

THE BACTERIOSTATIC EFFECTS OF  
SULFONAMIDE-UREA  
COMPOUNDS

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A thesis presented for the degree of Master of  
Science in the American University of Beirut.

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ABSTRACT OF THE THESIS ON THE BACTERIOSTATIC EFFECT OF

SULFONAMIDE-UREA COMPOUNDS.

The antibacterial properties of three new urea-sulfonamide compounds have been studied in vitro against the colon-typhoid-dysentery group, two strains of Staphylococcus aureus, two strains of Streptococcus viridans, four gram-positive spore bearing rods, three strains of Corynebacterium diphtheriae, and on Mycobacterium tuberculosis var. hominis.

Different techniques were used for the study of the various organisms. The method of Strauss and Dingle (186, 187) was used for testing the bacteriostatic effect of the sulfonamide-urea compounds upon Escherichia coli and Staphylococcus aureus. Other gram-negative rods, and the three strains of Corynebacterium diphtheriae were grown in veal infusion broth to which powdered crystals of the drugs to be tested were added so that the final concentration was 10mg per 100 c.c. Sixteen to twenty hour broth cultures of all the organisms were used; to 5 c.c. of the control and broth containing the various drugs 0.1 c.c. of varying broth dilutions were added. To test the bacteriostatic effect of the compounds upon Streptococcus viridans the same method as used in testing the bacteriostatic <sup>effect</sup> upon the gram-negative rods was used except the drug concentrations were variable and the inoculum consisted of Youmans (225) synthetic medium, containing 10 mg per 100 c.c. of the drugs to be tested, ~~was~~ was used in testing the effect of the compounds upon Mycobacterium tuberculosis. For inoculation 0.1 c.c. volumes containing the desired amounts, 0.4, 0.2, and 0.1 mg of tubercle bacilli were used. The method of Spray (183) was used to test the bacteriostatic effect of the compounds on Clostridium welchii, Cl. tetani, Cl. sporogenes and Cl. chauvei.

The results showed that sulfanilamido-urea and 4-homo-sulfanilamido-urea were superior to sulfathiazole in their inhibitory actions upon Clostridium welchii, Cl. tetani, Cl. sporogenes, and Cl. chauvei in concentrations of 15 mg. per cent. Sulfanilamido-thiourea showed much less activity than sulfathiazole in veal infusion broth;

A strain of Streptococcus viridans was completely inhibited by 10 mg. per cent sulfathiazole in veal infusion broth; sulfanilamide-urea and sulfanilamido-thiourea exerted a partial inhibition under the same experimental conditions but were completely bacteriostatic in concentrations of 100 mg. per cent. 4-homo-sulfanilamido-urea had no inhibitory effect. Sulfathiazole in concentrations of 10 mg per cent exerted a marked inhibitory action on the growth of virulent human Mycobacterium tuberculosis in a synthetic medium. Sulfanilamido-urea, sulfanilamido-thiourea and 4-homo-sulfanilamido-urea under similar conditions gave negative results. Sulfathiazole had a definite and marked bacteriostatic effect on one of the three strains of Corynebacterium diphtheriae tested, provided appropriate inocula were used. The sulfonamide urea compounds exhibited it irregularly and to a lesser degree than sulfathiazole. Two strains of Staphylococcus aureus exhibited no alteration of their growth in a synthetic medium containing the sulfonamides.

Sulfanilamide and sulfaguanidine, in concentrations of 3-5 mg. per cent were bacteriostatic upon Escherichia coli in a synthetic medium. This action was not shared by the sulfonamide-urea compounds. The sulfonamide were without effect on the various other gram-negative rods despite varying the size of inocula; sulfathiazole exerted slight bacteriostatic effect on some of the gram-negative rod..

The bactericidal effect of fresh, whole blood upon streptococci reported by Todd, was confirmed.

Suspensions of a virulent strain of Mycobacterium tuberculosis rapidly produced surface and subsurface growth when inoculated into the synthetic medium of Youman.

The thesis also includes a review on the in vitro testing of sulfonamide drugs.

### INTRODUCTION

Para-amino-sulfonamide was synthesised by Gelmo working in 1908, but its bactericidal power was not discovered until 1913 by Eisenberg. In 1932 Mietzsch and Klarer synthesized the original prontosil (the hydrochloride of 4-sulfamido-2'-4'-diamino-azobenzene), a red powder 0.25% soluble in water, and in the same year Domagk pointed out the streptocidal action of certain azo dyes in infected animals, and showed the high therapeutic activity of prontosil. Levaditi and Vaisman (1935) quickly confirmed these results. The next important finding, was that of Trefouel, Nitti and Bovet (1935), who noticed, following the observation of Heidelberger and Jacobs (1919) on the bactericidal action in vitro of sulphamido-benzene-azo-hydrocupreines, that azo-compounds not containing a sulphonamide group attached to one of the benzene nuclei were inactive.

Over a thousand derivatives of sulfanilamide have been introduced and some have been tried both clinically and experimentally. Some of the derivatives tested have proved better than sulfanilamide against certain diseases, for example, in infections due to staphylococci sulfathiazole and sulfapyridine are much more effective than sulfanilamide (20, 21, 28, 154). Following a report by Whitby (206), that sulfapyridine is a much more effective agent than sulfanilamide in the treatment of experimental pneumococcus infections in mice, numerous articles appeared on the use of this drug both experimentally and clinically (14, 42, 166, 153, 68). Sulfapyridine like sulfanilamide penetrates readily all the tissues and the body fluids but sulfapyridine is usually present in higher concentration in the liver than

in other tissues (120). Sulfanilylguanidine is therapeutically active against various bacteria but unlike sulfanilamide is poorly absorbed from the intestinal tract (122), allowing the attainment of a high concentration of drug in the intestinal tract and a low concentration in the blood and tissues. This property makes a drug useful in the treatment of bacterial infections which are mainly or entirely localized in the intestine.

Most papers on the action of these drugs have been concerned with studies in vitro, and since recent papers show a surprising parallel between resistance in vitro and unsuccessful clinical treatment, the in vitro tests seems to be of great value in the preliminary testing of new compounds. Cohn (39) demonstrated correlation between clinical and in vitro reactions of gonococcus strains to sulfathiazole. Spink and Vivino (182) showed that sulfonamide resistant staphylococci gave results which agreed in vitro and in vivo. Griffiths (69) tested the effect of sulfathiazole, sulfadiazine and sulfanilamide on cholera vibrio and got similar results; with the latter compound it was inhibition of growth in vitro and effective treatment of mice previously inoculated with lethal doses of cholera vibrios.

If a drug is effective bactericidal and bacteriostatic agent in the test tube, it may or may not be effective in vivo, but it cannot be expected that a drug ineffective against a certain bacterium in vitro will prove successful in combating a disease caused by that organism (124). Long and Bliss (110) expressed the belief that there is an appreciable degree of correlation but that parallelism ceases when relative susceptibilities of different organisms in cultures and their

reactions in the animal body are compared. Spray's (183) experiments showed that Clostridium welchii which is most effectively controlled in the animal body is least inhibited in the test tube; Clostridium sordellii which is least successfully controlled in the animal body was among the species most effectively inhibited in the test tubes, probably due to sulfonamide inhibiting substances produced by the organisms themselves. Read and Orr (157, 158, 159) observed the fact that species like Clostridium welchii which produce large amounts of inhibitor and against which the sulfonamides exert slight in vitro bacteriostatic action are effectively controlled in the animal body by the same sulfanilamides; or that species like Clostridium sordellii which produces little inhibitor and against which sulfonamides exert marked bacteriostatic action in vitro are much less effectively controlled in the animal body by the same sulfonamides.

There is great variation in the effectiveness in vitro of the sulfonamide drugs on a large number of strains of a single species of bacteria. Poston and Orgain (151) working with Streptococcus viridans demonstrated that out of a total of twenty-five strains, the growth of seventeen was inhibited by one or more drugs. Three were inhibited by one or more drugs. Three were inhibited by all seven drugs, while seven strains were not inhibited by any of the drugs, and the remaining fourteen showed considerable variation. Schmidt and his co-workers made extensive studies of the response of different types and strains of pneumococci to sulfapyridine (156, 157, 158). These investigators, working with strains of types I, II and III found that sulfapyridine was uniformly more effective against experimental infections with type I than against infections with type III, and that infections with

certain type II strains responded as type I, whereas infections with other strains of type II responded as type III. They suggested that these differences in type and strain response are related to differences in antigenicity. Certain strains of pneumococci are extremely resistant to sulfapyridine therapy and others are extremely susceptible, in addition there appears to be as much variability in therapeutic response as may occur between strains of certain types as between types (123).

Marked differences are seen in the reports of different investigators as to the effect of these drugs on bacteria in vitro; some workers find no effect, others a definite bactericidal effect. These differences are due mainly to differences in the conditions of the experiments. Much research has been expended upon the effect on sulfonamide activity of certain environmental conditions which are easily varied. It has been observed by numerous investigators (33, 89, 104, 77) that the inhibition is directly related to sulfonamide concentration, this relationship may not be strictly linear over a large range of concentrations (77). In the presence of a constant amount of sulfonamide, the inhibition of bacterial growth is inversely related to the number of organisms present, (33, 89, 104, 112, 127), that is, as the size of inoculum is increased a greater amount of sulfonamide is required to produce the same inhibition. In a small number of cases inoculum has little effect (9, 115). Nitti et al (142) claimed that the importance of inoculum size varies with the organism, being of less importance with meningococci and gonococci than with streptococci and pneumococci, and of no significance whatsoever in growth inhibition of the mold Aspergillus niger. Sulfonamide action is definitely influenced by the pH of the environment; this was first noted by those interested in sulfonamide



therapy of urinary tract infections, where the pH can be controlled within limits. Helmholtz et al (76) showed that sulfonamide activity in urine is increased as the pH is raised, e.g., from the range 5.5-6 to 7.5-7.8. Middlebrook et al (129) showed that sulfathiazole's bacteriostatic activity increases from pH 5.5 to pH 8.5 whereas sulfadiazine appears more active at the former pH of the medium.

The bacteriostatic and bactericidal action in vitro of the sulfonamides is definitely increased by an increase in temperature in vitro (100, 102, 207) and in vivo in gonococcal infections of the chicks chorio-allantoic membrane and in human gonococcal infections where artificial fever is used together with drug therapy ( , 7). The effect of temperature is probably due to enzyme inhibition (102, 100). The most exact experiments were those on streptococci reported by White (207), who found that at 30° C., sulfanilamide concentrations less than 0.058 M (1000 mg%) are inactive at 36° C., concentrations less than 0.0058 M (100 mg%) are inactive, and at 39° C., concentrations of 0.00058 M (10 mg%) or less are bactericidal.

The fifth environmental factor affecting the activity of sulfonamides is the composition of the medium. A good example of the role played by the medium is the observation by MacLeod and Mirick (114), that properly prepared fresh calf-liver infusion has no sulfonamide-counteracting action, and to produce the same results in plain broth and peptone broth, twenty to forty times as much sulfonamide is required. It has been stated by many investigators that the poorer the medium used for the growth of the organisms, the more effective is the action of the sulfanilamide; in other words if the medium is not optimal for growth, sulfonamide action is more pronounced (114, 182, 60, 93). Weld and

Mitchell (203) believed that peptone does not interfere with the bactericidal effectiveness of sulfonamides but peptone furnishes a richer medium for growth. Although this assumption probably contains a great deal of truth, it does not completely explain the effect of changes in the medium on the activity of sulfanilamide. Wolf and Julius (209) showed that streptococci in broth medium, where growth is extremely slow, were not influenced by sulfanilamide, but a change to a blood broth medium, where better growth occurs, resulted in bacteriostasis. They concluded that a certain rapidity of growth was essential for the action of sulfonamides. Harris (72) showed that the physical properties of a medium are of surpassing importance, and that a medium may appear to be actually bactericidal even when other factors are favorable for growth.

The finding that substances which inhibit sulfonamide action can be obtained from bacteria (53, 65, 184, 150), peptones, various animal tissues (114, 184, 211, 108), red blood cells and urine of the mouse (60), and yeast (211, 205); the statement that purified preparations, while keeping their antisulfanilamide potency have no effect upon growth, and the extreme variability in the amounts of sulfonamide-antagonists in various media, makes comparison of results in vitro practically impossible (110, 104, 114, 186, 187, 188). However, with the proper selection of media, careful checking of the concentration of drug in the medium, controlling the size of the inoculum and the temperature of incubation, in vitro tests have proved themselves very useful and have contributed to the advances achieved in chemotherapy.

Numerous experiments have been published on in vitro studies on the action of chemotherapeutic agents. The literature has been reviewed

by Marshall (123, 121), Findlay (49), and Henry (77).

The action of sulfonamides on the viruses has been investigated. The viruses for which there is adequate evidence indicative of a therapeutic response to sulfonamides are: trachoma (160), lymphogranuloma venereum (80, 173), inclusion blennorrhoea and the virus of psittacosis (77). Other virus infections have been investigated, some have been reported as susceptible to sulfonamide therapy; however, most of these claims have been disputed; others, such as the smallpox and yellow fever virus, have been found to be unaffected.

Protozoa have been tested by numerous investigators. Amebae, paramoecia, trichomonads (63), toxoplasma (164), Leishmania tropica (174), and Endamoeba histolytica (161), have been reported to be inhibited by sulfonamides. The effect of various sulfonamides against the malaria parasite in vitro and vivo has been investigated (36, 37, 38, 173, 119). Reports of the effectivity of sulfonamides on human malaria have been somewhat conflicting. There have been many uncertain or unfavorable and many favorable reports with regard to the therapeutic effectiveness of sulfonamides against human tertian malaria (Plasmodium vivax), quartan malaria (P. malariae), and estivo-autumnal malaria (P. falciparum). It appears in general that the sulfonamides are more effective against the more virulent plasmodia (77). Sulfonamides have been found repeatedly to be effective against the virulent P. knowlesi infection in rhesus monkeys, while they exert no effect on the milder P. cynomolgi and P. inui infections (36, 37, 38).

Other cells, hydra, mesostoma (flatworm), stenostoma (rotifer), Dero (annelid) (97, 47), chick embryo heart tissue culture, bone marrow, various yeasts (91), (Torulosporea, Torula, and Saccharomyces), various

fungi (Trichophyton gypseum) (103), Trichophyton purpureum (45), Blastomyces dermatitidis (144), and higher plants (algae) (35), a diatom (208), tomato roots (24), and pisum roots (118), are said to be inhibited by sulfonamides.

Numerous in vitro studies of the effect of sulfonamide drugs against different species of bacteria have been made. These have had two purposes: an elucidation of the mode of action of these drugs (105, 202, 210, 176, 74, 134, 137, 66, 192, 22) and their appraisal for clinical use.

The comparative action in vitro of sulfonamides, on representative organisms of the colon-typhoid-dysentery group has been studied. Long (117), Lawrence (97), and Libby (106), found that sulfathiazole was equal to, or, slightly superior to sulfanilamide and to sulfapyridine in its bacteriostatic action upon the colon-typhoid-dysentery group. Marshall, et al (122), showed that a new sulfacompound, sulfanilylguanidine, compared favorably with the in vitro effects of sulfanilamide and sulfapyridine upon micro-organisms, particularly those of the colon-typhoid-dysentery group the effect of sulfanilamide, sulfapyridine and sulfanilylguanidine were further studied by Lawrence upon additional organisms of the group mentioned and compared with sulfathiazole and sodiumsulfathiazole under corresponding experimental conditions (98). Rammelkamp and Jewell (155) compared the action in vitro of sulfanilamide, sulfapyridine, sulfamethylthiazole and sulfathiazole on the representative organisms of the typhoid-dysentery group and concluded that sulfathiazole was only slightly superior to sulfamethylthiazole and sulfapyridine, under their experimental conditions. The anti-

bacterial effect of thiazole compounds against Salmonella enteritidis was studied by Muir, Shanleffer and Jones (132); these authors also studied the use of a synthetic medium in the study of antibacterial effect of sulfathiazole. Hill (79), compared the action of sulfanilamide, sulfapyridine and sulfathiazole in a urine medium, Straus and Finland (189) demonstrated the bactericidal and bacteriostatic activity in vitro of sulfadiazine and sulfathiazole on the colon-dysentery-typhoid group of organisms in a simple medium. Schweinburg and Yetwin (190) tested the in vitro action of sulfamethazine as compared with that of sulfadiazine and sulfamerazine, and concluded that sulfamethazine in vitro is by far a more effective bactericidal and bacteriostatic agent against Eberthella typhosa, Escherichia coli, and the salmonella varieties than the other sulfonamides examined. Bémer tested the in vitro action of various sulfanilamide derivatives on Shigella dysenteriae and showed that sulfathiazole and sulfadiazine gave the best results while sulfaguanidine was relatively ineffective (13). The inhibitory effect of sodium sulfathiazole on Shigella paradysenteriae (flexner) and Salmonella pullorum in the developing chick embryo was shown by Weil and Gall (199).

The effect of sulfonamide drugs on Vibrio cholerae has been investigated; sulfathiazole, sulfadiazine and sulfanilamide (69), and sulfanylguanidine (10), inhibited the growth of Vibrio cholerae in vitro.

Sulfonamides are known to be excreted in the urine and their effect on bacterial infections of the urogenital tract, especially those due to Escherichia coli, has received considerable attention both clinically and experimentally, (186, 97, 189, 10, 89, 79, 141, 125, 11, 40).

Sulfonamide drugs have had an extensive trial, both in vitro and in vivo, against the tubercle bacillus. In vitro tests have given very variable results. Ballou and Guernon (2, 3, 4), showed that under specific conditions and in certain concentrations, sulfanilamide exerts an inhibitory effect upon the growth of virulent human tubercle bacilli in vitro. Fellis (54), who employed liquid media and large inocula failed to observe any appreciable bacteriostatic effect of either sulfanilamide or its acetyl derivative upon the growth of tubercle bacilli (var. hominis). Ballou and Guernon (5) in a later communication found that sulfathiazole and sulfamethylthiazole exerted a pronounced inhibitory effect upon the growth of virulent human tubercle bacilli on solid media, whereas sulfapyridine and sulfanilamide under similar conditions exerted a less striking inhibitory effect; this inhibitory effect was not observed when a liquid medium was employed. Working with liquid media Rist (160), showed that 4,4'-diamino-diphenyl-sulfone in dilution of 1:10,000 and 1:100,000 exerted a marked inhibitory effect upon the growth of human tubercle bacilli in vitro. Rist (160a) further showed that sulfonamides in a concentration of 1:500 exerted complete inhibition, and even in a concentration of 1:1000 a definite retarding effect upon the growth of tubercle bacilli was also shown in vitro. Giroux (61) confirmed these latter findings. Sulfapyridine was shown to possess more potent mycobactericidal properties in vitro than sulfanilamide (16). Green (64), further, showed sulfapyridine, in concentrations of 1:1000 and 1:2000 inhibited the growth of tubercle bacilli. The comparative action in vitro of sulfonamides on the tubercle bacilli was further investigated by Muller (135) and Youman (212). They

confirmed that sulfathiazole showed bacteriostasis in a concentration of 5 mg. per cent. Sulfanilamide had little effect on the tubercle bacilli under similar experimental conditions.

A large series of benzophenone derivatives, and other ketones (58), new derivatives of diamino-diphenyl-sulfone and naphthoquinones (107) were examined for tuberculostatic action in vitro. Of the compounds tested, the dichloro derivatives in the 2.2' and 2.4' positions showed a greater degree of inhibition against the tubercle bacilli than all the compounds investigated (58). Of the fifteen naphthoquinone compounds tested against the tubercle bacillus in vitro, only 3-sulfanilyl-1,4-naphthoquinone showed sufficient promise for further investigation (107).

The sulfonamide group of drugs were first shown to have a specific action on the streptococci. The use of mice infected with highly virulent culture of a group A-beta hemolytic streptococcus is still the most common method used for testing the chemotherapeutic activity of new compounds. The effect of the sulfonamide group of drugs has now been tested on almost every kind of streptococcus both clinically and experimentally (207, 108, 203, 139, 140, 151, 127, 177, 195, 146, 175, 166, 179, 59, 198, 18, 99, 67, 19, 113).

Most of the studies in vitro have been carried out with sulfanilamide and streptococci, (127, 207, 195, 108, 124, 25, 99, 18, 139, 203).

In vitro tests on the bacteriostatic effect of sulfanilamide and its derivatives on the hemolytic streptococci has given encouraging results (127, 207, 195, 108, 124). The most extensive papers are those of Marshall (124). He made a qualitative comparison of the in vitro

and in vivo activity on Beta-hemolytic-streptococci of one-hundred and twenty-six compounds consisting of sulfanilamide derivatives, sulfonamides, sulfones, sulfoxides and sulfides. White and Parker (207) showed the bactericidal action upon thirty-six strains of beta-hemolytic streptococci with 20 mg per cent sulfanilamide in vitro. Hamilton (71), determined the bacteriostatic effect of sulfathiazole (5 mg. per cent) on streptococci of Lancefield's Groups A, B, C, D, G and Streptococcus fecalis. Streptococci of Lancefield's Group A, B, C and G were inhibited whereas Group D streptococcus and Streptococcus fecalis were resistant to sulfathiazole in concentrations of 5 mg per cent.

The bacteriostatic effect of sulfonamide drugs upon the non-hemolytic streptococci has given variable results (151, 99, 18, 67, 127). Poston and Orgain (151) compared the bacteriostatic effect of the sulfonamide drugs on the growth of twenty five strains of Streptococcus viridans. A great variation in the effectiveness of these drugs against various strains and in the susceptibility of each strain to the various drugs has been noted. Neter (139) inhibited the growth of Streptococcus fecalis with sulfanilamide by retarding the growth of the hemolytic enterococci with the addition of 6.5-7 per cent sodium chloride or maltose 0.25 per cent. Weld and Mitchell (203), support the findings of Neter (130); these authors showed the sulfanilamide in moderate concentrations is bactericidal only for streptococci which are multiplying slowly. Long and Bliss (18), showed that sulfanilamide in concentrations of 1:10,000 markedly inhibited the growth of the alpha-hemolytic streptococcus. However, Britton (26), tested three strains of Streptococcus viridans and found that two were unaffected by a concentration of sulfanilamide as high as 1:500 and the third strain



was inhibited in its growth only when the concentration of sulfanilamide was 1:1000.

Experimental endocarditis by traumatizing the endocardium, or by the injection of particulate matter, in addition to bacterial inoculations, have been shown possible by Welch et al (201). Rosenow (163) and Horder (82), produced endocardial vegetations by simply infecting intravenously cultures of Streptococcus viridans. MacNeal et al (116) demonstrated that it is possible to transmit endocarditis lenta of man to the rabbit by repeated intravenous infections of pure cultures. Mueller and Kinsella (131) stated that they established bacterial endocarditis with persistent bacteremia in dogs, using a culture of non-hemolytic streptococcus. The objective of these experiments was to help resolve the problem of therapeutics in sub-acute bacterial endocarditis. However, the majority of reports concerning the efficacy of sulfanilamide are extremely pessimistic (131, 128, 179, 165).

Experiments in vitro have been made with pneumococcus. (19, 51, 52, 127, 113, 122, 120, 116a, 114, 170, 96). Nitti, Bovet and Depierre (145) found that sulfanilamide had an inhibitory effect on pneumococcus. White and Britton (26) noted a distinct but variable bactericidal effect on Type I, II, and Group IV pneumococci. Fleming (52) reported that sulfapyridine in concentrations of from 1:250,000 to 1:8,000 inhibited the development of large inocula of pneumococci in de-leucocyted human blood. It was shown by Finland et al (50, 51) that sulfanilamide and sulfapyridine in the concentrations ordinarily attained in therapy exerted a marked bacteriostatic action on Type III pneumococci when added to human blood in vitro. Long and Bliss

(113) further showed sulfathiazole to be as effective a bacteriostatic agent as sulfapyridine in broth cultures on Type I and II pneumococci. The bacteriostatic actions of three thiazole derivatives of sulfanilamide, namely 2-sulfanilamidothiazole (sulfathiazole), 2-sulfanilamido-4-methylthiazol (sulfamethylthiazol and 2-sulfanilamido-4-phenylthiazol (sulfaphenylthiazol) upon pneumococcus types I, II and III were studied. These compounds were found to be superior to sulfanilamide and sulfapyridine in their inhibitory actions in concentrations as low as 5 mg per cent (%). MacLeod (114) determined quantitatively the bacteriostatic effect of the sulfonamide drugs on pneumococci. The comparative in vitro action of sulfapyrazine (2-sulfanilamidopyrazine), sulfapyridine, sulfathiazole and sulfadiazine on pneumococci has been investigated by Schmidt et al (170). These same authors have made the most extensive studies on the response of different types and strains of pneumococci to sulfapyridine (168, 169) and have shown that sulfanilamide has considerable therapeutic value in pneumococcus infections in mice. They found that the therapeutic efficiency of sulfanilamide varied with the type of pneumococcus used as the infecting agent.

Studies in vitro have been carried out with sulfonamides and staphylococci (154, 156, 19, 113, 178, 83, 12, 187). Spink (178) studied the bactericidal effect of sulfanilamide upon fourteen strains of staphylococci, of which twelve belonged to the pathogenic (S.aureus), and seven were non-pathogenic (S.albus). All the strains were inhibited with a drug concentrations of 50 mg. per cent when suspended in urine. In a later communication Spink (180), in a comparative study on the antibacterial action of sulfanilamide and the

sodium salts of sulfapyridine, sulfathiazole and sulfadiazine obtained the greatest inhibition of growth with sulfathiazole and with sulfadiazine next. Several communications dealing with the activity of sulfathiazole and certain of its derivatives on staphylococci have appeared (99, 96, 78, 8, 76, 154). Harrell and Brown (78) found much greater in vitro bacteriostatic activity of sulfamethylthiazole than of sulfathiazole against Staphylococcus aureus. Lawrence (95, 96) found that sulfamethylthiazole had a greater bacteriostatic activity than had sulfathiazole. Barlow and Hamburger (8) reported results which indicated little, if any, greater activity of sulfamethylthiazole as compared with sulfathiazole. Rake et al (154), studied the activity of sulfathiazole and sulfamethylthiazole against Staphylococcus aureus as compared to that of sulfapyridine. The relationship of activity was found to be sulfamethylthiazole > sulfathiazole > sulfapyridine. Conflicting reports have appeared on the neutralization of staphylococcus toxins by sulfanilamide and allied compounds (12, 29, 30, 31). Carpenter and his coworkers (29, 30, 31) have reported an antitoxic effect of sulfanilamide and its derivatives on toxins formed by staphylococci and other bacteria. Bayliss (12) and Lavaditi et al (92), reported that the toxic manifestation of staphylococci are not inactivated in vitro by sulfanilamide and its derivatives.

The effect of sulfonamide drugs on the meningococci have been investigated. Favorable experimental and clinical effects of sulfanilamide have been reported with this organism (171, 117, 172, 152, 138, 84, 43). Studies were made on the effect of sulfanilamides upon the growth of meningococcus in vitro. Maergraith (117) showed the bacteriostatic effect of sulfonamide at a dilution of 1:60,000 and Schneiersen

(171) reported strong inhibitory effect of sulfanilamide and protosil upon the growth of meningococci in vitro.

The bacteriostatic action of sulfonamide drugs on gonococci have been studied. In vitro tests (117, 76, 204) indicate inhibitory effect of sulfanilamides upon the growth of gonococci. Bang and Betsy (6) determined the therapeutic efficacy of sulfathiazole and sulfadiazine upon gonococcal infections of the chorioallantoic membrane of the chick. Observations on gonococcal infections of mice have shown that they are readily curable by sulfanilamide and by a number of sulfonamide derivatives (93).

In vitro bacteriostatic action of sulfonamides against the gas gangrene clostridia have been reported. Spray (183), found the growth of Clostridium tetani, Cl. novyi and Cl. septicum to be inhibited by sulfanilamide and disulfonamide in low concentrations, but Cl. tertium, Cl. sporogenes and Cl. histolyticum to be inhibited only by high concentrations of disulfonamide. Reed and Orr (185) confirmed these latter findings. These authors showed that sulfanilamide, sulfapyridine, sulfaguandine, sulfadiazine and sulfathiazole are highly bacteriostatic for Cl. sordellii, Cl. septicum, Cl. carnis, a little less bacteriostatic for Cl. novyi and Cl. histolyticum; and, only slightly bacteriostatic for Cl. sporogenes and Cl. welchii. The in vitro antibacterial effects of sulfanilamidoindazoles upon Cl. welchii, Cl. tetani, Cl. histolyticum, and Cl. oedematiens have been successful (157, 158, 159).

The effect in vitro of sulfanilamide, sulfapyridine and sulfathiazole on Corynebacterium diphtheriae has been determined (147). These experiments confirmed and extended the observations made by Nitti

(142). Not only were the growth of these organisms retarded but marked bactericidal action on C. diphtheriae was observed. Of the three drugs tested sulfathiazole was active on the largest number of strains studied, and sulfanilamide least.

In vitro experiments on the bacteriostatic action of sulfanilamide on Brucella abortus are contradictory. Francis (57), believes that Br. abortus is more susceptible to the action of sulfanilamide in vitro than Streptococcus pyogenes under the same conditions; while Britton (27) reports that sulfanilamide and other allied compounds show no bacteriostatic action on Br. abortus, Br. suis and Br. melitensis. On a later communication Hamorn and Huddleson (70) showed the bacteriostatic effect of sulfapyridine on Brucella. The Brucellas tested were not inhibited by sulfanilamide-indazoles (99).

Most experiments dealing with the action of sulfanilamide and its derivatives in vitro were performed with pathogenic bacteria. However, experiments in vitro have been made upon aerobic spore-bearing rods (162), three of the subtilis-mesentericus group, and, one of the B. mycoides. Sulfanilamide inhibited spore formation and induced degenerative changes in the bacilli of subtilis-mesentericus-mycoides group.

The anti-bacterial properties of urea have been described by Peju and Rajat (148), Foulger and Foshay (56), Holder and Mackay (81) have reported a favorable response of infected wounds treated with mixtures of urea and sulfonamides. Tsuchiza, Tenenberg, Clark and Strakasch (196, 193,

197), showed that urea inhibited para-aminobenzoic acid and methionine, substances which antagonize the action of sulfonamides. These findings could not be confirmed by Kirby (87), but have been confirmed by Lee, Epstein and Foley (101).

It was shown by several investigators (193, 101, 196, 200, 87), that urea in conjunction with sulfonamides displays greater growth inhibitory effect than the later alone on Bacterium coli in vitro. Ecker (46) claimed to have cured a case of meningitis due to Bacterium coli, which failed to respond to sulfadiazine and penicillin, after receiving two grams of sulfadiazine and thirty grams of urea introduced into the stomach every four hours. Three strains of sulfathiazole resistant staphylococci were shown to be susceptible to urea-sodium sulfathiazole combinations whereas they were unaffected by these two agents used separately (197). Other studies (200) have shown that urea exerted both a bacteriostatic and bactericidal action, depending on the concentration used, and was active against Gram negative bacteria, Proteus vulgaris, Eberthella typhosa, Pseudomonas pyocyaneus, Salmonella schotmulleri, S. paradysenteriae (Flexner) and Bacterium coli; and also to a lesser degree against Gram positive bacteria, Staphylococcus aureus, pneumococcus and hemolytic streptococcus.

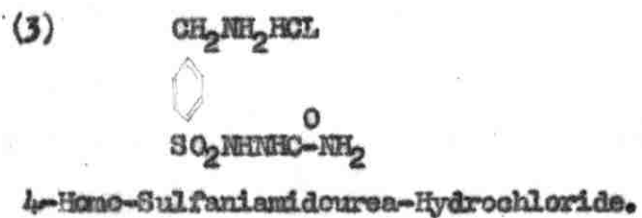
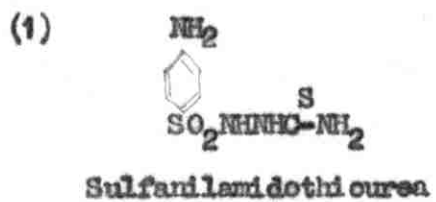
The bacteriostatic and bactericidal action of urea upon the tubercle bacilli in vitro has been shown by Symmers and Kirk (191) and Cummins (44). Mayer (126), made a comparative study of ten compounds including sulfanilamide, sulfapyridine,

sulfathiazole, thio-urea, phenylthiourea, sulfanil-amido-thiourea, ( $p\text{-H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NHCSNH}_2$ ), phenyl-sulfon-amido-thiourea ( $\text{PhSO}_2\text{NHCSNH}_2$ ) and sulfanilamido-ethyl-isothiourea ( $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{-NHC(=NH)SEt}$ ) on the tubercle bacilli in vitro. He found that the avian bacilli was less resistant than the human variety, that sulfanilamide and sulfapyridine possessed little activity, sulfathiazol was six to thirty times as active as sulfanilamide and sulfapyridine; thio-urea was less active than sulfanilamide and sulfapyridine. Sulfanil-amido-ethyl-isothiourea was inactive; while phenyl-sulfonamido-thiourea and sulfanil-amido-thiourea were very active.

The advantages of urea are: its relative non-toxicity either in the pure state or in saturated solution; its harmlessness to the tissues; it exerts marked solvent action on necrotic tissue, pus and debris, and thus chemically debrides contaminated wounds and mechanically remove inhibitors; it lyses bacteria (126, 148, 101, 197, 193); deodorizes foul smelling wounds rapidly and renders sulfonamides more soluble. Urea has been shown to have a marked beneficial effect in the treatment of septic wounds (44).

In view of these findings it seemed profitable to study the antibacterial properties of compounds containing sulfanilamide and urea, and sulfanilamide and thiourea.

The following compounds have been examined for their antibacterial properties using in vitro techniques :-





METHODS.

Different techniques were used for the study of the various organisms and these will be detailed under the organism concerned.

Escherichia coli.

Three strains of Escherichia coli isolated from the urine of patients with pyelitis were maintained by monthly transplants on agar slants and stores in the refrigerator (2°C.). The organisms showed typical cultural reactions, as defined by Bergey (1924).

Basal medium of Fildes (211) modified by Strauss, Dingle and Finland (1947) was prepared as follows :-

NaCl.....	1.0 g.
KH <sub>2</sub> PO <sub>4</sub> .....	4.0 g.
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.5 g.
NH <sub>4</sub> Cl.....	0.5 g.
FeSO <sub>4</sub> .(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .6H <sub>2</sub> O.....	0.02 g.
MgSO <sub>4</sub> .7 H <sub>2</sub> O.....	0.04 g.
Distilled water to.....	900. c.c.

The pH was adjusted to 7.2 with 1 N sodium hydroxide and the mixture heated in the Arnold steamer for 30 minutes. A granular precipitate, probably magnesium and iron salts, was removed after cooling by filtration through paper. The volume of the clear filtrate was adjusted to 900 c.c. with distilled water. The medium was distributed in 4.7 c.c. amounts into sterile test tubes and autoclaved at a pressure of 15 pounds

for 10 minutes.

Since the basal medium supported growth poorly or not at all, glucose and nicotinic acid solutions were added. Dextrose was made up in 25 per cent solution in distilled water and sterilized by Berkefeld filtration, the final concentration of dextrose in the medium was 0.25 per cent. A 5 per cent solution of nicotinic acid in distilled water was prepared; 5.8 g of sodium carbonate was added to the solution to dissolve the nicotinic acid and sterilized by Berkefeld filtration. The final concentration in the medium was 0.1  $\mu\text{g}$  per cent.

All solutions prepared separately were tested for sterility and added aseptically. Dextrose, nicotinic acid, the sulfonamide drugs and other test substances were added to the basal medium in 0.1 c.c. volumes in concentrations adjusted so that the appropriate amount of each substance was present in a final volume of 5 c.c. of medium.

Sulfanilamide, sulfaguanidine, sulfanilamido-urea, sulfanilamido-thiourea and sulfanyl-benzyl-hydrazide were dissolved in distilled water and heated in the Arnold steamer for 20 minutes before use. To account for the loss of water in the Arnold steamer the solutions were made in sterile volumetric flasks; after sterilization sterile water was added up to the mark. The stock solutions were 0.3 per cent from which the following lower concentrations were prepared by diluting these stock solutions with appropriate amounts of sterile distilled water. The concentrations used were: 0.25, 0.2, 0.15, 0.1 and 0.050

per cent. To inoculate the medium with organisms a small loopful of organisms was transferred from an agar slant to infusion broth and incubated for 6-10 hours at 37°C. The organisms were centrifuged at 2500rpm for thirty minutes, washed twice in the basal medium, to prevent the transfer of substances present in the agar or broth in which the organisms were grown, and then diluted in basal medium so that an inoculum of 0.1 c.c. added to 5 c.c. of test medium gave 2500 organisms per c.c. of medium. Hopkins tube method was used for counting.

Incubation was carried out at 37°C for five days; daily, the test tubes were examined for visible turbidity. Smears were prepared from the tubes showing cloudiness and stained with Gram's method in order to confirm growth and to rule out the Possibility of contamination.

#### OTHER GRAM NEGATIVE BACTERIA.

The organisms were stock laboratory strains of Eberthella typhosa (Rawling), Salmonella paratyphi (Schottmüller), Salmonella paratyphi (Sendai, 2216), Salmonella paratyphi (1853), Salmonella newport (London), Salmonella suispestifer (A. U. B., 1946), Salmonella schottmülleri B<sub>2</sub>, Salmonella schottmülleri (Tidy), Salmonella schottmülleri (5745, A. U. B.), Shigella paradysenteriae (Flemer W), Shigella dysenteriae (Shiga), Shigella dysenteriae (Shiga 4041 A. U. B.), Shigella paradysenteriae (Sonne 268), Salmonella enteritidis (Gaertner H<sub>3</sub> 3046), Salmonella typhimurium (aertrycke 3048), Salmonella pullorum (A. U. B.), Proteus vulgaris (448 A. U. B.), Vibrio comma (Qgans stock strain). The organisms showed typical cultural reactions as defined by Bergey

<sup>15</sup>  
(224). The cultures were stored at 15-20°C on plain agar slants and were transferred two times in the veal infusion broth before testing. The broth media containing the sulfonamides prepared by adding powdered crystals of the drugs to be tested (sulfathiazole, sulfanilamido-ourea, sulfanilamido-thiourea and 4-homo-sulfanilamido-ourea hydrochloride) to sterile veal infusion broth so that the final concentration was 10 mg per 100 c.c. The medium was then sterilized by heating at 56°C for two hours. The chemical broth media were distributed in 5 c.c. amounts into sterile test tubes and incubated for 24 hours at 37°C to test for sterility.

Sixteen hour broth cultures of all organisms were used. To 5 c.c. of the control and broth containing the various drugs 0.1 c.c. of 1:100, 1:200 and 1:500 broth dilution of the sixteen hour culture was added. Incubation was carried out at 37°C for 24 hours. Growth was estimated by the appearance of visible turbidity in the medium. Smears were made and stained with Gram's method from the test tubes showing cloudiness in order to confirm growth and to rule out the possibility of contamination.

Streptococcus viridans.

Two strains of *Streptococcus viridans* isolated from the blood of patients with subacute bacterial endocarditis were maintained by weekly transplants on Avery's culture media and stored in the refrigerator (2°). The organisms showed typical cultural reactions as defined by Bergey <sup>15</sup>(224).

Veal infusion broth containing 0.15 per cent dextrose, and the pH adjusted to 7.5 with 1 N sodium hydroxide was autoclaved

at 10 lb for 10 minutes; upon cooling 0.1 per cent sterile horse serum was added and the medium was distributed in 4.4 c.c. amounts into sterile test tubes.

A series of dilutions of each of the compounds, (sulfathiazole, sulfanilamidourea, sulfanilamidothiourea, and 4-homo-sulfanilamidourea), varying from 1:100 to 1:10,000 were prepared in sterile distilled water. The aqueous drug solutions were sterilized by boiling in a water bath for 30 minutes, and added to tubes containing 4.4 c.c. of nutrient medium in 0.5 c.c. amounts while still hot before recrystallizing upon cooling. The final drug concentrations were one tenth the original concentrations of the aqueous solutions.

Each tube was inoculated with 0.1 c.c. of a 1:200 broth dilution of a 24-hour culture of test organisms, the two strains of Streptococcus viridans. Two test tubes containing 5 c.c. of each concentration of drug and two control tubes containing no drug were used for each strain of Streptococcus viridans tested.

The inoculated test media, with controls, were incubated at 37°C and observed for visible growth after 24 hours. Growth was estimated by visible turbidity in the medium. Smears were made and stained with Gram's method from the test tubes showing cloudiness in order to confirm growth and to rule out the possibility of contamination.

The second method employed to test the bacteriostatic effect of sulfathiazole, 4-homo-sulfanilamido-urea, sulfanilamido-urea and sulfanilamido-thiourea upon the same strains of

Streptococcus viridans was that of Todd (224)<sup>194</sup>, modified by Hamilton (433)<sup>77</sup>, with a few further modifications.

Fresh, whole, human and sheepblood was used for the suspending medium. The optimum dilution, that is, the dilution that gives a colony count of approximately 200-300 colonies was determined. This was obtained as follows : A 24-hour culture of the organisms to be tested was diluted 1:10, 1:100, 1:1000, up to 1:100,000,000 in Difco-tryptose-phosphate broth. 0.1 c.c. and 0.050 c.c. of each dilution was put in 5 c.c. of fresh, whole citrated human blood and allowed to incubate at 37°C. for one hour and a half. At the end of this time the blood-streptococci mixture was thoroughly mixed and 1 c.c. transferred to a sterile Petri-dish and 10 c.c. of melted nutrient agar added. In a later experiment 1 c.c., 0.5 c.c. and 0.1 c.c. was transferred at the end of 1, 3, 6, 9 and 24 hours and pour plates prepared in the same way. The Plates were incubated at 37°C for 24 hours and the number of colonies were counted.

The drugs were prepared in the following way: A 1:650 dilution of each drug was made in sterile distilled water, this was sterilized by bringing the solution to boiling point.

Five c.c. of fresh whole blood was used for each dilution of drug to be tested, and 5 c.c. was used for the untreated control. 0.1 c.c. of the optimum dilution of the organisms was added to each of the tubes and the tubes were then incubated at 37°C. for one hour. At the end of an hour the drug solution was added in amounts to give the final concentrations desired. (example, 0.1 c.c.

of the 1:650 gives a concentration of 6 mg per cent when added to 5 c.c. of blood).

Plates were poured as soon as the drug was added. A plate was also poured from the control tube containing no drug, and again 6 and 24 hours after the drug had been added. These plates were made by placing 1 c.c. of the blood-streptococci mixture in a Petri dish and mixing with 10 c.c. of melted nutrient agar.

Colony counts were made on each set of plates (start plates, 6 hour plates and 24 hour plates), at 24 and 48 hours after they were poured.

#### Staphylococcus aureus.

The Oxford strain and a recently isolated strain of Staphylococcus aureus from a case of osteomyelitis were the test organisms. The organisms were coagulase positive. Stored on glucose agar slants in the refrigerator (2°C).

The basal medium, described by Knight (222)<sup>90</sup>, and modified by Strauss, Dingle and Finland (43)<sup>96</sup>, was prepared. Two ingredients were not available, therefore were prepared in the Biochemistry Department as follows:

The cystine hydrochloride was prepared as follows: 50 mg of cystine (Merck), was dissolved in 1 c.c. of 1 N HCL in a 10 c.c. volumetric flask and sterile distilled water was added up to the mark. Casein hydrolysate was prepared from Casein (Rochester), Mueller's method (223)<sup>120</sup>, was employed. To 20 gm of casein 34 c.c. H<sub>2</sub>SO<sub>4</sub> and 120 c.c. distilled water were added and boiled for 18 hours. The percentage of nitrogen in the solution was 0.3959 per cent.

The basal medium consisted of:

$\text{KH}_2\text{PO}_4$ .....	4.5	g.
Cystine hydrochloride.....	0.050	g.
Casein hydrolysate (0.395%).....	10.	g. (238 c.c.).
Distilled water to.....	700.	c.c.

The solution was obtained by boiling. The pH was adjusted to 7.3 with 1 N NaOH and the volume brought up to 900 c.c. with distilled water. The solution was distributed in 4.5 c.c. amounts in sterile test tubes and autoclaved at a pressure of 15 pounds for 10 minutes. The following were prepared in 100 c.c. volumetric flasks and sterilized by Seitz filtration and added aseptically, each in a volume of 0.1 c.c.

Glucose.....	12.5 per cent.
$\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ .....	M/250 in N/25HCl.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.2 per cent.
Tryptophane.....	0.1 per cent.
Thiamin Chloride.....	0.005 per cent.
Nicotinamide.....	0.005 per cent.

Sulfanilamide, sulfaguanidine, sulfanilamido-urea and sulfanilamido-thiourea were dissolved in distilled water and heated in the Arnold steamer for 20 minutes before use. From the stock solutions (0.3 per cent), the following lower concentrations were prepared by diluting with appropriate amounts of sterile distilled water. The concentrations used were: 0.25, 0.2, 0.15, 0.1 and 0.050 per cent. The drug solutions were added aseptically in 0.1 c.c. amounts.



Three test tubes containing 5 c.c. of each concentration of drug and three control tubes containing no drug were used for each strain of Staphylococcus aureus tested. To prepare the inoculum a small loopful of organisms were transferred to infusion broth, incubated for 6 to 10 hours and centrifuged at 2500rpm for 30 minutes. The sediment was resuspended and washed twice in basal medium and diluted so that 12500 organisms was contained in a volume of 0.1 c.c. (Hopkin's tube method). The organisms were added to the 5 c.c. drug-medium in 0.1 c.c. amounts.

The tubes were incubated at 37°C. Clouding of the medium was taken to represent growth. Smears were made and stained by Gram's method from the tubes showing turbidity, to confirm the presence of growth and to rule out contamination.

Mycobacterium tuberculosis.

Two virulent human strains were employed. One of the strains was imported <sup>from</sup> Turkey, the other was a local strain isolated from the sputum of a tubercles patient. The organisms were stored on Congo red medium at room temperature (15 to 20°C).

A synthetic medium described by Youmans (225)<sup>R13</sup>, was prepared as follows:

Asparagin.....	2.5 g.
Monopotassium phosphate....	2.5 g.
Magnesium citrate.....	1.25 g.
Magnesium sulfate.....	0.25 g.
Glycerol.....	40. c.c.
Redistilled water from glass to	500 c.c.

The pH was adjusted to 7.0 with 40 per cent sodium hydroxide and the mixture was autoclaved. The precipitate was removed, after cooling, by filtration through paper and autoclaved a second time at a pressure of 15 pounds for 10 minutes. The medium was distributed in 100 c.c. amounts into sterile 250 c.c. flasks aseptically, the powdered crystals of the drugs to be tested, sulfathiazole, sulfanilamido-urea, sulfanilamido-thiourea and 4-homo-sulfanilamido-urea hydrochloride, were added so that the final concentration was 10 mg per 100 c.c. The media were then sterilized by heating at 56°C. for two hours. The synthetic media with the sulfonamides were distributed in 2 c.c. amounts into sterile test tubes and incubated for 24 hours at 37°C to rule out the possibility of contamination.

Suspensions of tubercle bacilli were prepared by grinding aseptically 10-day surface growths from synthetic medium, third subculture, in an evaporating dish with the handle of an agate mortar, and suspending them in the synthetic medium. These suspensions were then standardized by centrifugation in Hopkin's vaccine tubes. For inoculation 0.1 c.c. volumes, containing the desired amounts, 0.4, 0.2 and 0.1 mg of tubercle bacilli were used. Two test tubes containing 2 c.c. of drug-synthetic media mixture and two control tubes containing no drug were used for each concentration of inoculum. The tubes were incubated at 37°C. for 10 days. Surface and sub-surface growth was observed, and smears were made and stained by Siehl-Neelsen's method to confirm growth of the organisms and to rule out the possibility of contamination.

Corynebacterium diphtheriae.

The two strains of Corynebacterium diphtheriae were local strains isolated from the throats of patients. The organisms were maintained by weekly transplants on Loeffler's media and stored at 10-16°C. The organisms showed typical cultural characteristics.

The drug-broth medium was prepared by adding powdered crystals of the drugs to be tested, sulfathiazole, sulfanilamidourea, sulfanilamidothiourea and 4-homo-sulfanilamidourea, to sterile veal infusion broth so that the final concentration was 10 mg per cent. The medium was then sterilized by heating at 56°C. for 2 hours. The chemical broth media were distributed in 2 c.c. amounts into sterile test tubes.

Two test tubes containing 2 c.c. of each drug and one control tube containing no drug were used for each concentration of inoculum.

24. hour broth cultures of the organisms were used. To one set of 0.1 c.c. of the undiluted 24. hour broth culture was inoculated; to the second set of 0.1 c.c. of 1:10 dilution; and to a third set 0.1 c.c. of 1:100 of the 24. hour culture were added. Incubation was carried out at 37°C. for 48 hours. Growth was estimated by the appearance of visible turbidity in the medium. Smears were made and stained by Gram's method from the test tubes showing turbidity in order to confirm growth and to rule out the possibility of contamination.

Anaerobes.

The organisms were stock laboratory strains of Clostridium welchi (London), Clostridium tetani (U.S.A. 229), Clostridium sporogenes (Canadian 534), and Clostridium chauvei (London). The cultures were stored at 15-20°C. in chopped meat infusion broth covered with 1 cm. deep of sterile paraffin.

The tests were conducted in a simple medium composed of 1 per cent Difco-Tryptone, 1 per cent Difco-Meoseptone and 0.2 per cent dextrose in distilled water (188). The drugs, sulfathiazole, sulfamidamido-urea, sulfamidamido-thiourea and 4-homo-sulfamidamido-urea were added to the basal medium, so that the final concentration was 15 mg per cent, except for the 4-homo-sulfamidamido-urea which was 20 mg per cent. The medium was tubed in 5 c.c. amounts, each covered with a sterile paraffin 1 cm. deep, and autoclaved at 15 lb. pressure for twenty minutes. The pH of the medium was 6.8.

24 hour cultures of the four organisms were used. To 5 c.c. of the control and basal media containing the various drugs 0.1 c.c. of 1:10, 1:100 and 1:1000 sterile distilled water dilutions of the 24 hour culture were added. Incubation was carried out at 37°C for 48 hours. Growth was estimated by the appearance of visible turbidity in the medium.

R E S U L T S .

The results of the study on each type of organisms are detailed under the organisms concerned.

Escherichia Coli.

The minimal bacteriostatic concentration of sulfanilamide and sulfaguanidine were determined and compared with that of sulfanilamido-urea, sulfanilamido-thiourea and sulfanylbengyl-hydrazide with inocula of 2500 Escherichia coli per c.c. No clouding of the medium during five days of incubation meant complete bacteriostasis. Clouding occurring after three days incubation meant partial bacteriostasis. The drug free controls grew up in 12-24 hours.

The results of the first experiment with Escherichia coli are recorded in Table I. (See Table I). Sulfanilamide and Sulfaguanidine were completely bacteriostatic in a concentration of 3 mg. per cent with 2500 organisms per c.c. Sulfanilamido-urea and sulfanilamido-thiourea were not bacteriostatic and showed no difference from the drug free controls.

The results of the second experiment are recorded in Table II. (See Table II).

Sulfanilamide and sulfaguanidine were completely bacteriostatic in a concentration of 5 mg per cent, sulfanilamido-urea, sulfanilamido-thiourea and sulfanylbengyl-hydrazide showed no bacteriostasis.

The experiment was repeated for the third time confirming the results in experiment one and two. The results of experiment three are summarized in Table III. (See Table III).

Table I. The Bacteriostatic Action of Sulfonamides on *Escherichia coli*.

Bacteriostatic Agent	Concentration of Drug in mg. per cent	Inoculum: 2500 <i>Escherichia coli</i> per c.c. of Medium.				
		Hours of Incubation at 37°C.				
		24	48	72	96	120
Sulfanilamide	6	----	----	----	----	----
	5	----	----	----	----	----
	4	----	----	----	----	----
	3	----	----	----	----	----
	2	----	----	----	+---	+++
	1	----	++--	++++	++++	++++
	0	++++	++++	++++	++++	++++
Sulfanilguanidine	6	----	----	----	----	----
	5	----	----	----	----	----
	4	----	----	----	----	----
	3	----	----	----	----	----
	2	----	----	----	----	++--
	1	----	----	----	+++--	++++
	0	++++	++++	++++	++++	++++
Sulfanilamide urea	6	---+	-+++	++++	++++	++++
	5	---+	-+++	-+++	++++	++++
	4	----	---+	-+++	-+++	-+++
	3	--++	-+++	-+++	++++	++++
	2	---+	-+++	-+++	-+++	++++
	1	---+	-+++	++++	++++	++++
	0	---+	++++	++++	++++	++++
Sulfanilamidothiourea	6	----	-+++	++++	++++	++++
	5	----	---+	-+++	-+++	++++
	4	----	----	-+++	++++	++++
	3	----	----	-+++	++++	++++
	2	----	---+	++++	++++	++++
	1	----	-+++	-+++	-+++	-+++
	0	---+	++++	++++	++++	++++

Summary of 4 experiments given side by side.

+, ++, +++, +++++, indicates abundance of growth  
 -, indicates no growth.

TABLE II

Bacteriostatic Action of Sulfanamide Drugs on *Escherichia coli*

Bacteriostatic agent	Concentration mg per cent	2500 <i>Escherichia coli</i> per cc of medium				
		Hours of Incubation at 37°C.				
		24	48	72	96	120
Sulfanilamide	6	----	----	----	----	----
	5	----	----	----	----	----
	4	----	----	----	++--	++++
	3	----	+---	++--	++++	++++
	2	----	+---	++--	++++	++++
	1	----	++++	++++	++++	++++
	0	++++	++++	++++	++++	++++
Sulfaguanidine	6	----	----	----	----	----
	5	----	----	----	----	----
	4	----	----	+---	++--	++++
	3	----	----	+---	++++	++++
	2	----	----	++++	++++	++++
	1	----	++++	++++	++++	++++
	0	++++	++++	++++	++++	++++
Sulfanilamidourea	6	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++
	4	++++	++++	++++	++++	++++
	3	++++	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++
	1	++++	++++	++++	++++	++++
	0	++++	++++	++++	++++	++++
Sulfanilamido-thio-urea	6	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++
	4	----	++++	++++	++++	++++
	3	----	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++
	1	++++	++++	++++	++++	++++
	0	++++	++++	++++	++++	++++
Sulfanil Benzyl Hydroxide	6	-+++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++
	4	++++	++++	++++	++++	++++
	3	----	++++	++++	++++	++++
	2	++++	++++	++++	++++	++++
	1	++++	++++	++++	++++	++++
	0	++++	++++	++++	++++	++++

Summary of 7 experiments given side by side  
 +, ++, +++, +++++, indicate abundance of growth  
 - indicates no growth

Table III The Bacteriostatic Effect of Sulfonamides on *Escherichia coli*

Bacteriostatic Agent Concentration mg %	Inoculum: 2500 <i>Escherichia coli</i> per c.c. of Medium					
	Hours of Incubation at 37°C.					
	24	48	72	96	120	144
Sulfanilamide 6mg %	---	---	---	---	---	--+
Sulfaguanidine 6mg %	---	---	---	---	---	--+
Sulfanilamide-urea 6mg %	---	+++	+++	+++	+++	+++
Sulfanilamidethiourea 6mg %	---	+++	+++	+++	+++	+++
Sulfanil-Benzil- Hydrazone 6mg %	---	+++	+++	+++	+++	+++
Control	---	+++	+++	+++	+++	+++

- indicates no growth  
+ indicates slight growth  
+++ indicates maximum growth.



Staphylococcus aureus.

The bacteriostatic action of sulfanilamide, sulfaguanidine, sulfanilamido-urea and sulfanilamido-thiourea was determined with 2500 staphylococci per c.c. The concentration of the sulfanilamide drugs was 6 mg per cent. The results of five repetitions are summarized in Table IV.

Table IV. The Bacteriostatic Effect of Sulfonamides on *S. aureus*.

Bacteriostatic Agent 6 mg per cent	Inoculum: 2500 <i>Staphylococcus aureus</i> per cc of Medium.					
	Oxford Strain.			A Local Strain.		
	Hours of Incubation at 37° C.					
	24	48	72	24	48	72
Sulfanilamide	++--	+++-	++++	++++	++++	++++
Sulfaguanidine	+++-	++++	++++	++++	++++	++++
Sulfanilamido-urea	++++	++++	++++	++++	++++	++++
Sulfanilamido-thio- urea	++++	++++	++++	++++	++++	++++
4-Homosulfanil- amido-urea	++++	++++	++++	++++	++++	++++
Control.	++++	++++	++++	++++	++++	++++

- indicates no growth  
+, ++, +++ indicates slight to heavy growth.  
++++ indicates maximum growth.

The two strains used in the test were resistant to the action of all the drugs tested. The Oxford strain showed very little inhibition with sulfanilamide in the medium.

Streptococcus viridans.

The experiments using human and sheep blood as medium for testing the bacteriostatic action of the drugs against Streptococcus viridans were not successful probably because both human and sheep whole blood alone were capable of sterilizing small inocula of the organism. Results are summarized in Table V.

A second method, using as medium veal infusion broth containing 0.15 per cent dextrose and 0.1 per cent horse serum was employed. The results of eight tests against a strain of Streptococcus viridans isolated from a case of subacute bacterial endocarditis from the heart valves of an autopsy case, are presented in Table VI.

The results presented in Table VI indicate that sulfanilamide-urea and sulfanilamide-thiourea have a slight bacteriostatic action on the test organisms but their effect was less than that of sulfathiazole under the same experimental conditions. The 4-homo-sulfanilamide-urea showed no bacteriostatic action.

Table V. The Influence of Leukocytic Activity on St. viridans.

Incubation Time In the Blood	Average Number of Colonies per Blood Plate				
	Dilution of Suspension of Organisms.				
Hours	1:1000	1:10000	1:100,000	1:1000,000	1:10,000,000
1	1272	80	13	6	1
3	402	60	4	2	0
6	265	39	2	5	1
9	169	3	1	1	0
24	0	0	0	0	0

A summary of ten experiments.

Table VI. The Comparative Bacteriostatic Action of Sulfonamides on *Streptococcus viridans*.

Drug Dilution in the medium mg. per cent	Inoculum: 0.1 cc. of a 1:100 dilution of a 24-hr. culture of <i>Streptococcus viridans</i> .				Control of Drug
	Bacteriostatic Agent				
	Sulfathiazole	S. Acetazolamidobenzenesulfonamide-urea	Sulfanilamide-thio-urea	Sulfanilamide-urea	
100 mg %	----- -----	++++ ++++	----- -----	----- -----	++++ ++++
20 mg %	----- -----	++++ ++++	----- -++	----- -+++	++++ ++++
10 mg %	----- -----	++++ ++++	----- -+++	----- ++++	++++ ++++
1 mg %	----- -+++	++++ ++++	++++ ++++	++++ ++++	++++ ++++

Summary of 8 experiments, given side by side.  
 - indicates no growth  
 + indicates growth.

Gram Negative Bacilli.

The test drugs showed no inhibition against the gram-negative bacilli tested. However, sulfathiazole was slightly bacteriostatic to some of the organisms tested.

The results of the test are shown in Table VII.

Table VII. Effect of the Sulfonamides on the Gram Negative Bacilli.

Organism.	Drug Concentration in the Medium: 10mg. per cent												Control No Drug.		
	Bacteriostatic Agent														
	Diaminosulfonamide			Sulfanilamide urea			Sulfanilamide-thiourea			Sulfathiazole					
	Dilution of Suspension of the Organisms.														
	1:100	1:200	1:400	1:100	1:200	1:400	1:100	1:200	1:400	1:100	1:200	1:400	1:100	1:200	1:400
<i>Escherichia typhosa</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Salmonella paratyphi</i> (Schaumüller)	+	+	+	+	+	+	+	+	+	±	±	±	+	+	+
<i>Salmonella paratyphi</i> (Sandai)	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella paratyphi</i>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella newport</i>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella sussex</i>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella schottmüller</i> B <sub>2</sub>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella schottmüller</i> (Fidy)	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella schottmüller</i>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	±
<i>Shigella paradysenteriae</i> (Flexner)	-	+	+	+	+	+	+	+	+	+	-	±	+	+	+
<i>Shigella dysenteriae</i> (Shiga)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Shigella dysenteriae</i> (Shiga)AB	-	+	+	+	+	+	+	+	+	+	+	±	+	+	+
<i>Shigella paradysenteriae</i> (Sonne)	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella enteritidis</i> (Bardoo)	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Salmonella typhimurium</i> (Centropus)	+	+	+	+	+	+	+	+	+	+	-	±	+	+	+
<i>Salmonella pullorum</i>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+
<i>Proteus vulgaris</i>	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
<i>Vibrio comma</i>	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+

Summary of three experiments:  
 - indicates no growth  
 ± indicates slight growth.  
 + indicates growth.

Tubercle bacilli.

The in vitro experiments with tubercle bacilli have shown that sulfanilamide-urea, sulfanilamido-thiourea and 4-homo-sulfanilamide-urea have no inhibitory effect on a human strain of tubercle bacilli. Under similar conditions, sulfathiazole exerted definite bacteriostatic action on the organisms tested.

The lowest concentration necessary to completely inhibit subsurface growth was usually higher than that necessary to completely inhibit surface growth.

The size of inoculum used was important. With 5 mg tubercle bacilli per 10 c.c. medium there was no surface growth. On the other hand with 6 mg tubercle bacilli surface growth occurred. With 1 mg tubercle bacilli per 100 c.c. of medium, growth did not appear even after 26 days following inoculation.

Growth was evident by turbidity. When the tubes were shaken flaky masses of tubercle bacilli could be seen which settled to the bottom where they continued to grow. That the growth evident in the cultures actually consisted of tubercle bacilli is indicated by the fact that strains made from them showed only masses of acid-fast bacilli. (See Table VIII).

Table VIII. Surface and Subsurface Growth of Tubercle bacilli and the Action of Sulfenamides.

Dilution of <i>Mycobacterium tuberculosis</i> per cent suspension in the medium.	Drug Concentration in the Medium: 10 mg per cent Bacteriostatic Agent.				Control No Drug.
	4-hydroxysulfanilamide-urea	Sulfanilamide-urea	Sulfanilamide-thiourea	Sulfathiazole	
5 mg	+ <sup>±</sup>	+ <sup>±</sup>	+ <sup>±</sup>	-	++
6.25 mg.	++ S	++ S	++ S	± --	+++ S
10. mg.	+++ S	+++ S	+++ S	+ --	+++ S
12.5 mg	+++ <sup>±</sup> S	+++ <sup>±</sup> S	+++ <sup>±</sup> S	1 --	+++ <sup>±</sup> S
20 mg.	++++ S	++++ S	++++ S	+ <sup>±</sup> --	++++ S

S, indicates surface growth  
+, ++, +++, ++++, indicates abundance of subsurface growth.  
-, indicates no growth.

*Corynebacterium diphtheriae*.

The relative growth inhibitory powers of sulfanilamidourea, sulfanilamidethiourea, 4-hydroxysulfanilamido-urea and sulfathiazole on three strains of *Corynebacterium diphtheriae* were tested. The results are summarized in Table IX.

Table IX. The Comparative Action of Sulfonamides on Three Strains of *C. diphtheriae*.

Organism <i>Corynebacterium</i> <i>diphtheriae</i>	Dilution of the Organism	Drug Concentration in the Medium: 10 mg. per cent				Control No Drug
		Bacteriostatic Agent:				
		4-homo-sulfanilamide-urea	Sulfanilamide-urea	Sulfanilamide-thiourea	Sulfathiazole	
Strain (1)	Undiluted	++++	++++	++++	----	++++
	1:10	++++	++++	++++	----	++++
	1:100	----	----	----	----	----
Strain (2)	Undiluted	++++	++++	++++	+++ -	++++
	1:10	+++ -	+++ -	++++	++ - -	++++
	1:100	+ + - -	+ + - -	+++ -	----	++++
Strain (3)	Undiluted	++++	++++	+++ +	+ + - -	++++
	1:10	+ + - -	+ + + -	++++	+ - - -	++++
	1:100	+ - - -	+ - - -	+++ -	----	++++

Inoculum: 0.1 c.c.      Summary of four experiments given side by side.  
 Organism: 24-hr. culture.  
 + indicates growth.  
 - indicates no growth.

From table IX it appears that sulfathiazole is more effective in inhibiting the growth of *Corynebacterium diphtheriae*. However, the other compounds, sulfanilamide-urea, sulfanilamide-thiourea and 4-homo-sulfanilamide-urea exhibited slight bacteriostatic action on the two local strains, strain (2) and (3) in table IX, but not the third strain; strain (3) was received from Teheran.

Sulfanilamide-urea and 4-homo-sulfanilamide-urea were more effective in inhibiting the growth of *Corynebacterium diphtheriae* strain (2) and (3) than was sulfanilamidothiourea.

Anaerobes.

Experiments to determine the bacteriostatic action of 4-homo-sulfanilamidourea, sulfanilamido-thiourea, sulfanilamido-urea and sulfathiazole on Clostridium welchi, Clostridium tetani, Clostridium sporogenes and Clostridium chauvei were carried out.

The results in Table X indicate that 4-homo-sulfanilamidourea and sulfanilamidourea were slightly superior to sulfathiazole in inhibiting the growth of the anaerobes tested. Very little, if any inhibition was observed with sulfanilamidothiourea.

Table X. The Bacteriostatic Action of Sulfenamides on Four Anaerobic Bacilli.

Organism	Dilution of the Organism.	Drug Concentration : 15-20 mg per cent.				Control No Drug.
		Bacteriostatic Agent:				
		4-Homo-sulfanilamidourea	Sulfanilamidourea	Sulfanilamidothiourea	Sulfathiazole	
<u>Clostridium welchi</u>	1:10	-----	-----	++++	++++	++++
	1:100	-----	-----	-----	-----	++--
	1:1000	-----	-----	-----	-----	+-
<u>Clostridium tetani</u>	1:10	-----	----++	++++	++++	++++
	1:100	-----	-----	++++	-----	++++
	1:1000	-----	-----	---++	-----	++++
<u>Clostridium sporogenes</u>	1:10	-----	-----	++++	++++	++++
	1:100	-----	-----	++++	-----	++++
	1:1000	-----	-----	++++	-----	++++
<u>Clostridium chauvei</u>	1:10	-----	+-	++++	+-	++++
	1:100	-----	-----	++++	-----	++++
	1:1000	-----	-----	+-	-----	+-

+ indicates turbidity  
- indicates absence of turbidity.



### DISCUSSION

The action of new sulfanilamides can be tested on bacteria against which the older drugs work satisfactorily. In this study the new drugs were tested against bacteria on which the older sulfanilamides exert little action, such as Staphylococcus aureus, Streptococcus viridans, Mycobacterium tuberculosis and the typhoid-dysentery group of bacilli, in addition to bacteria susceptible to the older drugs such as Escherichia coli spore bearing anaerobic bacilli and Corynebacterium diphtheriae.

To study the effect of the sulfonamide drugs upon bacteria in vitro, it is preferable to use a medium initially free of inhibitor, otherwise comparison of the results of different experiments with the same test organisms is not possible. The commonly used bacteriological media are not satisfactory since both the peptone and tissue infusion used in the preparation are known to vary from lot to lot in their content of inhibitor <sup>114</sup>(45).

In this study a synthetic medium was employed in testing the bacteriostatic effect of the drugs upon Mycobacterium tuberculosis and Staphylococcus aureus. The experiments upon the gram-negative rods, the clostridia, the diphtheria bacillus and the non-hemolytic streptococci were performed in the common bacteriological media but the peptone and tissue infusion used in their preparation were from the same batch throughout the experiments so as to avoid variation in their content of inhibitor.

An imitation of a streptococcus bacteremia in vitro using fresh, whole, human and sheep blood was tried, but the results

failed to confirm the findings of Hamilton (<sup>71</sup>433). Hamilton has used fresh whole blood for predicting the effect of drugs on streptococci, infecting the blood stream. The results of ten repetitions in this study agree in the main with those of Todd (<sup>194</sup>224), that fresh, whole, human and sheep blood are capable of killing streptococci. The difference in the results, <sup>presented here compared with those of Hamilton may be due to the variation</sup> in their response to the leukocytic activity of strains of Streptococcus viridans, the bactericidal action was slight in his experiments. Poston and Organ (<sup>151</sup>24), showed a great variation in the in vitro effectivity of the sulfanilamide drugs on twenty-five strains of Streptococcus viridans. They suggest that these differences in type and strain response are related to differences in antigenicity. Differences in antigenicity may explain the variation in their response to the action of leukocytes present in fresh, whole blood.

The older sulfanilamides exert little action on diseases caused by some gram-negative organisms and it has been shown, (<sup>193</sup>200, <sup>191</sup>203, 299, 209, <sup>87</sup>202), that urea in conjunction with sulfanilamides displays greater inhibitory effect than the latter on Escherichia coli and other gram-negative bacilli (200), Proteus vulgaris, Eberthella typhosa, Salmonella schotmulleri and Salmonella paradysenteria (Flexner). In view of these findings it seemed advisable to test the action of 4-homo-sulfanilamidourea, sulfanilamidourea and Sulfanilamido-thiourea<sup>17</sup> a number of gram-negative rods including two strains of Escherichiacoli in vitro. The results were disappointing because the drugs had no bacterio-

static effect upon any of the gram-negative rods tested; with very small inocula there was some inhibition with sulfathiazole but none with the compounds containing sulfanilamide and urea. Sulfanilamide and sulfaguanidine were completely bacteriostatic in concentrations of 3-5 mg per cent upon the two strains of Escherichia coli in a synthetic medium, under the same experimental conditions the sulfanilamide-urea compounds showed no bacteriostatic action.

It was shown by Tsuchiya et al (<sup>197</sup>204), that three strains of resistant staphylococci were susceptible to urea-sodium sulfathiazole combinations whereas they were unaffected by these two agents used separately. The bacteriostatic activity of 4-homo-sulfanilamidourea, and sulfanilamidothiourea on two strains of sulfanilamide resistant staphylococci were studied and the results showed that the organisms were more resistant to the sulfanilamide-urea compounds than to sulfanilamide alone.

There are reports on the bacteriostatic and bactericidal action of urea on the Mycobacterium tuberculosis in vitro (<sup>191</sup>209, <sup>196</sup>210), Mayer (<sup>126</sup>207) has shown in a comparative study of ten compounds on the Tubercle bacilli in vitro that sulfanilamide urea compounds, namely phenyl-sulfonamidothiourea and sulfanil-amidothiourea, having one NH less than the compounds tested in this study, were very active; he also showed sulfathiazole to be six to thirty times as active as sulfanilamide and sulfapyridine. The in vitro experiments with Mycobacterium tuberculosis var. hominis showed that sulfanilamidourea, sulfanilamidothiourea and 4-homo-sulfanilamidourea had no inhibitory effect on the growth of the organisms. Sulfathiazole under similar conditions exerted a definite bacteriostatic action on Mycobacterium tuberculosis.

Why sulfanilamidothiourea ( $p\text{-H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NHCSNH}_2$ ) is very active according to Mayer while sulfanilamidothiourea ( $p\text{-H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NHNHCSNH}_2$ ) is not active is not obvious, but the fact that sulfathiazole was active in both experiments showed the environmental conditions in the two experiments to be similar and the inactivity of the compounds in this experiment was not due to the environmental conditions but most probably the compound itself.

SUMMARY.

The antibacterial properties of three new urea-sulfonamide compounds have been studied in vitro against a variety of bacteria.

The studies were conducted firstly, to determine whether the drugs had any activity against various bacteria, and secondly, to compare their activities with common sulfonamides.

The results showed that sulfanilamido-urea and 4-homo-sulfanilamido-urea were superior to sulfathiazole in their inhibitory actions upon Clostridium welchii, Cl. tetani, Cl. sporogenes, and Cl. chauvei in concentrations of 15 mg per cent, Sulfanilamido-thiourea showed much less activity than sulfathiazole. A strain of Streptococcus viridans was completely inhibited by 10 mg per cent sulfathiazole in veal infusion broth, sulfanilamido-urea and sulfanilamido-thiourea exerted a partial inhibition under the same experimental conditions but were completely bacteriostatic in concentrations of 100 mg per cent. 4-homo-sulfanilamido-urea had no inhibitory effect. Sulfathiazole in concentrations of 10 mg per cent exerted a marked inhibitory action on the growth of virulent human Mycobacterium tuberculosis in a synthetic medium. Sulfanilamido-urea, sulfanilamido-thiourea and 4-homo-sulfanilamido-urea under similar conditions gave negative results. Sulfathiazole had a definite and marked bacteriostatic effect on one of the three strains of Corynebacterium diphtheriae tested, provided appropriate inocula were used. Sulfanilamido-urea, sulfanilamido-thiourea and 4-homo-sulfanilamido-urea exhibited <sup>1</sup>irregularly and to a lesser degree than sulfathiazole.

Two strains of Staphylococcus aureus exhibited no alteration of their growth in a synthetic medium containing the sulfonamides and the sulfonamide-urea compounds. Sulfanilamide and sulfaguanidine, in concentrations of 3-5 mg per cent were bacteriostatic upon Escherichia coli in a synthetic medium. This action was not shared by the sulfonamide-urea compounds. The sulfonamides were without effect on Eberthella typhosa, Salmonella paratyphi and various other gram-negative rods despite varying the size of inocula; sulfathiazole exerted slight bacteriostatic effect on some of the gram-negative rods.

The bactericidal effect of fresh, whole blood upon streptococci reported by Todd, was confirmed.

Suspensions of a virulent strain of Mycobacterium tuberculosis rapidly produced surface and subsurface growth when inoculated into the synthetic medium of Youman.

#### ACKNOWLEDGEMENTS.

I wish to express my sincere gratitude and indebtedness to Dr. Dikran Berberian, Professor of Bacteriology and Parasitology in the American University of Beirut, for his supervision throughout my graduate study, to Dr. Frank Alexander, Professor of Pharmacology, for his valuable suggestions given so generously, in connection with writing out the thesis; to Dr. Emory Dennis, ex-Professor

of Bacteriology for suggesting the problem, to Miss Eva Nemets for preparing the urea-sulfonamide compounds; to Mr. Artinian for photographing the tables and to the members of the Bacteriology laboratory for preparing some of the media and cleaning and sterilizing the equipment.

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