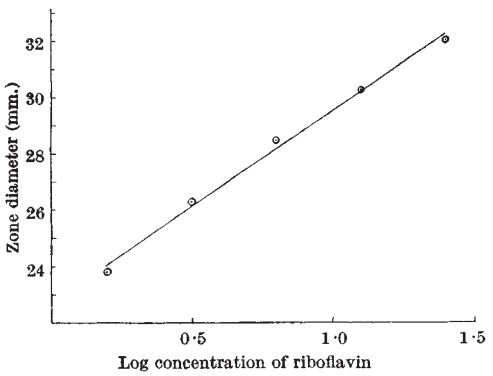
THE Petri dish technique for the microbiological assay of vitamins described by Mr. Bacharach[1] has been used in these laboratories for demonstration purposes since September 1946, and the disadvantage to which he refers, namely, the lack of sensitivity, can be fully confirmed.

The general technique we have employed follows closely that used for the cup-plate assay of penicillin. The suspension for inoculum is prepared exactly as for a riboflavin assay by the usual method, except that the cells are suspended in the original volume of saline and used without further dilution. To 12·5 ml. of the melted medium at 45° C., 1 ml. of this strong suspension is added, and the flasks are held at 45° C for 10 min. before pouring the plates. If a weaker inoculum is used, or the heating at 45° C. is omitted, the zones are less sharp and, in consequence, more difficult to read. Five cups are cut in each plate, and previously sterilized solutions of riboflavin containing 25, 12·5, 6·25, 3·125 and 1·56 μgm./ml. are distributed into the cups in approximately 0·05 ml. amounts. We can confirm Mr. Bacharach's observation that the zone diameter is proportional to the logarithm of the dose, as exemplified on the accompanying graph.



If further research makes it possible so to increase the sensitivity that the technique is suitable for the routine assay of riboflavin in foodstuffs, it will be interesting to examine the influence, on the sizes of the zones, of non-specific growth stimulants which are a source of difficulty with the assay of riboflavin as at present carried out. Since, in the Petri dish assay, the *diffusion* of the vitamin is a factor determining the magnitude of the response, it may be that stimulants of higher molecular weights than the vitamin under test will be without effect on an assay of this type.

Nevertheless the method is even now not without practical applications, as, for example, in demonstrating the qualitative and to some extent the quantitative differences between organisms with regard to their abilities to synthesize a given growth-factor. Instead of cutting cups in the bulk-inoculated, solidified riboflavin-free medium, the surface may be seeded in three or four places with suspensions of bacteria or mould-spores. Those organisms capable of synthesizing the vitamin may then be readily detected by the appearance of circular 'zones of exhibition', the diameter of the latter serving as an index of the amount synthesized. It is suggested that this might be an expeditious way of conducting a survey of organisms in order to differentiate them in this respect.

An obvious point of difficulty is the possibility of widely different requirements of the 'indicating' bacterium and the organisms under test, not only nutritionally but also with regard to *p*H and temperature. We have so far used only the usual basal media developed for microbiological assay (solidified with 1·5 per cent agar) and *Lactobacillus helveticus* and *Lactobacillus arabinosus* for the detection of riboflavin and nicotinic acid respectively. Such media permit growth of, and vitamin synthesis by, many moulds at 37° C., although they are scarcely likely to be optimal for this purpose. The ideal to be aimed at would be a medium and conditions of growth as nearly as possible optimal for both the indicating and the test organisms.

Pontecorvo[2] has described a technique, in some respects similar, for the identification of the nutritional requirements of a mutant, based on the addition of crystals of nutrilites to plates of 'minimal' medium inoculated with the mutant strain. The procedure outlined above, however, serves a different purpose, namely, the investigation of the synthesizing abilities of a range of organisms with respect to a given nutrilite.

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[1] *Nature*, **160**, 640 (1947).
[2] *Proc. Nut. Soc.*, **5**, 182 (1947).

Primary and Secondary Responses in Biological Assay

IT has already been shown that the irritant and paralysing effects of the pyrethrins are of material influence in determining the dose of insecticides accumulated by flying insects in pyrethrum-bearing sprays[1].

The influence of such primary responses on the dose of toxicant accumulated, and thus on the resulting secondary response (usually estimated as proportionate mortality), is now widely accepted as of importance in this system, and the purpose of this communication is to suggest that similar factors may be of more common occurrence in biological assay systems than is generally appreciated, and in particular to indicate their probable influence in fungicidal assay.

Dimond *et al.*[2] reported that tetramethyl thiuram disulphide tested as a fungicide against *Macrosporium sarcinæforme* yields a dosage-response curve exhibiting a maximum and a minimum. They offered an explanation, based on the spontaneous dissociation of the fungicide, which Finney[3] has shown could not account for the observed action without the additional hypothesis of drug antagonism between the two states of the molecule. Montgomery and Shaw[4] also criticized the dissociation hypothesis on physico-chemical grounds, and found similar effects, which they termed 'inversion', in the dosage-response curves for five thiuram and five dithiocarbamate derivatives tested against *Venturia inæqualis*. Parker-Rhodes[5], applying the theory of variability to both types of fungicide, demonstrated that absorption is necessarily preceded by reaction with the spore secretion.

The spray pick-up/toxicant concentration curves previously published for the flying insect system[1] may be converted into log (toxicant pick-up)/log (toxicant concentration) curves of the type shown