Limitations of the Use of 5-Fluorouracil as a Selective Agent for the Isolation of Leptospirae

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It has been shown that some component of Fletcher medium is able to annul the inhibitory action of 5-fluorouracil (400 µg/ml) on bacteria other than leptospirae. The most likely ingredient which can be implicated in this context appears to be beef extract.

The pyrimidine analogue, 5-fluorouracil (5FU), is known to exert a marked inhibitory effect on a considerable number of heterotrophic bacteria (2). A notable exception is leptospirae, the growth of which is evidently unaffected by concentrations of 5FU as high as 1,000 µg/ml (4). Consequently, Johnson and Rogers (4) proposed 5FU for use in the selective isolation of leptospirae from mixed bacterial populations.

Turner (5) and a WHO expert group on leptospirosis (6) recommended that media both with and without 5FU should be used in parallel, because certain strains of leptospirae might be inhibited by this compound. However, it was found that when 5FU (400 µg/ml) was added to Fletcher medium (1) containing sterile sheep serum in order to isolate leptospirae from bovine urine samples, suppression of contaminants was negligible (H. de Jong, personal communication). This paper concerns an investigation of this observation.

From a bovine urine culture in Fletcher medium without 5FU, six contaminant aerobic bacteria were isolated in pure culture on blood agar. They included a gram-negative spore former, a gram-negative nonmotile coccus, and organisms provisionally identified in each of the genera Staphylococcus, Escherichia, Micrococcus, and Citrobacter.

The contaminants were grown in Fletcher medium without agar and in the medium described by Johnson and Rogers (4). Both media were enriched with 10% sterile sheep serum and they were prepared both without and with 5FU at a final concentration of 400 µg/ml. One loopful of each contaminant was inoculated in three tubes of either medium, without and with 5FU, and the cultures were incubated at 37°C, at 30°C and at room temperature (15 to 20°C) for four days. Bacterial growth in the presence of 5FU then was compared with that arising in the same medium without 5FU under the same conditions. Growth was assessed visually as good (+++), fair (++), slight (+), and no growth (−). The presence of viable organisms in these cultures and their identity was confirmed by subculturing on blood agar.

The results (Table 1) indicated that the medium used and, incidentally, the temperature of incubation, had a pronounced effect on the inhibitory action of 5FU. Whereas addition of 5FU to Fletcher medium gave a poor suppression of most contaminants, it almost completely inhibited their growth in Johnson and Rogers medium. Evidently, ingredients of the more highly enriched Fletcher medium annulled the bacteriostatic action of 5FU in a manner that appears to be analogous to that which is exerted by pyrimidine bases (2).

Whereas sheep serum is used in both media, peptone and beef extract are present in Fletcher medium only. Peptones are less likely to be implicated in the reversal of 5FU’s activity, because they are used in other leptospirae media such as Vervoort and Korthof media (7) without beef extract, in which the reversal effect has not been observed (D. R. Ris, unpublished data).

It was notable that more contaminants survived in Johnson and Rogers medium containing 5FU at a lower incubation temperature than at a higher one. Perhaps the inhibitory activity of 5FU, like that of penicillin, may be related to the greater susceptibility of actively growing bacteria. Alternatively, a heightened metabolic requirement for pyrimidines and, therefore, an accelerated uptake of the analogue at the elevated temperature may provide an explanation. In any event, from a practical point of view, it now appears that one has to make the choice between either Fletcher medium without 5FU or another medium (suitable for the culture of leptospirae) with 5FU.
# Table 1. The growth of contaminants in two different media in the presence of 5FU (400 μg/ml)

| Contaminant | Incubation temperature |  |
|-------------|------------------------|---|
|             | 37°C | 30°C | 15-20°C |
|              | Fletcher and Johnson medium | Fletcher and Johnson medium | Fletcher and Johnson medium |
| A           | +++ | + + | + |
| B           | ++  | ++  | +  |
| C           | ++  | ++  | ±  |
| D           | −   | ±   | −  |
| E           | ++  | −   | +  |
| F           | ++  | −   | ±  |

*a Patterns of growth: ++++, good visible growth; ++, fair visible growth; +, slight visible growth; ±, no visible growth, but some (<10) colonies on blood agar culture; −, no organisms present. Contaminants A and B are, respectively, a gram-negative spore former and a gram-negative nonmotile coccus; C to F are organisms belonging, respectively, to each of the following genera: *Staphylococcus*, *Escherichia*, *Micrococcus*, and *Citrobacter*.

The Tween 80-albumin medium of Johnson and Harris (3) has been shown in this laboratory to be quite satisfactory, with added 5FU, for the isolation and routine culture of leptospiroae. Growth of most contaminants was suppressed by 5FU without any apparent effect on the leptospiral serotypes used.

### LITERATURE CITED

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