(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.

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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 experiments with males made reversibly anosmic by the insertion and subsequent removal of nasal plugs (*5*); anosmic male rhesus monkeys did not respond sexually to females receiving intravaginal estrogen until their olfactory acuity had been restored. These results were consistent with the notion that rhesus females treated with estrogen produce substances with pheromonal properties and that these increase the sexual activity and motivation of males by way of their olfactory sense. The active principles (copulins) are present in vaginal secretions collected from ovariectomized females treated with estrogen (donors), and when these secretions are applied to the sexual skin of untreated females ovariectomized at least 6 months previously (recipients), there is a very marked and immediate stimulation of sexual activity in their male partners (*6*).

It became of interest, therefore, to determine the chemical nature of the substances in vaginal secretions responsible for this powerful behavioral effect and, to do so, extraction and fractionation procedures were used in conjunction with methods of behavioral assay. Pairs of animals (one male, one female) were routinely tested together either daily or on alternate days in acoustically isolated cages, and observed from behind one-way mirrors. All tests were 1 hour long, during which many aspects of behavior were scored quantitatively by methods already described (*7*), particular attention being paid to (i) the number of mounting attempts made by males, (ii) the incidence of ejaculation, and (iii) the incidence of masturbation. Recipient females, ovariectomized at least 6 months previously and unreceptive, were trapped in a net immediately before each behavioral test, and extracts of vaginal secretions, fractions thereof, or control substances (*8*) were applied to the area of the sexual skin; the behavioral properties of the various extracts were thereby assessed.

Ovariectomized rhesus females given 10 μg of estradiol monobenzoate subcutaneously daily were used as donors; their vaginal secretions were collected by lavage with a vaginal pipette filled with 1 ml of distilled water. After being buffered at pH 4.5 with sodium dihydrogen phosphate, samples were extracted with 2 ml of diethyl ether. This extract was used for behavioral testing; it was highly active in 29 out of 32 tests with five pairs of animals (*9*). Thus, the active material was soluble in ether. We demonstrated the acidic nature of these behaviorally active constituents by applying to the sexual skin of recipient females a solution prepared by treating the ether extract with 2 ml of 0.01N sodium hydroxide, acidifying the alkaline layer, and again extracting into ether. Ether-soluble neutral and basic components of vaginal secretions, notably cholesterol, were removed by this procedure. Fractionation of the alkaline extract on columns of diethylaminoethyl-cellulose confirmed the acidic properties of the active constituents, and their elution volume corresponded to that of short-chain fatty acids.

After preliminary screening with different gas-liquid chromatographic media, Carbowax–terephthalic acid columns and temperature gradients between 50° to 200°C were used to study further the acidic, behaviorally active components of secretions. Chromatograms of extracts of individual secretions collected from five ovariectomized, untreated females indicated in each case that the amounts of volatile components were low (Fig. 1A). Chromatograms of extracts of individual secretions collected from five ovariecto-

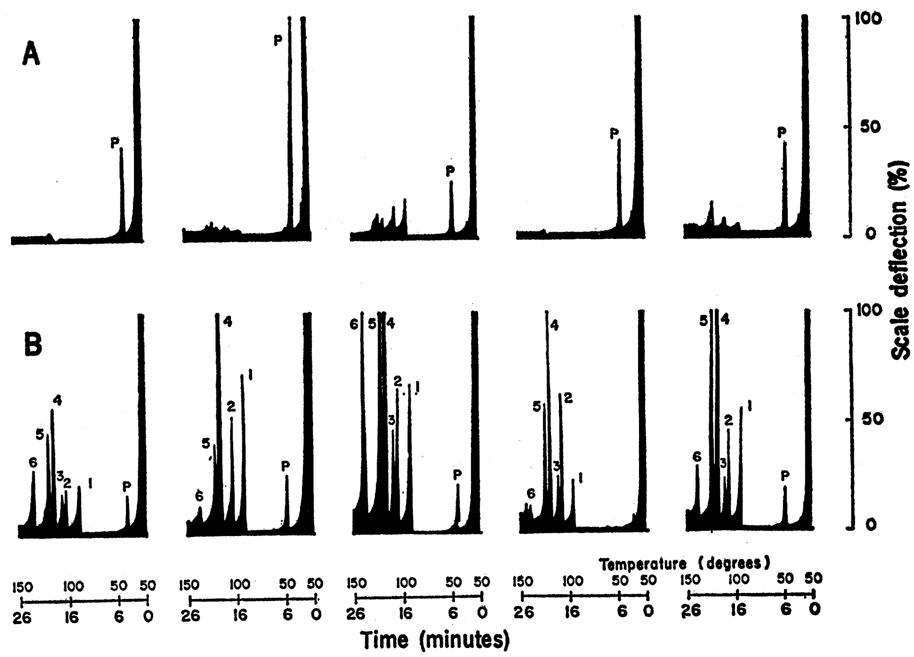

Fig. 1. Gas chromatograms of extracts of rhesus monkey vaginal secretions. (A) Chromatograms of secretions collected from five ovariectomized, untreated females. The volatile acid content of the secretions was low (0 to 3.3 μg per collection), and the four, from recipient females, that were tested (four at left) were without behavioral activity (Fig. 2, before treatment). (B) Chromatograms of secretions collected from five ovariectomized females during treatment with estradiol. The volatile acids in the secretions showed an eightfold increase (26.0 to 53.0 μg per collection), and the four, from donor females, that were tested (four at left) showed marked behavioral activity (Fig. 2, with treatment). The right-hand chromatograms (A and B) are from the same animal before and during treatment with estradiol. Peak P, after the solvent front, is authentic n-pentanol added to the extracting ether as a marker (*10*).

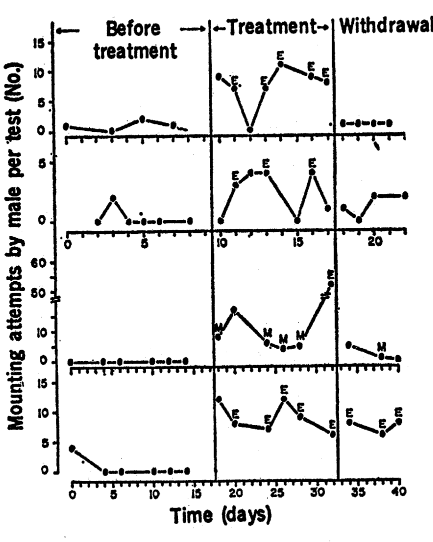

Fig. 2. Sexual stimulation of male rhesus monkeys by components of vaginal secretions fractionated by gas chromatography. In tests with four males each paired with a different ovariectomized (recipient) female, the application to the latters' sexual skin of material collected by trapping from the gas chromatograph resulted in a marked stimulation of the sexual behavior of their male partners. E, ejaculation; M, masturbation to ejaculation. Time scale of the lower two pairs tested on alternate days is half that of the upper two pairs tested daily.

mized females during treatment with estradiol showed that amounts of volatile components (peaks 1 through 6) were at least eight times greater (Fig. 1B). To determine whether the increased production of these constituents in animals treated with estrogen was responsible for the change in the secretions' behavioral properties, we trapped peaks 1 through 5 (80° to 130°C) into ice-cold ether; the material for the trapping procedure was obtained from a pool of 48 vaginal washings collected from three donor females. The trapped fraction was then applied daily to the sexual skin of four recipient females, each paired with a different male; an immediate, marked stimulation of the sexual activity of the males resulted in each case (Fig. 2). The four males made a total of ten mounting attempts during 22 tests before treatment compared with 213 mounting attempts during 26 tests with treatment (t-test, $P < .001$). There were no ejaculations during tests before treatment, whereas 14 occurred during treatment (χ^2 test, $P < .001$). In three pairs, stopping treatment resulted in an immediate return to baseline, but this did not occur in the remaining pair until the fifth test after treatment ended; similar carry-over effects have been reported previously (*6*). It should be emphasized that the recipient females were unreceptive and frequently refused the mounting attempts of males for which intromission was sometimes very difficult because of the atrophic state of the females' genital tracts. In one case (Fig. 2), where the female's persistent refusals generally prevented intromission and ejaculation, the male masturbated to ejaculation during several tests with treatment; here, the stimulation and expression of male sexual excitement in the presence of a totally unreceptive female was noteworthy.

These results establish that highly active copulins which sexually stimulate these male primates are trapped with peaks 1 through 5. Using gas chromatography and coinjections with authentic substances, together with mass spectrometry, we have made preliminary identifications of peaks 1 through 6 as follows: 1, acetic; 2, propionic; 3, isobutyric; 4, butyric; 5, isovaleric; and 6, isocaproic acids. The amounts of these acids in vaginal samples can therefore be calculated from the chromatograms (*10*). A synthetic mixture of authentic acids was then prepared to correspond in concentration and composition to the acids present in estrogen-stimulated vaginal secretions. When tested, this mixture possessed behavioral activity that was identical to that of the material trapped with peaks 1 to 5, a finding which excludes the possibility that a trace amount of an unknown substance was responsible for the activity of the fractions (*11*). The identification of these sexual pheromones, the first in any primate species, as simple aliphatic acids provides both a useful tool in behavioral research and also a specific olfactory input for neurophysiological studies on rhinencephalic-hypothalamic integration. The need for comparative studies in related primate species including the human is now obvious.

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One-Trial Learning and Biphasic Time Course of Performance in the Goldfish

Abstract. *Goldfish (N = 408) spontaneously swam against flowing water into a calm-water well. After a single trial punished by brief electric shock, the fish avoided the well, as indexed by increased latencies of reentry. Avoidance declined during the first minute after shock, then rose to a peak 1 hour later. The biphasic time course is compatible with the two-store theory of memory formation.*

Many phenomena of learning and retention have been demonstrated in fish (*1*). Goldfish have been useful in memory research that correlates deficits in the retention of active shuttlebox avoidance with inhibition of brain protein synthesis (*2*). The many trials required for such learning, however, mask the onset of consolidation of a memory trace and hamper precise determination of the consolidation gradient.

A useful one-trial learning method for fish is not generally available (*3*). We describe here a one-trial technique that uses electric shock to suppress a strong spontaneous behavior of the fish. Goldfish swim against a flow of water into a region of calm water. A single brief shock causes them to retreat into the water flow and to delay reentry into the calm-water well.

We tested 526 goldfish (Lambourne Industries Fish Hatchery, San Fernando, Calif.), measuring 3 to 6 cm from snout to caudal peduncle, during October to December 1970. Groups of 48 fish were kept for 2-weeks' acclimation in 40-liter tanks filled with aged tap water at 24° ± 1°C. Training and testing were done in a plastic foam trough, 38 cm long and 7.5 cm wide. Its floor was molded in a spinal curvature that ended in an overflow; the opposite end was formed into a calm-water well, 8 cm long and 3 cm below the adjacent curved floor. The outlet tube of a water-recirculating system