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The authors have commented as follows:

As we stated in our paper (Ellard and Greenfield, 1977), our urine-test method for monitoring the ingestion of isoniazid used the reaction procedure we reported previously for quantitatively determining isonicotinic acid and isonicotinylglycine (Ellard *et al.*, 1972). In this previous paper we described how the procedure had been modified from that originally described by Nielsch in order to improve its reproducibility and sensitivity. The results set out in our paper demonstrated that, employing this modification, concentrations of down to about 0·75 μg/ml of isonicotinic acid and isonicotinylglycine could be reliably detected in urine. This indicates that our isonicotinic acid urine-test method is considerably more sensitive than the Belles and Littleman, and Kasik modifications (to about 2·5 and 10 μg/ml isonicotinic acid, respectively).

Notwithstanding Dr Eidus' surprise with some of our results, there can be no doubt from the results given in our paper and the experience of others who have tried both methods that our new isonicotinic acid method is much more sensitive than the Eidus and Hamilton acetylisoniazid procedure. The acetylisoniazid method has been of great use in the past for monitoring the regularity of ingestion of isoniazid but it is now being replaced by our more sensitive isonicotinic acid method in current British Medical Research Council studies for the practical reason that it enables test urine samples to be collected at any time. The centres that have made the decision to use the new method have felt that this advantage outweighs the slightly more elaborate nature of the procedure as compared to the Eidus and Hamilton acetylisoniazid method.

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Anaerobic blood culture media

The article by Collee *et al.* (1977) raises some interesting problems. There must be better methods of obtaining an anaerobic headspace than by the method suggested, which seems to be erratic. The commercial method used by Difco, which seems to be the best so far, is to fill the vacuum in the autoclave with carbon dioxide and then screw a false lid down onto the bottles, forcing the bungs into place. As the autoclave cools still further, a vacuum is created in the bottles. After taking these elaborate procedures to produce anaerobically sterilised media, it seems a curious practice carefully to remove the bungs, thereby losing the vacuum the media had. Consequently, poor isolation of anaerobes may occur and the chances of contamination are much greater. This is due, firstly, to the shortness of the bung and, secondly, to the vacuum being released, thereby sucking air rapidly into the bottle. The bungs are invariably wet around the neck and this liquid will be contaminated fairly easily. The suggestion of using syringes and needles to subculture is a very practical method and has been practised in this department since the introduction of Thiol broth for the past four years. This difference in inoculation and sampling procedures may account for the differing results obtained by Collee *et al.* (op cit) compared with my own (Szawatkowski, 1976).

I agree with the authors that it is very difficult to compare the costs of laboratory-made media and commercially produced media. Firstly, Thiol broth is £31·00 for 100 (Difco) bottles; any media transported from London to Edinburgh (including Oxoid's Brain Heart Infusion broth) incurs a carriage fee. Southern Group Laboratories (a non-profit making laboratory) sell 50 ml of cooked meat medium at £16·80 for 100 bottles. Gibco Biocult will supply Brain Heart Infusion broth with cooked meat particles (as suggested by Collee *et al.*) at £48·00 per 100 as well as Thiol at £27·00 for 100; therefore Thiol is not the most expensive.

Nowhere in my article (Szawatkowski, 1976) did I suggest that Thiol should be used on its own as a general purpose blood culture medium. Thiol has several advantages. Firstly, in this laboratory we have found that it yielded anaerobes far more rapidly than the other media tested. Secondly, it inactivated the antibiotics listed in the article; and, thirdly, it was found that Thiol grew facultative anaerobes on average 24 hours earlier than the aerobic or vented bottles.

In this laboratory, as a routine 'set' of blood cultures, three bottles are used: Thiol, tryptic soy broth, and Southern Group nutrient broth with a castenada slope. It is very difficult to find a medium which will universally support the growth of the variety of organisms that can be cultured from blood. Consequently, our set can be changed at will, depending on the type of patient who is being screened.

As Collee *et al.* (op cit) say, it is very difficult to simulate clinical microbiological problems in a carefully controlled laboratory model. I would suggest that 'the proof of the pudding is in the eating' and the only way to compare the media is in clinical cases of bacteraemia.

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Diagnosis of Candida vulvovaginitis

Jones and Warnock (1977) comment that Stanley and Hurley (1974) omitted to determine the incidence of candida precipitins in pregnant women harbouring yeasts as commensals in the vagina. The 200 patients whom we studied were selected because of the high probability of mycotic vulvovaginitis among them; *all harboured yeasts* and many had symptoms suggestive of vaginitis (Table II). In Table III we contrasted the percentage incidence

of precipitins (64%) in 75 women with undoubted candida vulvovaginitis with the incidence (32%) in 65 women, 55 of whom harboured *C. albicans*, but in whom the aetiological diagnosis was not certainly established. The latter may be regarded, if one is so inclined, as a group in whom yeasts are, or may be, commensal, thus answering the comment of Jones and Warnock.

We have previously demonstrated (Carroll *et al.*, 1973) that, during pregnancy, *C. albicans* is isolated only rarely from women who are free of signs of vulvovaginitis or other indication of morbidity of the lower genital tract, this occurring but once in 50 times. We do not subscribe to the notion of commensalism of the fungus in the vagina of pregnant women, although, doubtless, it may be present on occasion as a contaminant or transient. Clearly, interpretation of the significance or otherwise of candida precipitins depends in part on the stance taken with respect to commensalism, both in the vagina and elsewhere.

Jones and Warnock record that 20% of 289 women harboured yeasts in the vagina without clinical signs of infection, yet they omit reference to the clinical criteria used to establish the presence or absence of vulvovaginitis. Since firm clinical diagnosis is crucial to interpretation of data accruing from laboratory tests on populations, the omission is not without significance. We believe that the clinical diagnosis should be established prospectively, according to defined criteria, and that the clinical and mycological observations should be double blind if conclusions are to be validly drawn. We have summarised the criteria for diagnosis of candida vulvovaginitis (Carroll *et al.*, 1973) and have drawn attention to the importance of accurate grading and recording of signs if clinicopathological correlation is to be attempted (Stanley *et al.*, 1975). We have reported a significant difference ($P < 0.001$) among clinical observers and have emphasised the unreliability of relying on diverse observers in studies of this kind, and the importance of genuine collaboration between clinician and microbiologist. In the absence of supportive clinical evidence, the rate of commensalism of 20% reported by Jones and Warnock is certainly questionable.

The distribution of yeasts reported by these authors in a population wherein only 25/289 suffer from mycotic vulvovaginitis is contrary to our experience (Hurley *et al.*, 1973), since only *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were isolated. The absence of *Torulopsis glabrata* and an occasional isolate of a non-pathogenic yeast is remarkable.

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The authors have commented as follows:

Altogether 82 of our 300 patients were found to be harbouring yeasts in the vagina; 25 of these women (30%) had clinical signs of vaginitis or vulvovaginitis (the clinical criteria of Carroll *et al.* (1973) were used to establish the presence or absence of vulvitis and vaginitis). The incidence of clinical signs of infection in these 82 women with yeasts in the vagina was thus similar to that (38%) found in another unselected group of non-pregnant women attending a VD clinic in London (Oriel *et al.*, 1972) and contrasts with the incidence found in pregnant women attending an antenatal clinic (Carroll *et al.*, 1973).

The absence of *Torulopsis glabrata* from our patients is rather remarkable. Nevertheless it is a fact and not a result of any inadequacy in mycological technique. During the period over which our investigation took place, *T. glabrata* was isolated and identified 19 times in the same laboratory from other patients.

In our paper we noted that Stanley and Hurley (1974) had not compared the incidence of Candida precipitins in pregnant women harbouring yeasts in the vagina with the incidence in similar women harbouring yeasts in sites other than the vagina. Our results showed that precipitins could be detected in only 20% of non-pregnant women with vaginal candidiasis, and this incidence was similar to that (23%) observed in women harbouring yeasts in the vagina without clinical signs of infection, and *also* to the incidence (21%) observed in women who were harbouring yeasts in sites other than the vagina. It was these observations that led us to conclude that the double diffusion test has little to offer in the diagnosis of vaginal candidiasis.

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