Technique. A solution of 60 per cent cane sugar and 1·6 per cent gelatine is sterilized by autoclaving. One platinum loopful of this solution is mixed on a cover-slip with a loopful of the antiseptic to be tested. Ripe stamens of the flower selected are stroked with a sterile needle, held just over the cover-slips; a control of 30 per cent cane sugar is set up from the same flower.

Strips of adhesive plaster are applied on either side of the depression in a hollow-ground slide. The surface of the plaster is smeared with 'Vaseline' and a 'hanging drop' preparation made. The slides are placed in moist chambers, prepared by placing moistened filter paper disks in petri dishes; they are examined with 2/3 objective and × 8 ocular with micrometer scale after fifteen minutes at room temperature.

Liberal sowing is desirable, as a number of grains do not germinate in controls. White dead nettle is a convenient pollen, as this plant flowers all the year round, and is very common; the grains germinate in fifteen minutes. However, it shows no protoplasmic streaming.

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Chemical Production of Mutations

IN a previous letter in *Nature*[1], chemical substances were mentioned which are as effective as X-rays in inducing mutations and chromosome rearrangements. The chemical nature of the main substance used can now be stated. It is dichloro-diethyl-sulphide, or mustard gas. Three other substances of similar efficiency were found, all of them chemically related to mustard gas. Lewisite, on the other hand, gave negative results. The results were first described in a report sent to the Ministry of Supply on March 14, 1942.

In a large-scale test on the production of mutations by mustard gas carried out in April 1941, *Drosophila melanogaster* males were exposed to volatilized mustard gas and afterwards tested for sex-linked lethals by the standard ClB method. 7·3 per cent lethals were obtained in more than 1,000 chromosomes, as compared with 0·2 per cent in the controls. In later tests, even higher mutation-rates (up to about 24 per cent) were obtained. The limit for the increase in mutation-rate by increase of dosage is given by the equally increased rate of dominant lethality in the F_1: doses which produce more than 20 per cent sex-linked lethals practically sterilize all the treated males.

The mutagenic action of mustard gas appears to be exercised directly on the chromosomes, and not by way of a change occurring primarily in the cytoplasm; for the mutation-rate is not increased in untreated spermatozoa which have been introduced into treated eggs. Various rearrangements (inversions, large deletions, translocations) have been produced by the treatment. But the frequency of translocations is lower than would be expected after a dose of X-radiation which produces the same percentage of sex-linked lethals; it is, however, still considerably higher than after ultra-violet treatment. Thus, in one experiment with mustard gas in which the frequency of sex-linked lethals was 8·6 per cent, only seven translocations involving the *X*-chromosome and/or the two large autosomes were found in 812 treated chromosome sets.

The visible mutations which have so far been produced by chemical treatment have, with one exception, been previously described. In the progeny of chemically treated males, 30–50 per cent of visible mutations occur as 'fractionals', a proportion which is considerably higher than that observed in progenies of X-rayed males.

Full details of these results will be published elsewhere.

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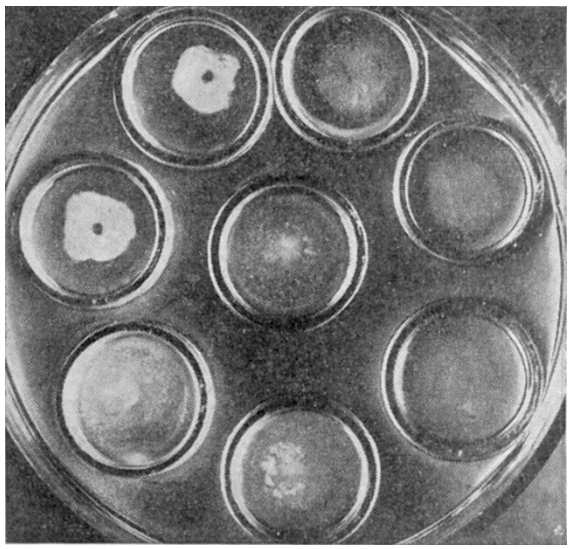
[1] Auerbach and Robson, *Nature*, 154, 81 (1944).

Silica Jelly as a Substrate for Counting Holozoic Protozoa

THE study of selective feeding[1–5] of soil protozoa, especially amœbæ, on a large number of bacterial strains, led to the development of an improved method[6] of counting soil Protozoa. In the past, dilution methods[7] or estimating Protozoa numbers have suffered, first from having too few replicates, and secondly from the use of unspecified bacterial food. These two difficulties have been met in the method described, the first by making a number of separate cultures enclosed by glass rings in each petri dish, and the second by using, as food supply, pure cultures of bacteria known to be edible to Protozoa. These food bacteria have in the past[6] been spread on the surface of washed agar made up with 0·5 per cent sodium chloride. It has been found, however, that silica jelly may be more advantageously used for two main reasons. First, it further reduces the growth of organisms coming in with the inoculum; and secondly, it has another advantage over agar because it does not allow the growth of agar-liquefying organisms which may be abundantly present in certain substrates.

Silica jelly is easy to make, and can be sterilized for the purpose of counting Protozoa. A 10 per cent solution from liquid sodium silicate (B.D.H.) is made in distilled water. The sodium silicate quickly dissolves in water by gently warming it. 4 c.c. of 2*N* hydrochloric acid is poured into a petri-dish and 2 3 drops of brom-thymol blue are added. Now a 10 per cent solution of sodium silicate is slowly added to the hydrochloric acid and the petri dish is gently rotated to mix the sodium silicate until a grass-green colour is obtained. About 15–20 c.c. of 10 per cent sodium silicate is required and the *p*H of the medium should then be about 6·8–7·0. Eight glass rings are quickly put in the silica jelly before it solidifies; the rings are firmly embedded in the jelly when it has set. With a little practice it takes about two minutes to make a plate. The plates are then put, with the lids removed, in a tank under running water for 24 hours or longer to get rid of most of the salt. Then they are immersed for 24 hours in



normal saline solution, which provides an osmotic pressure in the silica jelly suitable for Protozoa. The water is drained from the plates and they are sterilized with the lids on by dry heat in an oven at about 80°–100° C. for an hour or more. This temperature is sufficient to kill Protozoa, both active and cystic, if they happen to have fallen into the plates during the course of preparation. The plates are now ready for use. A strain of a pure culture of one or a mixture of several suitable bacteria (2–7 days old culture) is spread over the silica jelly contained in the glass rings, in the form of a thick disk or 'bacterial circle'. In the count of soil Protozoa fifteen two-fold dilutions are made ranging from 1/5 to 1/81,920, with eight replicates at each level and with an inoculum of 0·05 c.c. added at the centre of the 'bacterial circle'. The number of Protozoa per gram of soil is then estimated from the count of negative cultures (that is, showing no Protozoa) by applying the method of Fisher and Yates[8], Table VIII, 2. The accompanying photograph shows two negative and six positive cultures.

A few counts that have been made on silica jelly from a decomposing compost heap of straw and sludge set up at Rothamsted farm are given in the accompanying table.

Date	Samples	Total amœbæ per gm.	Cystic amœbæ per gm.	Active amœbæ per gm.
April 16, 1945	*A*, taken from 3 in. depth	467,000	70,500	396,500
	B, taken from 6 in. depth	211,000	17,300	193,700
April 21, 1945	*A*, taken from 2 in. depth	256,000	—	—
	B, taken from 4 in. depth	121,000	—	—

It seems that the number of amœbæ decreases as the samples are taken from a greater depth. This is probably due to either an increase in temperature or the lack of oxygen or both.

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[7] Cutler, D. W., Crump, L. M., and Sandon, H., *Phil. Trans.*, B, 211, 317 (1922).
[8] Fisher, R. A., and Yates, F., "Statistical Tables", 2nd. ed. (Oliver and Boyd, Ltd., 1943).

Nature of the Cyst Wall of the Potato-Root Eelworm *Heterodera rostochiensis*, Wollenweber, and its Permeability to Water

EELWORM cysts are white and soft when first formed on the roots of potato plants: afterwards they turn yellow and eventually become brown and hard. I have been able to demonstrate that this process is due to the activity of polyphenol oxidase*; white cysts will oxidize catechol and tyrosine, and the action of the enzyme can be inhibited by carbon monoxide, hydrogen sulphide, potassium cyanide, potassium ethyl xanthate, and sodium diethyl carbamate, substances known to

* With material kindly supplied by Mr. F. G. W. Jones, School of Agriculture, Cambridge, I have since been able to show that this enzyme is also present in the beet eelworm, *H. schachtii* Schm.