tions by the method of Ceriotti (1955). On a dry weight basis, DNA and RNA contents of Reiter treponeme were found to be 0.7% and 1.48%, respectively, and the RNA : DNA ratio was closer to that of bacteria than to that of yeast. Base ratio analysis and thermal transition experiments were carried out on each individual nucleic acid. Nielsen [1] mentioned studies by Rathlev on the structure of treponemes, concentrating on the RNA and DNA of the Reiter treponeme.

9. EFFECT OF PHYSICAL, CHEMICAL AND BIOLOGICAL AGENTS

PHYSICAL AGENTS

Bednova (1957) demonstrated that the effect on cultured treponemes of different chemicals, antibodies and immune serum, as well as refrigerating to −10°C to −15°C and heating to 45°C to 60°C, was to produce a speedy formation of granules and the disappearance of spiral forms. Hardy & Nell (1961a) found that Reiter treponemes under suitable conditions behave like protoplasts, and that they are extremely sensitive to osmotic-pressure changes in the medium. When suspended in a 0.15 M to 0.10 M sodium chloride solution the treponemes retained spiral morphology, whereas organisms suspended in more dilute salt solution and distilled water rapidly became spherical. In solute concentrations of 0.3 osmolality, treponemes in lactose, sodium chloride and some other salts, retained spiral shape, while in glycerol, sucrose and some other sugars, the treponemes formed spheres. Aeration and heating the treponemes to 40°C or above were found to induce sphere formation. A variation from pH 6 to pH 9 had little effect. Those findings demonstrated the selective permeability of the plasma membrane of the Reiter treponeme.

Hampp (1947b) devised a liquid medium for the cultivation and drying of certain treponemes from the frozen state. The subsequent darkfield examination of dried specimens showed organisms with typical morphology, but without motility. Viable organisms were obtained from lyophilized " English Reiter strain " stored for periods of 24 hours, 16 days, and 7 months. Gastinel et al. (1958) stated that lyophilization had not preserved the motility and pathogenicity of the virulent *T. pallidum*, but that Reiter treponemes, particularly young cultures, had been preserved after lyophilization for from 92 to 130 days. Although immobile after 215 days, it could be subcultured. The following species or strains of spiral organisms were tested by Hanson & Cannefax (1964) for viability, both microscopically and culturally, at 24 hours and at 30-day intervals for one year after drying from the frozen state: Kazan 2, 4, 5, and 8, Reiter treponeme, English Reiter, Nichols, and Noguchi; *T. microdentium*, strain FM; *T. phagedenis*; *Borrelia vincentii*, strain N-9; *T. zuelzerae*; and *Borrelia refringens*. Growth obtained in 72 hours from lyophilized cultures stored for one year was equivalent to that obtained from organisms preserved by storage at −70°C for the same length of time. Lyophilization of *Treponema* and *Borrelia* strains was found to provide a convenient and inexpensive method of maintaining viability for a minimum of one year. Annear (1962a, 1962b) reported the successful preservation of leptospires by drying from the liquid state and then applied the same method to the preservation of the Reiter treponeme. He placed 0.04 ml of equal parts of the treponeme suspension and 20% glucose solution in 150 mm × 8 mm tubes and dried these *in vacuo* over P_2O_5. The suspension dried as a coarse foam in the bottom of the tubes. The dried suspensions were stored at room temperature for 12 months, rehydrated with freshly prepared fluid thioglycollate medium with agar, and incubated at 37°C. All tubes showed flourishing growth in 4-5 days, active forms beginning to appear after 1-2 days' incubation.

CHEMICAL AGENTS

Rose & Morton (1952a) found that Tween-80 inhibited the growth of Nichols, Kazan, and Reiter treponemes, and Keller & Morton (1952) reported that the same organisms had an unusually high susceptibility to soaps. Exposure to dilutions of at least 1 : 1000 of Ivory soap and of Dial soap with 2% hexachlorophene killed all treponemes within 5 minutes. Mutermilch, Gerard & Delaville (1959) demonstrated that different agents, such as mercury, arsenic, bismuth and vanadium, had the same activity on Reiter treponemes as on Schaudinn treponemes.

Kawata & Kaizo (1959) found that Reiter treponemes are rapidly lysed in a phosphate-buffered

[1] Unpublished working document WHO/VDT/RES/45 of December 1963.

saline after addition of toluene or irradiation with ultraviolet light. Cells were lysed in a buffered saline within 2-4 hours at 37°C. The optimum pH for autolysis was pH 7.0-8.0, no lysis occurring at pH 5.3. Lysis was inhibited by heating at 80°C to 100°C for 10 minutes, but not by heating at 50°C to 60°C. Lysis did not occur in distilled water. When cells were suspended in buffered saline with 25 000-50 000 units penicillin per ml, rapid lysis was observed within 15-30 minutes. Lysis of the Reiter treponemes was inhibited by adding 10 mg to 50 mg per ml of dihydrostreptomycin.

Costa & Casciano (1960a) studied the effect of lysozyme on the Reiter treponeme. They were concerned with the following: (*a*) morphological changes of Reiter treponemes when brought into contact with lysozyme solutions of 1.5%, 1% and 0.5% for 1 hour at 37°C (after staining these by the method of Fontana-Tribondeau, it was not possible to find any well-preserved treponemes, all microscopic fields being occupied by masses of granules; less concentrated solutions of lysozyme produced lesser changes); (*b*) tests for agglutinability of Reiter treponemes suspended in 1% and 1.5% lysozyme (these organisms underwent complete agglutination with precipitation of the granular masses to the base of the tubes); (*c*) turbidimetric test. Reiter treponemes suspended in 1.5% lysozyme solution were added to tubes of Michaelis buffer from pH 2.62 to pH 9.64, incubated for 1 hour at 37°C, and examined with a colorimeter. Turbidity curves showed maximum turbidity of 80 units at pH 4.13. Controls showed maximum turbidity of 60 units at the same pH. Another peak of 45 turbidity units appeared at pH 7.0 with lysozyme. Del Carpio (1962) investigated the morphological alterations of pathogenic *T. pallidum* (Nichols) and Reiter treponeme brought about by lysozyme under the following conditions: (*a*) concentration of lysozyme and time of exposure; (*b*) composition of suspending medium; and (*c*) aging and storage of treponeme. Studies were done by darkfield, contrast, and electron microscopy. The fibril bundle which twists the treponeme remained intact under the action of lysozyme, although this enzyme caused profound alterations of the treponeme body.

PENICILLIN

Eagle & Musselman (1948) demonstrated a significantly decreased net rate of multiplication of Reiter treponemes in culture medium containing crystalline penicillin G in a concentration of 0.016 μg per ml. The minimal concentration with a net bactericidal action was 0.032 μg per ml and almost maximum effect was produced by concentration of 0.25 μg per ml to 1.0 μg per ml. Thereafter, a 2000-fold increase in penicillin concentration, up to 2040 μg per ml, only slightly accelerated the rate at which organisms were killed. The Reiter treponemal cultures were killed at a slow rate, as compared with *Streptococcus pyogenes* C-203. To kill 99.9% of Reiter treponemes, at the maximum effective level of 1 μg per ml, required from 23 to 33 hours, as against 1.4 to 2.2 hours for the streptococcus strain. Scarpa (1949) confirmed the work of Eagle & Musselman, in that immobilization of Reiter treponemes occurred at doses of 400-600 IU of penicillin after 24 hours, and at doses of 5000-7000 IU after 3 hours. The Reiter treponeme could not reproduce in tubes containing only 10 IU of penicillin. No lysis of the organisms was observed at the doses employed. According to Morton & Ford (1953), Reiter treponemes and avirulent Nichols treponemes cultivated in medium containing 0.001 IU and 0.002 IU of penicillin per ml were morphologically similar to those in the control without penicillin. However, in the tube with 0.004 IU per ml the treponemes were definitely elongated, and with 0.009 IU per ml there was no visible growth. Vjaseleva (1957a, 1957b), working with *T. pallidum* isolated from blood of patients with syphilis, Kazan II strain, and Reiter treponeme, showed that a high concentration of penicillin was necessary to affect the morphology and motility, immobilization depending on the concentration and duration of action. After the strain had been in penicillin medium for 2 hours, subcultures could be obtained, demonstrating that loss of motility and partial deformity of the treponemes were not necessarily signs of death. Vjaseleva stated that it was possible that treponemal granules were less damaged by penicillin and were able to give rise to cultures of typical forms. Very small doses of penicillin (0.055-0.008 IU per ml) exerted a stimulatory action on growth. The minimum spirochaetostatic dose averaged between 0.015 and 0.039 IU per ml, and maximum doses, verging on a spirochaetocidal dose, averaged between 1.84 and 3.16 IU per ml. After prolonged passage through media containing penicillin for 6 months, the treponemes became poorly developed and underwent morphological changes, but fermentative properties were only slightly affected. Gastinel, Collart & Dunoyer (1959) found that the presence of sub-

inhibitory doses of penicillin in medium favoured the growth of elongated forms and inhibited the transverse division of the Reiter treponeme. No great differences in bacteriostatic or bacteriocidal doses for the normal or elongated forms were observed.

Tung & Frazier (1946) could not develop in Reiter treponemes an increased tolerance to penicillin after 15 passages. After a 72-hour incubation, the sub-lethal level of penicillin was 0.05-0.1 IU per ml and the spirochaetostatic level approximatively 0.125 IU per ml. Under identical conditions, *Staphylococcus aureus* 209 became about 100 times more resistant to penicillin than the original strain. Penicillin apparently inhibited cellular division and resulted in elongated forms which were short-lived. The increase in treponeme length was from an average of 20 μ to an average of 40 μ to 60 μ, and occasionally to 100 μ to 150 μ. In electron micrographs the long forms appeared to have lost flagella. Mutermilch, Gerard & Delaville (1959) found it impossible to obtain *in vitro* an antibiotic-resistant strain of Reiter treponeme, and they observed that the morphological alterations induced in Reiter treponeme by penicillin were similar to those observed in old cultures. Berger & Marggraf (1960) were able to increase the resistance of Reiter treponeme to penicillin over 100 times in *in vitro* immobilization tests. The initial resistance of Reiter treponeme to penicillin was to 0.06 IU per ml, and after 20 passages in cultures the Reiter treponeme was resistant to 7.0 IU per ml. Ustimenko (1963) isolated L-forms of *T. pallidum* strains—Stavropol VII and Stavropol IX—morphologically resembling the L-form of bacteria (spheres, vacuoles, granules) through the action of penicillin. The most favourable medium for the cultivation of *T. pallidum* L-forms was one suggested by Kagan & Levashev (1961) for isolation of L-forms of streptococci with an optimal concentration of 15.62 IU of penicillin per ml of medium.

OTHER ANTIBIOTICS

Keller & Morton (1953) indicated that the Kazan, Nichols, and Reiter strains were very susceptible to action of erythromycin. Vandeputte (1951), working with the Nichols, Noguchi, and Reiter cultured strains and the Gand strain in rabbits, found that bacitracin and terramycin had only slight or no action on motility and that tyrothricin was more active. Tyrothricin inhibited growth considerably in Nichols and Noguchi cultures and only slightly in Reiter cultures. Bacitracin inhibited growth completely. Berger & Marggraf (1960) increased the resistance of Reiter treponeme to terramycin 8 times, the initial value being 2.0 μg per ml; after 20 passages, the final value was 16 μg per ml. Berger (1960) noted that high resistance of the Reiter treponeme to streptomycin had not developed. Meinicke (1961) found that griseofulvin at concentrations of 0.0000375 μg per ml to 375 μg per ml showed no influence on motility of Reiter treponeme or *T. pallidum*. Mutermilch & Gerard (1957a, 1957b), working with Reiter treponemes *in vitro*, and Turner & Schaeffer (1954) with *T. pallidum in vivo* in rabbits, obtained similar results on the effect of antibiotics on those organisms. Antibiotics without action on Reiter treponemes were: soframycin, viomycin, polymixin, neomycin, tyrothricin, and streptomycin. Streptomycin had some action on *T. pallidum*. Antibiotics with action on Reiter treponeme were: penicillin, erythromycin, magnamycin, terramycin, rovamycin, tetracycline, chloramphenicol, aureomycin, and bacitracin. The bacteriostatic dose of penicillin was 0.3 IU or 0.18 μg, and the bactericidal dose was 600 IU. Mutermilch & Gerard concluded that apparently easy and economical *in vitro* experiments on Reiter treponemes were able to serve as a test in the study of therapeutic action of antibiotics in syphilis.

Sole-Vernin & Vieira (1961) recovered Reiter treponemes from a culture contaminated with a *Sarcina* species by first determining the antibiotic sensitivity of the contaminating bacteria, and then by serially inoculating the contaminated culture on media to which the antibiotic, kanamycin, had been added. They verified the selective bactericidal effect of the antibiotic on the contaminating organism and demonstrated that it did not interfere with the normal vitality and morphology of the Reiter treponeme.

10. ANTIGENIC COMPONENTS

Eagle (1937) suggested that the treponemes might well contain an antigenic factor immunologically similar to a lipid present in normal tissue. Beck (1939) postulated that the treponemes contained the ubiquitous lipoidal substance representing the Wassermann antigen and a specific antigen. Kolmer, Kast