during this period for the group with borderline elevations of pressure tends to support Obrist's (*9*) contention that mild elevations of BP may be necessary among the aged to maintain adequate cerebral circulation. Our failure to duplicate this pattern for the older subjects (those initially examined at ages 70 to 79) suggests that even in the face of mild elevations of BP other factors may be operating to compromise cerebral circulation. Perhaps the duration of cardiovascular disease, with consequent structural change or other interacting pathology relating to more advanced age, may intervene.

These results raise questions about adaptation to the effects of high BP. Since none of the older subjects with high BP completed the follow-up study and those of younger age who completed it were superior to their counterparts who were subsequently lost to the study, it might be appropriate to ask whether some individuals develop compensatory mechanisms to hypertension and therefore adjust to it with minimum difficulty for protracted periods, whereas others who do not develop adequate physiologic or anatomic compensations manifest central nervous system difficulties with cognitive deficits and progressively severe physical pathology.

An earlier cross-sectional study (*10*), in which the same subjects were tested, suggested that socioeconomic status may be a contaminant in any investigation of cardiovascular disease difficulty, since the incidence of cardiovascular disease is high in the low socioeconomic group. In this context, it should be noted that in this 10-year longitudinal study, approximately 70 percent of the subjects within each BP group were of a nonmanual occupational background, and, furthermore, the amount of intellectual change over time was approximately the same for all subjects within each BP category regardless of whether the subjects were of a manual or nonmanual occupational background.

It is also of particular significance that in contrast to cross-sectional studies (*11*) which report a decline in intelligence across the later decade of life, longitudinal studies of intellectual ability (*12*) have raised some doubts as to the generality of such decline in relatively healthy aged at least through age 75. To this end, the presence of large numbers of aged with cardiovascular illness suggests that the basis for the cognitive decline associated with aging after maturity should be considered secondary to some pathologic processes and not merely as a "normal" aging process.

FRANCES WILKIE
CARL EISDORFER
Center for the Study of Aging and
Human Development,
Duke University Medical Center,
Durham, North Carolina 27706

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Intersexes and Sex Determination in Chickens

Abstract. *Chickens sexed as females in commercial flocks show a low frequency of intersex individuals. Gross anatomical examination of the intersexes revealed malformed gonads. Histological examination proved that these gonads were not ovotestes but were composed of testicular tissues. Karyotype analysis of 15 intersex individuals showed 13 of them to be 3A-ZZW triploids (normal females are 2A-ZW) while the other 2 were mosaic for male and female karyotypes. Red blood cell volume accurately reflects the ploidy level and is suggested for use in screening for polyploids. Intersex triploids may develop parthenogenetically or from fertilization between reduced and unreduced gametes.*

In commercial flocks, hybrid chickens developed for egg production are sexed at hatching so as to retain only the females. In addition to the occasional males observed before or at sexual maturity, recent observations indicate that intersex chickens appear in these flocks in a low frequency of about 1/2000 of the commercially sexed females.

Phenotypically, the secondary sexual characteristics of most intersexes are intermediate between normal males and females. Their body size and feathers on the neck, saddle, sickle, and tail are usually intermediate. However, some exhibit a definite trend toward masculinity, since they show a much larger size of head, comb, and wattles than the females, as well as possessing spurs which are lacking in the females.

Fifteen intersex individuals were secured from a number of commercial flocks. Seven of these intersexes as well as a rooster and a laying hen were killed and their internal anatomies, especially that of their reproductive systems, were compared. Tissues were fixed in buffered 5 percent formalin for histological examination. Wax-embedded blocks were sectioned 8 μm thin and stained with hematoxylin and eosin and finally prepared as permanent slides.

All intersexes had both right and left gonads except two (B130 and Y147) that lacked the right gonad. Their gonads looked like ovotestes and varied in shape and size, ranging between 0.08 and 9.30 g (Table 1). They lacked vasa deferentia and all had a left oviduct similar in shape to that of laying hens but smaller in size, ranging in weight between 1.91 and 44.5 g. None had a right oviduct except for P2 and P47, in which the duct was similar to the rudimentary right oviduct of adult females.

Karyotype analysis of these seven intersexes revealed two separate groups, one being triploid 3A-ZZW and the other mosaic (Table 1). One of the latter (G32) was diploid, but 70 percent of its mitotic plates represented the male karyotype, the remaining 30 percent the female karyotype. In the other mosaic (G68), 70 percent of the cells were 3A-ZZZ, that is, of the male triploid karyotype, while 30 percent were representative of the normal 2A-ZW female. Both had a large right gonad similar in size to that of the normal male (Table 1). The development of the intersex phenotype is easily explained in the mosaics, but is not so readily understood in the homogeneous triploids with ZZW sex chromosome complement.

Table 1. Comparison between intersexes and normal chickens in karyotype, gross internal morphology (weights), and red blood cell (RBC) volume.

Designation	Karyotype	Right gonad (g)	Left gonad (g)	Right gonadal duct (g)	Left gonadal duct (g)	Mean RBC volume (10^{-7} mm^3)*
B130	3A-ZZW		4.03		44.5	1.4
P2	3A-ZZW	0.27	3.11	2.14	24.89	1.6
P47	3A-ZZW	1.82	0.08	0.24	2.49	1.5
P64	3A-ZZW	0.34	2.51		14.7	1.4
Y147	3A-ZZW		1.53		1.91	1.3
G32	70% 2A-ZZ 30% 2A-ZW	7.25	1.39		2.43	1.0
G68	70% 3A-ZZZ 30% 2A-ZW	9.30	5.10		9.30	1.2
Rooster	2A-ZZ	8.54	8.13	0.55	0.77	1.1†
Laying hen	2A-ZW		47.09	2.19	46.90	1.0†

* Values determined from ten counts for each bird, five from each wing. † Mean values calculated from five individuals.

Histologically, both right and left gonads of intersex individuals were invariably composed only of testicular tissues. Thus, their gonads were not ovotestes but rather malformed testes. Their seminiferous tubules varied in size and shape between and within individuals and were usually spaced with connective tissues or marked Leydig-cell hyperplasia, or both, as opposed to the uniform testicular hypoplasia observed in the rooster (Fig. 1). Furthermore, they were usually lacking in observable spermatogenic activity. With the exception of P64 where very few spermatozoa were observed, none of the triploid intersex group exhibited spermatozoa in their gonads. By contrast, the relative content of spermatozoa per seminiferous tubule in the mosaics was about one-half that observed in the rooster. All intersexes showed considerable internal folding in their gonadal ducts which was lacking in male vasa deferentia, but which was similar to that found in the infundibulum and the uterus of a laying hen's oviduct.

Compared with normal males and females, the homogeneous triploid group of intersexes had distinctly larger red blood cells. The diploid mosaic G32 showed red blood cells of normal size, whereas in the triploid-diploid mosaic G68, 22 percent of the erythrocytes were of normal size and the rest were of the large size.

Average erythrocyte cell volume was slightly higher in males than females but much larger for the triploid intersex group. The difference was highly significant (*1*). Interestingly, 1.5 multiples of the mean red blood cell volume determined for the control group (five males and five females) fall within the range of 99 percent confidence interval limits for the red blood cell population volume determined on the five triploid intersexes, that is, the latter is statistically equal to two-thirds of the former. Thus, mean red blood cell volume in chickens is an accurate indication of the ploidy level, as it corresponds very closely to the number of chromosomal sets in somatic nuclei. The difference in ploidy level was also reflected in the average DNA content of single erythrocytes, since the triploid intersexes averaged 1.4 times that of the normal rooster.

Since determination of red blood cell volume is much easier and far less time-consuming than karyotype analysis, the former is recommended as a new screening method for the ploidy level in chickens.

In addition to these seven intersexes, a group of eight other intersexes was later secured, and all proved to be 3A-ZZW triploids. The significance of these results lies in the fact that beyond our basic knowledge that females are the heterogametic sex (ZW) and males are the homogametic sex (ZZ) in birds, there is very little known about the mechanism of their sex determination. In mammals, especially humans, the Y chromosome is clearly male-determining, XO individuals having a female, and XXY a male, phenotype. In *Drosophila*, sex is determined by the balance between the number of X chromosomes and the number of autosomal sets of chromosomes.

Recently, the first case of triploidy in man was reported: a male child born alive after a normal gestation period

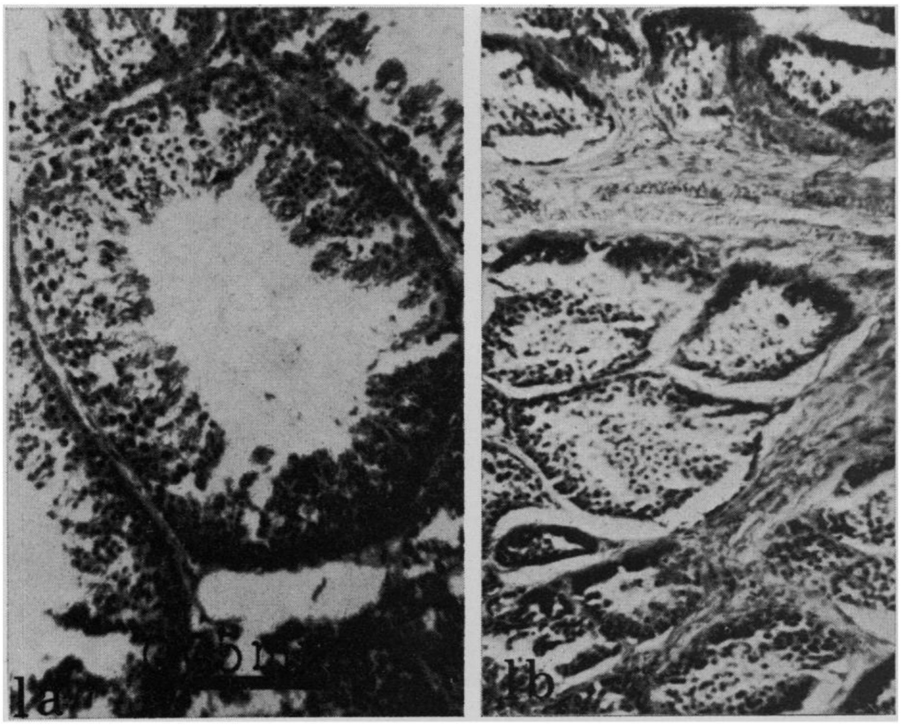

Fig. 1. (a) Section of rooster testis, showing active spermatogenesis and uniform testicular hypoplasia. (b) Section of the gonad from mature intersex chicken. The seminiferous tubules vary in shape and size and are spaced with large patches of connective tissues and interstitial cells. Scale marker represents 0.05 mm.

(*2*). His karyotype proved to be 3A-XXY, and histologically his gonads were similar to those of our intersex chickens. This case provides good evidence that balance between autosomes and the X chromosome has little effect on sex determination in humans.

In the literature, only two cases have been reported of adult triploid chickens (*3, 4*). The first was described by Ohno *et al.* (*3*) as a 3A-ZZ intersex. However, it is likely that its karyotype was actually 3A-ZZW, since Ohno and co-workers (*5*) reported difficulty in identifying the W chromosome with certainty in the domestic fowl. The recent development of the feather-pulp technique in Shoffner's laboratory (*6*) has made it possible to ascertain the presence of the W chromosome in the females of many avian species (*7*), including chickens. The second case was a parthenogenetic 3A-ZZW chicken (*4*). Even though Sarvella did not refer to it as an intersex, its external and internal morphology as well as the histology of its gonads were essentially identical to those of our intersex chickens.

So far, no serious attempt has been made to elucidate the problem of sex determination in chickens and other avian species. Such determination has been difficult because of the small size of the microchromosomes and the lack of adequate cytological techniques to identify both autosomes and sex chromosomes, as well as the lack of studies on sexual deviants. Therefore, the recent advances in karyotype analysis, in addition to the data presented here on intersex chickens, represent an exceptional opportunity to characterize the mechanism of sex determination in this group.

If the W chromosome is strongly female-determining in chickens, we would expect the 3A-ZZW individuals to be phenotypically females. Even though observations on the phenotypes of 2A-ZO and 2A-ZZW chickens are lacking, we can still conclude that the W chromosome is not a strong female-determining element. The present study indicates that sex determination in chickens, and probably birds in general, is unlike that in mammals, and most likely is similar to that operating in *Drosophila*, that is, dependent upon the ratio between the Z chromosomes and the number of autosomal sets of chromosomes. Thus the observed intersex phenotype and oviduct-type tissues in intersex chickens, especially those with 3A-ZZW complement, could be explained, at least partially, in the light of the data from recent sex-reversion experiments. Male chicken embryo sex reversion following estradiol benzoate treatment occurs for a limited period of embryonic development. Afterward, the rapidly proliferating testicular tissues replace the degenerating ovarian-type tissues (*8*). Intersex chickens probably develop in a similar fashion under the effect of indigenous male-female hormones during early embryonic stages. The production of such hormones is probably under the influence of the somatic–sex chromosome balance.

While triploidy is well known in plants and lower vertebrates, it has only been observed in a few instances in higher vertebrates (*3, 4, 9*). Polyploidy has been observed recently by Bloom (*10*) in certain chicken embryos. Triploid intersex chickens may develop parthenogenetically (*4*). It has been shown that parthenogenesis in chickens may be increased in frequency after viral infection by live fowl pox (*11*). It may also arise from fertilization between a normal male sperm (1A-Z) and an unreduced female gamete (2A-ZW). Or it may originate as a result of unreduced male sperms (2A-ZZ) or by diandry through fertilization of a haploid egg by two haploid spermatozoa. Here, however, one might expect a 1:1 ratio of 3A-ZZW:3A-ZZZ individuals. If both are equally viable, the fact that none of the 13 triploids examined in this study were 3A-ZZZ could be accounted for if such individuals are phenotypically males. Thus they would be discarded in commercial flocks at the time of sexing or later. The origin of mosaic intersexes is not clearly understood at present, and polyembryonic development (*12*) seems the most probable.

F. ABDEL-HAMEED
Department of Biological Sciences,
Northern Illinois University,
De Kalb, Illinois 60115
R. N. SHOFFNER
Department of Poultry Science,
University of Minnesota, St. Paul

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Pheromones: Isolation of Male Sex Attractants from a Female Primate

Abstract. *Fractionation of vaginal secretions from rhesus monkeys by partitioning and chromatographic procedures, combined with behavioral studies, demonstrates that short-chain aliphatic acids are responsible for stimulating the sexual behavior of males. Injection of estradiol into ovariectomized females increases the concentration of volatile acids in secretions which will then sexually stimulate these male primates.*

An increasing amount of evidence implicates olfactory mechanisms in communication between the sexes in prosimians (*1*) and also in their social organization (*2*), but to date there is only scant information that olfaction plays a similar role in higher, Old World primates (*3*). When small doses of estrogen were administered intravaginally to ovariectomized rhesus monkeys, stimulation of mounting activity by their male partners was greater than when the same doses were injected subcutaneously (*4*). One interpretation of this finding was that local application of estrogen to the vagina resulted in the production of substances capable of affecting the male's behavior other than by changing the sensory input to his penis. We confirmed this view in ex-