susceptible as a group, requiring fairly intensive and prolonged treatment before they are completely and permanently eradicated, and there is a higher proportion of resistant cases. The high susceptibility of the haemolytic streptococcus (especially the A group) to sulphonamide compounds is well known, but I have convincing evidence that certain non-haemolytic streptococci—for example, *Strep. viridans* and *Strep. faecalis*—can also be partly or completely inhibited by both sulphanilamide and uleron. I have also seen cases of mixed genito-urinary infections in which such organisms as *B. proteus, B. aerogenes,* and diphtheroid bacilli are concerned completely clear up with both those substances. The staphylococcus and the pneumococcus, on the other hand, have proved almost entirely resistant in my experience.

It is now generally realized that whatever the exact mode of action of sulphonamide compounds may be they act only as blood-borne antiseptics, and their direct application to an infected focus is of comparatively little value. Reaching the blood and tissues quickly, they are also rapidly excreted, and in practice unless they are correctly administered their concentration will soon be found to fall to a subefficient level. An adequate blood supply to a focus of infection is, of course, an essential condition for success; avascular foci, such as blood clots, cardiac vegetations, necrotic areas, and chronic foci embedded in scar tissue or sclerosed bone, will resist chemotherapeutic attack no matter how susceptible the enclosed organisms may be.

One cannot help feeling that all these substances exert a purely bacteriostatic action, and that they do nothing to stimulate the defence mechanisms of the host. The final result of any infection must largely depend on this defence mechanism, and many failures with chemotherapy are probably to be attributed to its faulty operation. Again, chemotherapy does not appear to have any action in neutralizing existing toxins or those liberated by the bacteria which these drugs destroy. Above all, it in no way protects the patient against relapse or recurrence, as is shown by the high relapse rate in the present series and in my other groups of cases. Indeed, I have reason to believe that the opposite effect is sometimes produced, and that chemotherapy at the very onset of an infection may actually inhibit those anti-bacterial processes which confer immunity against recurrence or relapse of an infection. This would explain the fact observed in practice that a higher incidence of ultimate success is obtained when infections are treated some time after rather than at their onset.

Reflection on these and many other problems connected with the great therapeutic possibilities which have been opened by the discovery of sulphonamide compounds shows that a great deal of bacteriological and immunological work will have to be done before the new chemotherapy finds its proper level in the treatment of disease.

Summary

1. Clinical experiences are reported with several sulphonamide-containing chemotherapeutic substances (one of which, recently introduced under the trade name of uleron, is a di-sulphonamide).

2. Fifty-seven cases, with illustrative records, of genito-urinary infection are reviewed.

3. In a group including four cases of epididymitis and twenty-six of urethritis chemotherapeutically treated (in both instances non-gonococcal) there were respectively one and three failures.

4. Twelve cases, similarly treated and constituting a mixture of acute and subacute pyelitis and cystitis, showed one failure and three relapses.

5. Fifteen cases of "secondary" urinary infection, nine of them with senile prostatic enlargement, four with urethral stricture, one with epididymitis, and one with vesico-colic fistula, were treated with these drugs. In the prostatic cases two-thirds of the patients were benefited by chemotherapy, which proved of value in combating pre-operative and post-operative urinary sepsis, although the relapse rate was again high. Two prostatic and two stricture cases—all with chronic retention and heavy alkaline mixed infection—completely failed to respond.

6. The selective action of these drugs on various organisms and strains and their mode of action *in vivo* are discussed.

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A NEW CONTRAST STAIN FOR GONOCOCCI AND MENINGOCOCCI IN SMEARS

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The examination of smears for gonococci, particularly from women, is facilitated by a method of staining described elsewhere (Sandiford, 1937). It consists of Gram's method with Pappenheim's stain in place of a simple red counterstain. The organisms stain as in the usual Gram stain; while the polymorph cells show blue-violet nuclei and rose-violet cytoplasm, the red gonococci standing out much more clearly against the blue-violet background of cells than against the red background afforded by the ordinary counterstain. While the method was independently evolved, an acknowledgment was given in the paper to Fauth (1918), who, it was found, had previously described a similar method of staining. Recently I have received reprints of papers by Scudder and Lisa (1931) and Scudder (1931), who, apparently unaware of Fauth's paper, described such a stain and recommended it for Neisseriae in sections and smears.

The present paper is the outcome of an attempt to devise a method of staining which would not diminish the contrast between cells and Gram-positive organisms as does the Gram-Pappenheim combination, yet which would retain and, if possible, still further improve the contrast between Gram-negative organisms and cells. To this end attention was directed to the staining of the Gram-positive organisms and to the staining of the cells.

The Gram-positive Organisms

Effect of Acid.—It was found that when, as sometimes happened in dealing with large batches of routine specimens, the counterstain was left on rather too long, the Gram-positive organisms tended to decolorize. In view of the reported effect of acid vaginal secretion on the Gram-staining of organisms (see below), it seemed probable that the 2 per cent. carbolic acid which forms the bulk of Pappenheim's stain was responsible for this decolorization. Experiments on stained films with 2 per cent. carbolic acid alone showed this supposition to be correct.

The Violet Stain.—Improvement was effected by the use of crystal violet with ammonium oxalate as the initial stain and acetone as the decolorizer. (I am indebted to Major Beamish, R.A.M.C., for drawing my attention to the method, which seems to be the same as that described

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by Hucker, 1922.) This stain gives a deep purple-black to Gram-positive organisms, and is more "fool-proof" in use than other violet stains. Various authors mention the effect of acid vaginal secretion in producing spurious Gram-negative results, and recommend the use of either alkaline violet (Burke, 1921) or alkaline iodine (Scudder and Lisa, 1931). Scudder (1931) advocates the use of a buffer with the initial violet stain, and says that the ammonium oxalate mixture acts as a buffered solution. Its advantages for vaginal smears seem to be obvious.

The Decolorizer.—Whatever method of Gram is used, acetone is much superior to alcohol for decolorizing films containing pus. With acetone, complete decolorization of the cell nuclei may rapidly be obtained and the Gram-positive organisms be left unaffected—a result which is difficult, or impossible, of attainment with alcohol unless the smears are very thin and well spread.

The Cells

It seemed that if the violet element could be eliminated from the counterstain and the cells be stained green the contrast between them and both Gram-positive and Gram-negative organisms would be improved. According to Lee (1937) this metachromatic effect of methyl green is due to the presence of methyl violet as an impurity. With a view to obtaining a pure sample, Messrs. Grübler were communicated with, but the one they sent gave no better results than their product which we were using. A sample from British Drug Houses gave, just perceptibly, a purer green. Scudder (1931) says that an over-ethylated methyl green prepared by the National Aniline and Chemical Co. was found to be practically free from violet impurity. The results she got with it—dark blue-purple nuclei and faint lavender cytoplasm—however, suggest that they were much the same as those given by Grübler's product. This is borne out by her statement that Grübler's iodine green produced similar results, for I have found it to give the same colour as their methyl green.

Various other dyes, as substitutes for methyl green, were tried in admixture with pyronine and other substances, but without success. Malachite green had seemed a hopeful substitute, as both it and methyl green are "basic" colours. This had been discarded, however, as the sample we had available—pre-war, made by Lautenschläger—was practically insoluble. However, in view of our failure to find any other satisfactory substitute, and in view of the fact that according to McIntosh and Fildes (1931) malachite green is highly soluble in alcohol or water, a sample was obtained from Messrs. Grübler. This proved to be freely soluble, and an aqueous mixture of it with pyronine in the proportions given below produced excellent results. Used as the counterstain with the Hucker–Gram stain it gives a beautiful contrast between the bluish-green cells, the purple-black Gram-positive organisms, and the red gonococci. Overstaining with it does not affect the Gram-positiveness of organisms.

In view of this success with malachite green it was surprising to find in Scudder's paper (1931) that Pappenheim (1899) was quoted to the effect that one can use methyl green or iodine green but not malachite green in his stain.

Other Uses of the Stain.—I have found it useful also for detection of meningococci in cerebrospinal fluid, and Gram-negative organisms in centrifuged urine deposits, faeces, and sputum. It does not seem, however, to be applicable to *any* sort of pus containing *any* sort of organism. I have had the opportunity of trying it on only a few specimens of "surgical" pus and on only one containing Gram-negative bacilli, which did not take on the pyronine well. This substance is said to have a special affinity for Neisseriae (Scudder, 1931; Walton, 1936). My experience with vaginal smears has been that all Gram-negative organisms stain quite well with it. Experiments with the counterstain below and films of *Bact. coli* and *Bact. typhosum* suggest that their affinity for pyronine is a function of their age. In young cultures most of the organisms stain well with it, while in old cultures most of the bacilli take on the malachite green. This factor of ageing and degeneration probably affects the affinity of Neisseriae for pyronine, as we have had irregular results with stale specimens of cerebrospinal fluid. It should be noted that all the gonorrhoea specimens used in this work have been smears made by the clinician at the time of taking the specimen.

The following is the method of staining now employed in these laboratories and in the British Military Laboratory in Cairo.

Method of Staining

Any Gram technique to which the worker is partial may be followed up to the end-point of decolorization, but the following is strongly recommended, especially the use of acetone, which is essential in order to obtain complete decolorization of the cell nuclei before counterstaining.

INITIAL STAIN

Crystal violet	1 gramme
Alcohol (98%)	20 c.cm.
Ammonium oxalate (1% aqueous ..	30 c.cm.

(i) Leave this mixture on the heat-fixed film for half a minute.
(ii) Flood off with triple strength Lugol's iodine (as used in Jensen's modified Gram stain) and leave on for half a minute.
(iii) Pour off the excess iodine and blot once.
(iv) Decolorize with acetone for three or four seconds.
(v) Wash.
(vi) Put on the counterstain for two minutes.
(vii) Flood off with water—do not wash—and blot.

CONTRAST COUNTERSTAIN

Malachite green	0.05 gramme
Pyronine	0.15 „
Aq. dest.	ad 100 c.cm.

N.B.—There are two varieties of pyronine, "B" and "G," the former being almost insoluble in water. Pyronine (Grübler) is apparently pyronine "G," as it is freely soluble in water.

Keeping Qualities.—The following statements are based on haphazard observations only, during the use of solutions kept in ordinary bottles at room temperatures under 20° C.

Crystal violet stain: keeps for about a month; is found to have deteriorated after six weeks. Counterstain: keeps for at least three weeks, possibly longer; 100 c.cm. in a staining-pot may be used for at least 150 small smears.

Summary

A method of contrast staining which greatly facilitates the detection of gonococci and meningococci in smears is described.

By this method the cells and nuclei are stained bluish-green, Gram-positive organisms purple-black, and Neisseriae red.

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