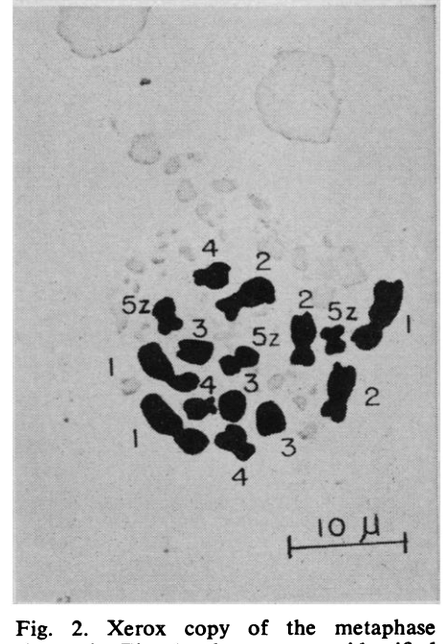

Fig. 2. Xerox copy of the metaphase shown in Fig. 1; chromosomes identified as numbers 1, 2, 3, 4, and 5 have been darkened with ink.

Table 1. Arm lengths of the chromosomes numbered 1, 2, 3, 4, and 5 in Fig. 2.

Chromosome number	Long arm (cm)	Short arm (cm)	Arm ratio*
1	0.85	0.48	1.77
1	.83	.54	1.54
1	.77	.46	1.67
2	.61	.35	1.75
2	.60	.32	1.88
2	.61	.32	1.91
3	.55		
3	.55		
3	.55		
4	.40	.15	2.66
4	.36	.11	3.28
4	.39	.12	3.26
5Z	.24	.24	1.00
5Z	.28	.26	1.08
5Z	.25	.24	1.04

* Arm ratio = long ÷ short.

In all, 30 well-spread metaphase and prometaphase figures were counted. Eleven, or 36.7 percent, of the metaphases were diploid, with a ZZ constitution. Nineteen, or 63.3 percent, were triploid, with a ZZZ constitution. A metaphase showing the 3A-ZZZ chromosome constitution is shown in Fig. 1, and a reproduction of the metaphase, in which the largest chromosomes are identified by number, is shown in Fig. 2. Measurements of arm lengths for the chromosomes numbered 1, 2, 3, 4, and 5 in Fig. 2 are given in Table 1. The measurements were made from Fig. 1.

Ohno (*3*) demonstrated that triploidy can be compatible with viable postnatal life in the chicken. In the present study, the triploid (3A-ZZZ)–diploid (2A-ZZ) condition was found in an embryo exhibiting retarded development and little mitotic activity. It may be suggested that chromosome number deviations of the type reported here for the chicken may be one of several possible factors causing embryonic mortality. However, before such a relationship may be accepted, additional cases should be found.

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16 March 1966

Porotic Hyperostosis, Anemias, Malarias, and Marshes in the Prehistoric Eastern Mediterranean

Abstract. *Porotic hyperostosis, formerly called osteoporosis symmetrica, is an overgrowth of the spongy marrow space of the skull. In children, other bones may also be affected. The disease is a consequence of one of the thalassemias or sicklemia. These anemias are balanced polymorphisms which are apparently maintained by falciparum malaria. Falciparum malaria spread over the anopheline belts of the Old World in coincidence with porotic hyperostosis, but did not penetrate the New World. Here some other parasitism or deficiency anemia must have been the cause of porotic hyperostosis in ancient times. In Anatolia, Greece, and Cyprus from the seventh to second millennia B.C., porotic hyperostosis occurred frequently in early farmers who lived in marshy areas, but rarely in inhabitants of dry or rocky areas or in latest Paleolithic hunters. As shown by skeletal samples from Greece, the frequency of the disease decreased as farming methods improved. However, from Hellenistic to Romantic times it again increased together with increases in the incidence of malaria and in poorer farming. There are correlations between porotic hyperostosis and adult stature and fertility. The mutations producing falciparum malaria therefore must antedate seventh millenium B.C. and I think may have an Eastern Mediterranean origin.*

Skulls of the earliest farmers from Western Anatolia [6500 B.C. at Çatal Hüyük (*1*)] and from Macedonia [6000 B.C. at Nea Nikomedeia (*2*)] often have extraordinary thickening of the diploë together with thinning of the outer layer of compact bone. In ten skulls which have medium to pronounced porotic hyperostosis, the parietal boss area averages 12 mm in thickness; this is about 4 to 5 mm thicker than the normal bone. Since the inner and outer layers of compact bone make up only about one-quarter of the thickness of the skull vault, the diploic thickening must reflect a red cell production by the hyperostotic bone almost double that of normal diploë. Skulls with a slight degree of porotic hyperostosis have less thickening of the diploë, but the outer table is always irregular and somewhat porous. Apparently the disease in these bones is vascular in origin and affects either the outer table ["symmetrical osteoporosis" (*3*)] or the orbit roofs ["cribra orbitalia" (*4*)], or usually both the vault and the orbits. Porotic hyperostosis of the skulls of young children and older infants often extends to sphenoid and zygomatic bones and sometimes affects the long bones. Some of the long bones from a child from Bamboula in southern Cyprus, an infant from Lerna in southeastern Greece [both Bronze Age (*5*)], and an infant from Nea Nikomedeia (Early Neolithic) have an "inner shell" of bone attached to the cortex by a few trabeculae. This finding indicates that hypertrophy of the red marrow may have prevented normal remodelling of the bone as it grew (Fig. 1).

These observations point to anemias as causes of the hyperostosis. The lesions produced by thalassemia and sicklemia (*6*) match those found in these skulls and long bones. However, the extreme ballooning of the bone marrow cavities seen in modern children hospitalized with thalassemia (*6*) is very rare in these samples because under prehistoric conditions ill children died at an earlier age. Moseley (*7*) shows that hereditary spherocytosis or iron deficiency anemia coupled with prolonged suckling, as in Bahima disease, can cause changes in the skull very similar to those found in patients with abnormal hemoglobin. Anemia caused by chronic amebiasis, severe bacillary dysentery, hookworm, any one of the malarias, or even altitude anoxia might cause identifiable bone changes.

I believe the infants and children from which the severely affected skeletons came were homozygous for one of the thalassemias or sicklemia and that the adults represent a spectrum of heterozygotes which were severely to very mildly affected by the disease. Such heterozygotes are apparently more resistant to falciparum malaria than normal people (*8*). This advantage allows a reproductive increase in the genes for abnormal hemoglobin which is balanced by the deaths of homozygotes from anemia. I know of no studies yet which show that some other chronic parasitism may also maintain these polymorphisms.

Williams (*9*) and Vogt (*10*) first suggested that "osteoporosis symmetrica" in American Indians might be Cooley's anemia (*11*). Then Chini and Valeri (*12*) and Neel (*13*) showed it probable that the "osteoporosis" in