BACTERIAL GROWTH FACTORS IN SOIL[1]

IN a study of the nutritional requirements of indigenous soil bacteria, isolated by non-selective plating methods, special attention was given to organisms unable to grow in a basal salts-glucose medium to which was added a mixture of ten amino-acids (or peptone) plus seven growth factors, or even in one containing yeast extract supplement. Such organisms, comprising approximately 20 per cent. of the isolates from soil, were found to grow well in yeast extract medium upon the addition of a heated aqueous extract of field or garden soil, more than 80 per cent. of them showing good or sub-maximal growth without the yeast extract. For the great majority of the 63 strains studied the growth-promoting properties of soil extract were dependent upon a factor or factors (not concerned with the ash constituents) present in the acetone, but not in the ether extract, and capable of being adsorbed by Norit and eluted with ammoniacal alcohol.

It has already been shown that certain vitamins may be present in soil. Thus Lilly and Leonian[2] have demonstrated the presence of thiamin, while the occurrence of biotin in plant and animal tissues readily accounts for its presence, particularly in well-fertilized soils. Furthermore West[3] has shown that measurable quantities of thiamin and biotin may be excreted by the roots of seedlings. Since the organisms studied by us, however, showed no growth in the presence of the growth factor supplement, which included thiamin, biotin, riboflavin, pyridoxin, pantothenic acid, nicotinic acid and inositol, it is suggested that the growth-promoting properties of soil extract for this group of bacteria are to be ascribed to factors other than those listed.

More than one such growth-promoting factor appears to be present in soil extract, one or more of which are capable of being synthesized by certain other soil organisms having simpler nutritional requirements. Thus it was found that for certain strains the nutrilite effect of soil extract could be replaced by a filtrate from cultures of bacteria capable of maximum development in the basal salts-sugar medium. For other organisms similar filtrates were ineffective, indicating distinctly different growth requirements. In the case of the relatively small number of isolates requiring yeast in addition to soil extract, it was found that the effect of yeast could be likewise supplied by filtrates of certain bacteria growing in the basal medium. The associative influence of organisms producing such by-products doubtless explains the ability of certain more fastidious bacteria to grow on the original plating medium which contained soil extract without additional nutritive supplements.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MICROSCALPEL FOR USE IN EXPERIMENTAL EMBRYOLOGY[1]

A MICROSCALPEL that has been found to be very suitable for many kinds of operations and dissections may be made from the cutting edge of a razor blade. Such small scalpels have been used with success, principally in the microsurgery of experimental amphibian embryology, but have also proved valuable in the dissection of annelids, copepods and insects. The scalpels are easily made and are usually prepared in lots of ten or more so that they may be discarded when dull without loss of time.

The razor blade used is of the thin, double-edged type. A narrow strip of steel, which bears one of the cutting edges is broken off by clamping the blade firmly in a hand vise, then bending the protruding part of the blade sharply against a glass plate or other smooth, flat surface (Fig. 1). It is important to clamp (*i.e.*, break off) as narrow a strip of the blade as can be firmly held in the hand vise for this will minimize the time required to finish the scalpel. Care should be exercised upon inserting the edge of the blade into the hand vise to avoid dulling or nicking the cutting edge.

The shaft of the microscalpel is made from a fine embroidery needle, the tip of the eye of which is ground away on a fine emery wheel or oil stone, leaving two prongs (Fig. 2). Between these prongs, the small portion fractured from the razor blade is fastened by means of solder (Fig. 3). In order to make a delicate joint, the soldering is best done with the use of a rosin base soldering flux and by first coating the prongs thinly with a layer of solder (tinning). One end of the razor edge is then coated with flux (only) and inserted between the tinned prongs of the needle. The prongs are pressed lightly upon the point

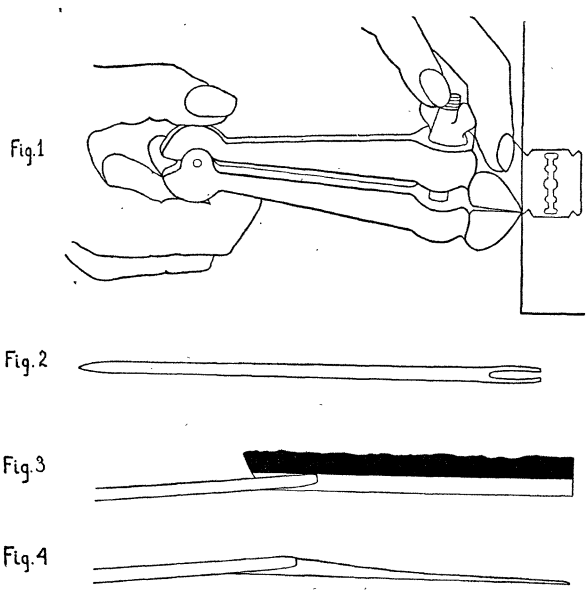
[1] Contribution No. 156 (Journal Series).
[2] V. G. Lilly and L. H. Leonian, SCIENCE, 89: 292, 1939.
[3] P. M. West, *Nature*, 144: 1050–1051, 1939.

[1] From the Division of Anatomy, University of California Medical School.

of a hot soldering iron, and a smooth, sweated union between the prongs and the strip of razor blade is obtained.

The shaft of the scalpel (*i.e.*, the needle) may now be clamped by its pointed end in a needle holder or pin vise, in order to facilitate shaping the blade of the microscalpel. The shaping is accomplished by grinding away the back of the small blade with a fine emery wheel or oil stone to the desired shape, exercising care to avoid grinding the cutting edge of the blade.

The shape of the blade and the angle which the blade makes with the shaft of the scalpel depend upon the use to which the scalpel is to be put. For the purposes of experimental embryology, the blade is ground to a gradually tapering, delicate point of nearly microscopic dimensions. A moderately fine blade is shown in Fig. 4.



In use, the shaft of the scalpel is held in a needle holder or pin vise. Incisions are made in amphibian embryos by inserting the tip of the blade into the tissue of the embryo and then gently stroking the surface of the tissue above the edge of the blade with a fine glass needle or hair loop. For coarser work, the scalpel may be used in conjunction with fine forceps or jeweler's tweezers.

With practice, a dozen of these small knives can be made in a half hour. It has been found most practical to store extra scalpels by sticking the pointed ends of their shafts into a large cork and then inserting the cork into a wide-mouthed bottle so that the blades are protected from moisture and mechanical damage. They also may be coated with oil or grease until ready for use.

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THE INSECTICIDAL ACTION OF PHENOTHIAZINE

PHENOTHIAZINE, an organic compound which is non-toxic to humans, is effective as a urinary antiseptic,[1] anthelmintic,[2] fungicide[3] and insecticide.[4] The compound offers promise as a substitute for arsenicals, especially in codling moth control.[4]

The effect of phenothiazine upon the American cockroach, *Periplaneta americana* (L.) was investigated. The compound is toxic to the roach, acting entirely by contact with the body surface. No toxic effect results when the chemical is taken into the alimentary canal. When applied to the body surface phenothiazine passes through the exoskeleton and is converted internally to a compound believed to be a conjugate of thionol, present in leuco form. The latter compound must reach a definite concentration in the haemolymph before the toxic effect is produced. The effective concentration of the thionol conjugate in the haemolymph is correlated with the particle size and with the quantity of phenothiazine in contact with the exoskeleton. The most rapid kill at the lowest concentration is produced with particles of the smallest size. When an equal amount of phenothiazine in a larger particle size is in contact with the body surface, the lethal concentration of the thionol conjugate in the haemolymph is not reached. In this case only a slight uncoordinated leg movement is evident, and recovery is rapid as the thionol conjugate is eliminated through the Malphigian tubules.

Ingested phenothiazine has no effect upon the roach, although undergoing oxidation primarily during its passage through the mid-intestine. The wall of the intestine is impermeable to phenothiazine and to the oxidation products formed.

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[2] P. Manson-Bahr, *The Lancet*, 239: 808–809 1940.
[3] M. C. Goldsworthy and E. L. Green, *Phytopathology*, 29: 700–716, 1939.
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