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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[7] The skilful assistance of Miss G. MAISCH is gratefully acknowledged.

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.

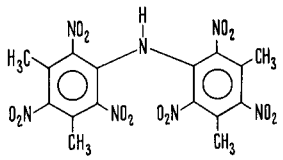

Fig. 1

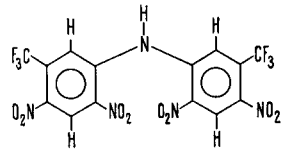

Fig. 2

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.

[1] T. S. MEYER, C. E. MOORE and P. F. FRANK, Nature *215*, 312 (1967).
[2] These findings were also confirmed by Dr. D. IVLER, University of Southern California Medical School by personal communication.
[3] A. J. FRITSCH, C. E. MOORE and T. S. MEYER, Nature *217*, 350 (1968).
[4] S. S. SMITH, J. Org. Chem. *15*, 1125 (1950).

Fifteen sulfadiazine resistant and 8 sulfadiazine sensitive strains of *Neisseria meningitidis* of groups B, C, and Y[5] were tested. The assay was carried out on Mueller-Hinton agar as described by FRANK, WILCOX and FINLAND[6].

The minimal inhibitory concentration of the compound ranged from 0.156–1.25 µg/ml medium for both the sulfadiazine resistant and sensitive groups.

In addition to the *Neisseria meningitidis* strains, 4 Gram-negative and 9 Gram-positive microorganisms were tested. The Gram-negative organisms consisted of *Pseudomonas aeruginosa, Proteus vulgaris, Herellea vaginicola* and *Aerobacter aerogenes*. The following Gram-positive organisms were used: *Staphylococcus aureus* No. 198, 201, and 204[7], *Streptococcus sanguis* (SBE), *Streptococcus sp. HHT, Streptococcus FA-1.* ATCC No. 19645, *Lactobacillus casei*, and *Bacillus subtilis* ATCC No. 9372.

The organisms were grown on Todd-Hewitt agar supplemented with 1% dextrose. Filter paper disks (Schleicher-Schuell No. 740-E, diameter 12.7 mm) were impregnated with the compound (10 µg/ml) and placed on inoculated Petri dishes. The organisms were grown for 24 h at 37 °C and the extent of inhibition around each disk was noted. The Gram-negative organisms were not inhibited by the compound, but all of the Gram-positive organisms tested were inhibited. The inhibition noted at this concentration was found to be bacteriostatic. Using the tube dilution technique, the compound was found to be bacteriocidal in the range of 50–100 µg/ml.

Zusammenfassung. Eine neue organische Substanz, 3, 3'-*bis*(trifluoromethyl)tetranitrodiphenylamine ist synthetisch hergestellt worden. Die antibakteriellen Eigenschaften gegen Gram-positive Bakterien und gegen Sulfadiazin resistente und empfindliche Arten von *Neisseria meningitidis* werden beschrieben.

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[5] D. G. HOLLIS, G. L. WIGGINS and J. H. SCHUBERT, J. Bact. *95*, 1 (1968).
[6] P. F. FRANK, C. WILCOX and M. FINLAND, J. Lab. clin. Med. *35*, 188 (1950).
[7] NDRI strains.

Cardiac Ganglia in Spiders (Arachnida: Araneae)

It has long been known that neurogenic hearts occur in some of the arthropods, notably the decapod crustaceans and *Limulus*, but it has been only recently that the nature of the heartbeat has been investigated in any detail in any spider[1–3]. In each of the 3 species of spiders that have been studied thus far there is a cardiac ganglion. Since the presence of a cardiac ganglion is one indication of a neurogenic heart, it would be of interest to know if cardiac ganglia are of general occurrence in the Araneae. In order to determine this, we have made a histological study of the hearts of 28 species of spiders representing 11 families.

In everyone of the 28 species there is a cardiac nerve running the length of the heart on its mid-dorsal external

Spiders investigated for the presence of a cardiac ganglion[5]

Agelenidae
- *Agelenopsis naevia* (Walckenaer)
- *Agelenopsis pennsylvanica* (C. L. Koch)
- *Coras medicinalis* (Hentz)

Amaurobiidae
- *Amaurobius bennetti* (Blackwell)

Clubionidae
- *Chiracanthium inclusum* (Hentz)
- **Clubiona tibialis* Emerton

Epeiridae
- *Araneus cornutus* Clerck
- *Araneus trifolium* (Hentz)
- *Argiope aurantia* Lucas
- *Argiope trifasciata* (Forskal)
- **Mangora gibberosa* (Hentz)
- *Neoscona domiciliorum* (Hentz)

Linyphiidae
- *Pityohyphantes phrygianus* (C. L. Koch)

Pholcidae
- *Pholcus phalangioides* (Fuesslin)

Pisauridae
- *Dolomedes tenebrosus* Hentz
- *Pisaurina mira* (Walckenaer)

Salticidae
- *Evarcha hoyi* (Peckham)
- *Habronattus borealis* (Banks)
- **Zygoballus bettini* (Peckham)

Tetragnathidae
- *Tetragnatha* (Eugnatha) *straminae* Emerton
- *Tetragnatha versicolor* Walckenaer

Theridiidae
- *Steatoda borealis* (Hentz)
- *Theridion* (Parasteatoda) *tepidariorum* (C. L. Koch)

Thomisidae
- *Misumena vatia* (Clerck)
- *Misumenoides formosipes* (Walckenaer)
- *Misumenops asperatus* (Hentz)
- **Xysticus elegans* Keyserling
- **Xysticus funestus* Keyserling