Partial Masculinization of Genetic Female Chickens (*Gallus gallus*) After Incorporation of ^{3}H-Thymidine During the Period of Oogenesis

^{3}H-Thymidine is very intensely incorporated in the germ cells of 9-, 10- and 11-day-old female chick embryos, both in vitro[1] and in vivo[2]. After successive applications of ^{3}H-thymidine of high specific radioactivity during this period, complete sterilization was found almost of the left ovary of the female chicks at the moment of hatching[3]. The experiment was performed with embryos from sex-linked matings (FI generation from Rhode Island Red *X* White Plymouth Rock; females have brown feathers, males have a majority of white feathers). Six of these female chicks which survived treatment with 8 or 9 successive applications (every 8 h) of 50 μc ^{3}H-thymidine (specific radioactivity: 14 C/m*M*) were reared to the age of 7–8 months. At the age of 4–5 months, 2 of them showed initial signs of masculinization. At the age of 8 months (Figure 1) typical male comb, gills and ear lobes could be seen, together with glossy oblong feathers on the neck. The remaining parts of the body seem to have a normal female appearance. One of the two chickens was able to crow. No semen could be obtained from this chicken and egg laying was not observed.

After killing, their abdomens were opened for investigation of the genital tract (Figure 3). In both cases on the surface of the left ovary pedonculated vesicles, filled with an opaque white fluid, were seen. Under the ovarian surface, yellow deposits were obvious. The left oviduct was approximately half as large as normal. On the right side, an elongated rudiment of the mesonephros (approximately 1.5 cm long) and a Wolffian duct were visible. After fixation the ovaries were embedded in paraffin and sectioned at 5 μ thickness. After deparaffination and rehydration the sections were coloured with Groat's iron hematoxyclin and eosin; under the microscope the structure of the ovary appeared to be completely disturbed through proliferations of fibrous tissue. No oocytes could be discerned. At the hilus of the ovary, cord-like formations were visible. These formations have some resemblance to sterile testicular tubes. Because of this peculiar aspect of the ovary, the reason of the masculinization could not yet be found. Since the phenomenon of masculinization did not appear in all of the treated and subsequently sterilized females, it is possible that in only 2 of them did treatment with ^{3}H-thymidine also interfere

Fig. 1. 8-month-old genetic female chicken treated during the period of oogenesis with successive applications of ^{3}H-thymidine.

Fig. 2. 8-month-old control hen.

Fig. 3. Genital tract of the chicken of Figure 1.

[1] M. CALLEBAUT and R. DUBOIS, C. r. hebd. Séanc. Acad. Sci., Paris sér. D. *261*, 12 (1965).
[2] M. CALLEBAUT, J. Embryol. exp. Morph. *18*, 299 (1967).
[3] M. CALLEBAUT, Experientia *24*, 944 (1968).

with the normal induction of ovarian differentiation. Let us point out that a certain degree of sex reversal from female to male has also been induced in the chicken after X-irradiation[4,5].

Résumé. L'apparition de caractères mâles localisés à la tête et à la nuque a été observée chez des poules génétiquement femelles après des applications successives de thymidine tritiée pendant la période de l'ovogenèse.

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[4] J. M. ESSENBERG and A. ZIKMUD, Radiology *31*, 94 (1938).

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A Microscopic Test for Rapid Detection of Antibodies Against *Mycoplasma pneumoniae*

Morphological studies on *Mycoplasma pneumoniae* in coverslip chambers have shown this *Mycoplasma* species growing in colony-like structures and filamentous forms[1]. The filaments, which seem to be separate, single organisms, were destroyed by homologous antiserum[2]. In the following experiments this reaction is tested for specificity. Additionally, human sera are used in the test.

PPLO-broth supplemented with horse serum, yeast extract[3] and 0.002% phenol red was inoculated with *M. pneumoniae* strain FH. Sterile coverslip chambers were filled with about 0.2 ml each of the inoculated medium (pH 7.8). They were incubated at 36 °C for 1 day or longer, until the change of colour indicated a pH of 7.2–7.5 (about 10^7 colony-forming units (cfu) per ml). Then the chambers were opened, the broth replaced by 0.2 ml of antiserum diluted in PPLO-broth with 20% active horse serum, and incubated at 37 °C for 1 h. After this, the structures on the coverslip were observed by phase contrast microscope with a long distance condensor. If such condensor is not available, the test can be read by darkfield or normal phase contrast after mounting the coverslip (without glassring) on a microslide.

In the presence of antiserum, the filaments were destroyed and rounded up (Figure 1). In the control chambers, incubated with negative serum or only with the diluent, the structures were preserved (Figure 2). The specificity of this phenomenon was tested by addition of antisera against *M. orale* type 1, *M. salivarium*, *M. fermentans*, and the GDL group. In a dilution of 1:10 these sera did not alter the form of filaments. In contrast to this, antiserum against *M. pneumoniae* (Baltimore Biological Laboratories) destroyed the filamentous structures up to a dilution of 1:1600. Preliminary results on 86 unselected human sera, tested in a dilution of 1:10 by the microscopic test, showed a correlation in the results between metabolic inhibition test (MIT)[5] and this new method. 63 sera were negative and 10 positive in both methods, 11 sera were negative in the microscopic test but positive in the MIT in a dilution of 1:2, and 2 sera showed different results.

The immunological destruction of the filamentous structures seems to depend on the presence of active fresh horse serum. In control experiments with homologous antiserum and heat-inactivated horse serum (56 °C 30 min), a destruction of filaments was not visible, though some change in the structures occurred. This result supports the assumption that heat-labile factors, possibly complementary, are participating in the reaction. The number of organisms at the time of testing seems to influence the result of the reaction. Dense growth after prolonged incubation (pH < 7.0, $> 10^8$ cfu/ml) resulted in a reduced titer. In the test described here the antiserum reacts on the surface of intact living mycoplasma cells.

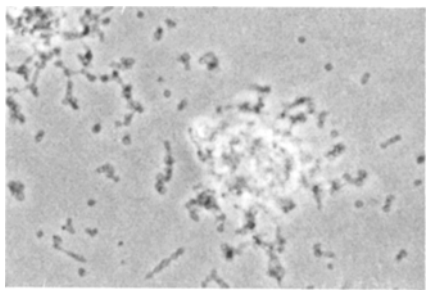

Fig. 1. Filaments incubated with homologous antiserum 1:400 (× 1200).

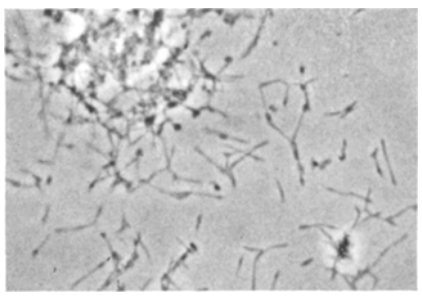

Fig. 2. Negative control chamber (× 1200).

[1] W. BREDT, Proc. Soc. exp. Biol. Med. *128*, 338 (1968).
[2] W. BREDT, Zbl. Bakt. I, Orig. *208*, 549 (1968).
[3] L. HAYFLICK, Tex. Rep. biol. Med. Suppl. 1, *23*, 285 (1965).
[4] R. H. PURCELL, D. TAYLOR-ROBINSON, D. C. WONG and R. M. CHANOCK, Am. J. Epidem. *84*, 51 (1966).
[5] D. TAYLOR-ROBINSON, R. H. PURCELL, D. C. WONG and R. M. CHANOCK, J. Hyg. *64*, 91 (1966).