benefit of the Indian farmer, aviator, engineer and the public at large.

Apart from the problems of organisation, procedure, communication, etc., referred to above, the Commission also considered some of the important research aspects of pure and applied Meteorology. In the field of Agricultural Meteorology the Indian workers under the Director of Agricultural Meteorology at Poona have played a leading role and it is pleasing to note that the Indian Crop-Weather Scheme has been adapted for international use by the Commission. A permanent Sub-Committee on Agricultural Meteorology, with the Director of Agricultural Meteorology, Poona (India), as Chairman, has been constituted for the Asian Region for developing and co-ordinating the work on this subject in the various countries of Asia.

Another permanent Sub-Committee on Hydrology, a subject of such vast importance for India where many multi-purpose irrigation schemes are under way, was also constituted.

It is obvious that these meetings at Delhi have been very fruitful and we congratulate both the Government of India and its Meteorological Department for the important role they have played in furthering the cause of Meteorology in the service of the Asian Region.

THE MICROMANIPULATOR

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EARLY in February 1948, I had the pleasure of giving to Prof. M. Sreenivasaya during his stay in my Laboratory, a comprehensive demonstration of the Micromanipulator technique and its applications to problems in bacteriology and cellular physiology. On this occasion he suggested that I should contribute a note on this subject to *Current Science*; the following article has been written in response to his kind request.

The introduction of the gelatine medium by Robert Koch, represents one of the epochal landmarks in the development of bacteriological technique and has been responsible for the phenomenal advances in microbiological research during the past sixty years. This we certainly owe to the simplicity, ease and elegance of what has now come to be known as "plating method". The clarity of the medium facilitates the unmistakable location of microbes and the development of colonies as a result of their growth. No wonder that the method has remained unchallenged and is universally employed as a routine in bacteriological research.

Many workers, however, unwilling to confine themselves to routine investigation, observed that this method had certain serious limitations. When plating river water, we may be justified in assuming that by vigorous stirring of the molten nutrient medium, the individual cells would be completely separated, and the resulting colonies developed in the petri-dish, would represent integrally pure cultures. In the case of slimy material, however, *e.g.*, pus, blood, fæces, filthy ditch water, this assumption would not be justified. In such samples the cells adhere together, so that it is usually extremely difficult to separate them by stirring. We encounter the same difficulty with dermatomycoses. Even after pulverising with quartz powder one finds the cells adhering together in great numbers.

The investigator is often confronted with a mixture of different bacilli, from which he is obliged to isolate and examine one of them in pure culture. The "plating method" does not give him any reliable result; none of the colonies in the plate would be representative of the species he is seeking. This difficulty is experienced by all workers. Plating—I might say—is a blind method. An unknown quantity of unknown species is plated, but you never know which of them develop, neither the way of developing (only think of the lag-time!) nor the antagonistic influence of the different associates. From bacilli, found in fæces, it is often found that only one per cent. of them develop.

The inherent drawbacks which characterise gelatine or agar-agar affect the development of certain organisms, *e.g.*, nitrifying bacteria, but such organisms are fortunately few and occur only rarely. But what is of real importance is described in the next para.

The basis of all biological work consists in the integrity of the individual. In problems of heredity, in determining vari-

ability of a given strain, the experimenter has to carefully select his organisms individually. Should such a selection be not possible, scientific research would not be considered unimpeachable. In microbiology, selection and isolation of individual cells was considered impossible and till the end of the preceding century, investigators had reconciled themselves to this state of affairs. How could it be possible to experiment with individual cells of bacilli ; Yet it is the first condition to be satisfied, if one is to follow the hereditary factors and the spontaneous variability of the organism.

In 1899, I demonstrated at the Scientific and Medical Congress at Haarlem a method which I had discovered in 1897, for isolating a single cell under the microscope. This technique which was further developed and applied, formed the subject of my dissertation. Since then I have continued my researches on the micromanipulator ; during the last fifty years this work has developed in a direction which renders possible the adaption in principle of the same type of investigations with microorganisms as those practised in the domain of the more highly organised organisms.

The method of isolating is, in brief, as follows :—On a coverslip, which has been smeared with a little Tangkallak fat and then passed through a flame, a drop of the material is placed from which it is desired to isolate a cell. At a distance of about 3 mm. apart drops are placed in which it is desired to grow the pure culture. The coverslip is then placed on a moist chamber on the microscope stage. The lateral walls of this chamber are provided with a horizontal slit, closed by a viscid fluid and through which the isolation needles project into the chamber. By means of a simple mechanism the needles can turn round a support, so that their ends can touch the corner surface of the coverslip. Any mechanical shake renders it possible to do this in any part of the field. The space in the moist chamber is kept saturated with water vapour by a drop of water on the floor. The vapour condenses on the lower surface of the coverslip, and since the latter has been treated with fat, the condensation takes place in small rounded droplets, which do not coalesce. Before use the needles are sterilised by dipping their ends in sulphuric acid and in ammonia. As micro-organism to be isolated is now sought at the edge of the so-called material drop. The needle is moved up so that the end touches the edge of the drop near the cell that is to be isolated. Then, when the needle is moved sideways, the cell with a tiny droplet will be drawn out of the large drop. This cell is now taken up in the eyelet of the needle. This eyelet is then brought down somewhat, so that it no longer touches the coverslip, and then the moist chamber is moved sideways, under a low power, so that the eyelet, by an upward movement, comes near to the edge of one of the sterile drops and deposits the isolated cell, in a small drop (about 6 μ) on the coverslip. Under high power this small drop with the cell is moved into the sterile drop, in which the culture is made. This is repeated with other cells, and when all the sterile drops contain each a cell, the coverslip is placed on a moist chamber and incubated at the required temperature. If the culture drop is solid, the colony grows on its edge, and with the aid of a high power microscope, its development can be followed from the beginning. If the culture drop is fluid, the colony generally spreads itself over the whole drop.

So much for an outline of the method of isolating by means of eye-shaped needles. When pointed needles are used, the method is somewhat modified ; then the material (*e.g.* bac., *tuberculosis*, *granula*) is smeared on the coverslip, and the cells are simply picked up. In micro-operating fine glass point needles, for bacilli, a point of about 0·2 μ is used; glass knives also can be employed.

Sometimes it is considered that these manipulations require a special aptitude and practice. But with good prepared coverglasses and clean and properly made needles, the work is agreeable and not too difficult. Isolation of a bacillus and transferring in a sterile drop requires two minutes, and cutting into two of a bacillus, taking up unfolding the double-folded, takes 15 seconds.

As to the making of the needles, I know from experience that a worker, endowed with common skill, will be able to make a good eye needle after two days, on condition that he can practise under expert personal guidance.

Note —The cost of printing this contribution has been met from a generous grant from the Indian Council of Agricultural Research, New Delhi.