A Simple Method for the Establishment of Geometrical Progressions by Diluting with the Pipette

IN serology and biochemistry it is a daily task to ascertain by so-called serial tests the effective dose of any substance; for example, to establish the titre of the agglutinating power of a serum, to find the strength of a hæmolytic amboceptor, or the smallest concentration of salt that just precipitates a colloidal solution, etc.

We can carry out the serial tests by preparing solutions of the substance in question in the form of an arithmetical or geometrical progression. Michaelis and Rona[1], and many others, have shown, however, that this procedure as it is often carried out is the source of various errors, when the series are laid out in the form of arithmetical progressions.

Let us, for example, examine the sodium chloride series which was prescribed by Kafka for the establishment of the smallest gold-sol precipitating salt concentration (or mastic-sol ones).

Salt conc. (%)	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9	1·0	1·1	1·2
Difference (%) between various tubes		100	50	33	25	20	16	14	12·5	11	10	9

The variation in percentage differences between consecutive concentrations in the above series is clearly undesirable for quantitative estimations. In the great majority of cases it is necessary to estimate, with approximately the same percentage error, at whichever part of the series of dilutions the indicating tubes occur. This is ensured by the use of serial dilutions, in which the ratio of concentrations of the reagent in any two adjacent tubes is the same throughout the series. The preparation of such a geometrical series with any required ratio of concentrations between the contents of adjacent tubes is much facilitated by the use of a simple algebraic formula.

A series of tubes is placed in a row. Into the first tube a convenient amount (V) of the reagent is accurately measured. Into each of the remainder of the tubes the same volume of the diluent is measured. Into the second tube is measured also a volume (K) of the reagent, where

$$K = \frac{V}{Q - 1},$$

Q being the ratio of concentration between the contents of adjacent tubes, which is appropriate for the experiment. The contents of tube 2 are then thoroughly mixed and a volume K of the mixture transferred from tube 2 to tube 3. After mixing, a volume K is transferred from tube 3 to tube 4, and so on through the series.

As an example, we may revise the Kafka salt concentration series cited above. If we arrange the twelve dilutions in a geometrical series, the value of Q to cover the range of dilutions from 0·1 to 1·2 per cent is 1·2535. This is arrived at by dividing the difference of the logarithms of 1·2 and 0·1 by 11 (the number of intervals between tubes) and taking the antilogarithms. If we use $Q = 1{\cdot}25$, the highest concentration should be 1·16 per cent instead of 1·2 per cent. Then with $V = 0{\cdot}5$, $Q = 1{\cdot}25$, $K = \frac{0{\cdot}5}{0{\cdot}25} =$ 2 c.c.; 0·5 c.c. of the 1·16 per cent solution is put into tube 1 and 0·5 c.c. of the diluent into each of the other tubes. The 2 c.c. of 1·16 per cent is added to tube 2, mixed, and 1 c.c. of the mixture transferred to tube 3; 2 c.c. is then transferred from tube 3 to tube 4, and so on.

If the same pipette is used for each of the successive dilutions, it is important that it should be a very accurate one, as any errors in its calibration cumulate rapidly. If, for example, a 5 per cent deficiency occurs at every stage of successive dilution, the last tube of a series of twelve will have little more than half its true concentration.

In conclusion, I should like to express my sincerest thanks to Dr. J. W. Trevan, director of the Wellcome Physiological Research Laboratories, for criticisms and advice.

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[1] Michaelis and Rona, "Pratikum der physikal. Chemie" (1932).

Potato Dry Rot and Gangrene as Soil-borne Diseases

DRY rot was described as a storage disease of the potato nearly forty years ago[1,2], and has since become of increasing economic importance; yet only recently has attention been paid to the soil as a source of infection. The control of the disease obtained by the use of organo-mercury dips at lifting time[3] and the marked variation in its severity in stocks of the same variety from different farms lent support to the assumption that the causal fungus (*Fusarium caeruleum*) was soil-borne. No attempt had, however, been made to provide direct experimental evidence of this until last year, when Small[4,5] showed that *F. caeruleum* was often present in the soil adhering to healthy tubers both at lifting time and during storage.

We have carried out experiments to determine the presence of the fungus in soils from selected fields in Scotland and to discover whether any variations could be detected in the degree of infestation. In the autumn of 1943, bulked random samples of soil were taken, with aseptic precautions, from twenty fields. Potatoes of the susceptible variety Doon Star were inoculated with small quantities of the soils, twenty-five tubers, each inoculated in two places, being used for each sample. A fresh sterilized inoculator was used for each tuber.

RESULTS OF INOCULATING POTATO TUBERS WITH SOIL TAKEN FROM TWENTY SCOTTISH FIELDS IN OCTOBER 1943.

Date of last potato crop	Number of fields	Percentage of inoculations causing dry rot
1941	1	32
1942	11	52, 22, 16, 10, 6, 6, 4, 2, 2, 0, 0
1943	8	88, 24, 20, 4, 4, 4, 2, 0
Control: sterilized soil		2
Control: spore suspension of *F. caeruleum*		100

The infection of one wound in the first control series was presumably due either to imperfect sterilization or to chance air-borne contamination.

Isolations from random samples of infected tubers showed that the causal organism in almost every case tested was *F. caeruleum*.

The results summarized in the table show that *F. caeruleum* may be present in the soil at least two years after the last potato crop, and that marked variations in the degree of infestation of the soil samples were detected by the method used. Experiments on a larger scale are now in progress to ascertain whether such variations can be correlated with