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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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.

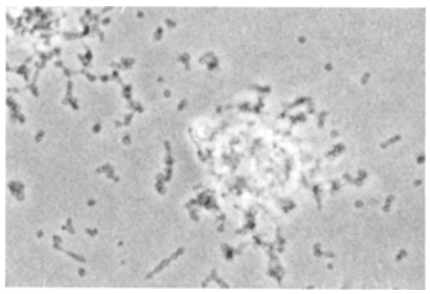

Fig. 1. Filaments incubated with homologous antiserum 1:400 (× 1200).

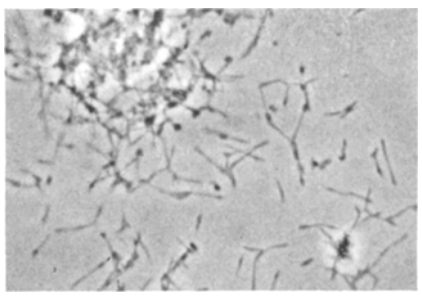

Fig. 2. Negative control chamber (× 1200).

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Therefore it seems possible that the antibodies responsible for the destruction of filaments are related or identical with the antibodies causing growth inhibition or metabolic inhibition. This test could therefore provide a tool for rapid detection of such specific antibodies against *M. pneumoniae*. Since tetracyclin does not alter the morphology of filaments after 1 h of incubation[2], serum levels of tetracyclin, a possible cause of error in the metabolic inhibition test[4], should not interfere with this immunological reaction. Further experiments are in process to investigate the nature of the reaction[6,7].

Zusammenfassung. Die Filamentform der an Glas wachsenden Zellen von *M. pneumoniae* wird durch homologes Antiserum in Gegenwart von aktivem Pferdeserum zerstört. Diese Reaktion ist spezifisch und kann auch zum Nachweis von Antikörpern in menschlichen Seren verwendet werden. Ein Zusammenhang der an der Reaktion beteiligten Antikörper mit den im Stoffwechselhemmtest nachzuweisenden Antikörpern wird diskutiert.

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The Preparation and Antibacterial Activity of 3,3′-*bis*(Trifluoromethyl) Tetranitrodiphenylamine

Work in our laboratories on tetramethyldipicrylamine (Figure 1) has shown that this compound possesses antibacterial activity against a number of Gram-positive organisms and sulfadiazine-resistant and sensitive strains of *Neisseria meningitidis*[1–3]. A number of nitrated methyl analogs of the parent compound have been synthesized and found to have no significant antibacterial activity. However, a trifluoromethyl analog, 3,3′-*bis*(trifluoromethyl)tetranitrodiphenylamine (Figure 2) was also found to show antibacterial properties and is the subject of this report.

The 3,3′-*bis*(trifluoromethyl)tetranitrodiphenylamine was synthesized as follows: 3-aminobenzotrifluoride was converted to 3,3′-trifluoromethylacetanilide; this was reacted with 3-bromobenzotrifluoride and the product hydrolyzed[4]. The hydrolysis product, 3,3′-*bis*(trifluoromethyl)diphenylamine, a light brown oil (34% yield), was purified by treatment with 14% hydrochloric acid and extracting the secondary amine with benzene. The NMR-spectrum in carbon tetrachloride gave a singlet at 5.92 δ tms (amino hydrogen) and an aromatic multiplet at 7.25 δ with an intensity ratio of 1:8, indicating the reported product.

This material was nitrated as follows: 13.0 g of 3,3′-*bis*(trifluoromethyl)diphenylamine was dissolved in 150 ml of concentrated sulfuric acid and heated for 15 min at 80 °C. The solution was cooled, immersed in an ice bath, brought to 5 °C and maintained at this temperature with stirring while 150 ml of concentrated nitric acid was added dropwise over a three hour period. The solution was stirred for an additional hour and then heated for one half hour at 75–80 °C, cooled and poured over ice to give a yellow flocculant precipitate. The precipitate was filtered, washed with distilled water, dissolved in warm 1 molar sodium carbonate solution and the resulting red filtrate treated with excess hydrochloric acid to precipitate the nitrated product (Figure 2) in 52% yield. Purification was effected by dissolving the product in acetone and adsorbing it on a column containing neutral Woelm alumina (100 g/g of solute) and eluting the adsorbate with chloroform. The major fraction was concentrated by evaporation. The purified product was recrystallized from acetone and chloroform (50:50 v/v). Bright yellow trapezoidal prisms were obtained with a melting point of 218–219 °C.

The nuclear magnetic resonance and IR data seem to confirm that nitration proceeds in the expected manner giving 3,3′-*bis*(trifluoromethyl)4,4′,6,6′-tetranitrodiphenylamine. The deactivating influence and the steric effects of the 2 trifluoromethyl groups appear to have hindered the complete nitration of the aromatic rings. The compound is acidic and of low solubility but the sodium salt is soluble and has been used for all tests.

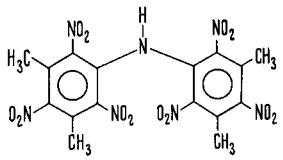

Fig. 1

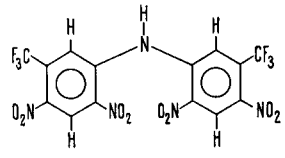

Fig. 2

Fig. 1. Tetramethyldipicrylamine.

Fig. 2. 3,3′-*bis*(trifluoromethyl)tetranitrodiphenylamine.

Elemental analysis		Calculated	Found
for $C_{14}H_5F_6N_5O_8$	C	34.67	34.69
	H	1.04	0.95
	N	14.43	14.48
	F	23.49	23.05

Instrumental data

The NMR-spectrum in deuterated acetone gave 2 aromatic singlets at 8.62 δ tms and 9.15 δ with an intensity ratio of 1:1.

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