Although the aetiology of the condition is not clear, further trial of sulphanimide therapy is urged for the acute forms of lupus erythematosus without clinical evidence of tuberculosis. In view of the leucopenia accompanying the condition, the simultaneous administration of pentnucleotide seems to be indicated.

I wish to thank Dr. Henry MacCormac for his encouragement, and for permission to publish this case, for which I am also grateful to Surg. Rear-Admiral Whiteside, medical superintendent.

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Medical Memoranda

An Improved Pappenheim Stain for Detection of Gonococci in Smears of Pus

In 1938 Sandiford described a malachite green-pyronine counterstain for use in Gram's method. He stated that with it pus cells were bluish green, gonococci bright red, and Gram-positive cocci purple-black. It was in fact a modification of the original Pappenheim and Saathof stain (Pappenheim, 1899), which consisted of methyl green, pyronine, ethyl alcohol, phenol, and glycerol. Walton (1936) used the Pappenheim formula recommended by Conn (1929) and got good results in the examination of smears for gonococci; but Sandiford (1937) could not reproduce them, possibly because he used the original Pappenheim formula. Sandiford found that bacteria and cells and their nuclei were not well differentiated on account of a violet coloration, and in 1938 suggested that this coloration was a metachromatic effect due to the presence of methyl violet as an impurity in methyl green. He therefore substituted malachite green.

According to Conn, methyl green is largely used in stain technology for its metachromatic effect, and is actually crystal violet into which a seventh methyl group has been introduced. This seventh methyl group is very loosely attached and thus there is always some methyl violet present, either because it is not all converted into the higher homologue (methyl green) or because it has broken down. In order to obtain pure methyl green Conn suggested that the commercial dye should be shaken up with chloroform, in which substance methyl violet is soluble.

Walton (1938-9) increased the stability of Pappenheim's stain by incorporating in it methyl alcohol instead of ethyl alcohol. He held that methyl alcohol by virtue of its extra CH₃ groups prevented the seventh methyl group of methyl green from dissociating itself.

THE NEW METHOD

In preliminary work at the Liverpool laboratories it was found that Pappenheim's stain deteriorated within a week even after the substitution of methyl alcohol, and gonococci stained more of a violet colour than a red. It was decided, therefore, to shake up methyl green with chloroform before incorporating it in the Pappenheim stain; this resulted in stabilization of the stain. In the method now used a 1% aqueous solution of methyl green is shaken in a separating funnel for a few minutes with about one-third of its volume of chloroform. The chloroform becomes deep violet and is discarded after separation. A modified Pappenheim solution is then prepared which has the following composition:

Methyl green (Gurr), chloroform-treated, 1% ..	100 c.cm.
Methyl alcohol, absolute	10 c.cm.
Pyronine G (Gurr)	0.6 g.
Phenol	1.0 g.
Glycerol	20 c.cm.

The solution is allowed to mature for 24 hours and is then filtered into a dropping-bottle before use. The optimum staining period is 1/2 to 1 minute.

If used as a counterstain in Gram's method for smears of pus, Gram-positive organisms stain normally and gonococci brilliant red. Most other Gram-negative organisms are a less intense red. Nuclei of the leucocytes are bright blue, but the cytoplasm of the polymorphonuclears behaves differently from that of the mononuclears. The cytoplasm of the latter is red, whereas that of the former is unstained. Endothelial cells have blue nuclei and a cytoplasm of a variable pink.

Sandiford found that the phenol in Pappenheim's solution, normally 2 g. in 130 c.cm., tended to decolorize Gram-positive bacteria after more than 2 minutes, and therefore omitted it from his modification. This fact was confirmed by me, and 1 g. of phenol was substituted. With this amount the decolorizing action was minimized, although it was of little importance because the staining

period was much shorter than that used by Sandiford. The staining intensity of Pappenheim's solution is lessened if phenol is omitted.

In a comparison of Sandiford's modification of Pappenheim's stain with that described by me it was found that gonococci were more distinctly red by the latter and easier to detect against the clear unstained background of the polymorphonuclear cells. Moreover, the contrast of the brilliant-red gonococci and the bright-blue nuclei by my modification was greater than that by Sandiford's, with which gonococci were red and the pus cells green with bluish-green nuclei.

The modified Pappenheim stain here described was primarily intended for use in the examination of smears for gonococci, either directly or as a counterstain, but it is very suitable for blood films, particularly in the differentiation of the mononuclear leucocytes. The stain is also excellent for cerebrospinal fluid containing meningococci, and any amount of pyronine from 0.5 to 1 g. will yield good results.

The keeping quality of the modified stain has not yet been determined, but it is longer than 3 months and the solution is effective after Sandiford's has begun to deteriorate.

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The Vitamin C Saturation Test

This test, as modified by Harris and Abbasy (*Lancet*, 1937, **2**, 1429), affords useful and reliable information concerning the vitamin C reserves of men. During my year as lieutenant in the R.A.M.C. I carried it out on 1,200 soldiers according to a scheme drawn up by the Directorate. I performed several thousand titrations, and in the first tour each involved the usual simple calculations, taking into account the period of retention, the volume of the sample of urine, the burette reading, and the variable strength of the reagent—2:6-dichlorophenol-indophenol. These were tedious. The following is the procedure followed in the second tour; it is described here because it saved so much time both in the titrations and calculations.

As before, the men were dosed with 0.75 g. of ascorbic acid in water at 7.30 a.m. They emptied their bladders at 10.30 a.m., and a two-hour period of retention was adhered to rigorously. This is better than three hours, which in winter is too long. The samples were collected at 12.30 p.m. in numbered 2-lb. jam-jars, and instead of measuring the volume, which for 100 samples took about 1½ hours, each sample was made up to 500 or 1,000 ml. with tap-water. While an assistant did this, 10 ml. was pipetted off from each and run into a 6-oz. medicine bottle, numbered on shoulder and on cork. These bottles had previously received 1 ml. of glacial acetic acid.

This method obviates the addition of large quantities (30 to 50 ml.) of reagent in titrating the more concentrated urines. By taking 2.20 ml. of acidified sample for titration (namely, 2 ml. of urine), using a pipette graduated to the tip, with reagent adjusted so that 1 ml. was equivalent to 0.1 mg. of the pure vitamin, convenient readings were obtained. These were around 0.2 ml. for the initial low values of the first day, rising finally to about 6 to 8 ml. Saturation was taken to be when 50 mg. was excreted in a 3-hour period, or correspondingly for two hours. It is convenient to use the numbered corks to identify the 2-ml. portions removed to test-tubes for titration.

The reagent was adjusted to correspond to 0.1 mg. of vitamin, using 100-mg. portions weighed before the tour (not a bought tablet or a 1-g. tube as sold—these are not prepared as standards, and do not justify the implicit faith in their exactness that one sometimes finds). Tap-water had to be used, but this introduced no appreciable error. The standard must be titrated immediately after preparation.

The method appears to be entirely reliable, and will differentiate groups of men living under slightly different conditions. This may best be appreciated by plotting the number of daily doses required to saturate on the vertical axis and the number of men saturated by that day on the horizontal axis; namely, the sum of those saturated on first, second, and third days—say 60%—would be plotted at 60 for third day. Another way of assessing is to plot for each group the number unsaturated on some selected day; this shows up deficiencies. Thus, for example (an entirely hypothetical case), suppose one group of men had 5% remaining unsaturated after six doses and another had 50%, the difference could be strikingly shown in a graph. If, however, the two groups were close together they might best be differentiated by choosing a smaller number of doses for a comparison. Thus two groups which gave the same percentage of unsaturated after, say, five doses might be separated by one of them having a larger number unsaturated after four doses.

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