# TECHNICAL NOTE

# ORGAN CULTURE METHOD OF PRODUCING A LEPROSY-BACILLUS SUSPENSION (NIMORPEL) FOR USE AS A LEPROMIN

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A method of producing a leprosy-bacillus suspension for use as a lepromin is described which has some advantages over conventional methods, and may be of interest in view of the large demand for lepromin today. The lepromin-type antigen obtained by this method, for which the name Nimorpel has been introduced, consists of a suspension of leprosy bacilli obtained from explants of biopsy material maintained in *organ culture* for about five to six weeks.

## MATERIALS AND METHODS

Explants from biopsy specimens from untreated cases of lepromatous leprosy were maintained *in vitro* culture, using organ-culture techniques. (Two modifications of the original procedure of Fell and Robinson ([4]) were used.

1. Lepromatous nodules were removed aseptically and explants were immediately prepared by cutting the tissue into small cubes of about 1-3 $mm^3$ volume. After several washings with Tyrode or Hanks' solution they were transferred to rayon strips 1 cm. square. These were placed on clots of chicken embryo extract (Difco) and plasma (Difco), equal parts, in watch glasses enclosed in petri dishes with damp cotton wool at the bottom (Schaffer ([5])). The rayon strips were transferred to new clots at regular intervals, about twice weekly. Some explants were removed at each change of medium and were either fixed for histologic examination or minced to make smears for preliminary investigation.

2. Alternatively, a platform of tantalum wire mesh (Trowell ([6])) was placed in a dish about 1½ inch in diameter. The explants were placed on top of the wire supported by squares of lens paper, and the fluid medium selected was carefully pipetted onto the bottom of the dish until the fluid was level with the lens paper. The fluid medium was changed about twice weekly. Various combinations of nutrients were used, mainly the serum-embryo extract medium, or 2 parts of serum to 1 part of a solution containing 0.6 per cent glucose and 0.2 per cent sodium chloride.

## RESULTS

In previous work by one of us (E.M.B.) it was shown that in explants made from early tuberculous lesions which were cultivated for only a fortnight *in vitro* by either the tissue or organ-culture technique, bacilli, which were rare at the beginning of the culture period,

had multiplied to such an extent that in sections the whole microscope field was covered with colonies of acid-fast organisms (Brieger, Fell and Smith [2]; Brieger [1]). In parallel experiments done at the Africa Inland Mission leprosarium at Oicha, explants were made from biopsy specimens from lepromatous cases, and the impression was gained that the bacilli had multiplied in some of the explants that had been cultivated for four to six weeks. The microscope field was covered with colonies of acid-fast bacilli.

It might be argued that this appearance is due to digestion (autolysis) and consequent concentration of the tissue and redistribution of bacilli in the explant. This does not seem likely when one considers the very great difference in bacillus content of the material expressible from the explants before and after culture, and in histologic preparations of the tissues before and after culture. In any case, however, a suspension made from minced six-weeks-old explants consisted almost entirely of bacilli, singly or in clumps.

An attempt is now being made to apply quantitative methods to ascertain if multiplication of the bacilli actually takes place in such explants. The final results of this investigation, which was made during a visit (E.M.B.) to Oicha in 1959, have not yet been fully analyzed, but an electron microscope study of the fine structure of the bacilli present in explants after several weeks of incubation showed a well-preserved internal structure in many bacilli, some of which were apparently undergoing division (Brieger, Glauert and Allen [3]).

An explanation of why our results differ from those of Hanks, who found a decrease in number of bacilli over the culture period in tissue culture, may be found in the fact that conditions in an organ culture differ from those in tissue cultures. (Tissue from borderline or reactional tuberculoid cases, although reasonably rich in bacilli, are not suitable for this work.)

## PREPARATION OF NIMORPEL

A piece of fine-meshed nylon cloth is spread out in a glass funnel. The explants are transferred to the apex of the cloth. They are then crushed and squeezed with a glass rod. While this is being done normal saline is poured over the tissue. This washing is continued as long as a definite degree of turbidity is maintained. The concentration of bacilli is then assessed by one of the conventional counting methods. The preparation is finally diluted as required. The dilution is controlled by counting. Phenol is added to a concentration of 0.5 per cent. The suspension is placed in suitable vials and sterilized by autoclaving.

## CONCLUSION

The product which we have called Nimorpel is a suspension of heat-killed leprosy bacilli in 0.5 per cent phenol-saline, which is practically

free from tissue debris. It can be easily standardized. Its immunologic effect is comparable to that of the Mitsuda-Hayashi lepromin prepared by conventional methods. This has been shown, not only in observations at Oicha, but also by Dr. J. Cap at Léopoldville in controlled experiments and by Dr. J. M. B. Garrod at the East African Leprosy Research Centre at Alupe, in Kenya, using Heaf's multipuncture technique.

RESUMEN

El producto, al cual se la ha dado el nombre de Nimorpel, es una suspensión de bacilos leprosos matados al calor en 0.5 por ciento de fenol-solución salina, que se encuentra prácticamente exenta de tejidos. Puede además ser normalizada fácilmente. Su efecto inmunológico es comparable al de la lepromina de Mitsuda-Hayashi preparada por los métodos aceptados. Esto ha quedado demostrado, no sólo por las observaciones verificadas en Oicha, sino también por el Dr. J. Cap en Léopoldville en experimentos fiscalizados y por el Dr. J. M. B. Garrod en el Centro de Investigación Leprosa del Africa Oriental en Alupe, Kenia, usando la técnica de multipunción de Heaf.

REFERENCES

1. BRIEGER, E. M. Tubercle bacilli in infected tissues grown on tissue culture. Ciba Foundation Symposium on Experimental Tuberculosis. J. & A. Churchill, Ltd., London, 1955, p. 225-236.
2. BRIEGER, E. M., FELL, H. B. and SMITH, B. R. Comparative study of reaction in vivo and in vitro of rabbit tissues to infection with bovine tubercle bacilli; observations on cultures of spleen and lymph glands from infected rabbits. J. Hyg. (Cambridge) **49** (1951) 189-200.
3. BRIEGER, E. M., GLAUERT, A. M. and ALLEN, J. M. Cytoplasm structure in *Mycobacterium leprae*. Exper. Cell Res. **18** (1959) 418-421.
4. FELL, H. B. and ROBINSON, R. Growth development and phosphatase activity of embryonic avian femora and limb-buds cultivated in vitro. Biochem. J. **23** (1929) 767-784.
5. SHAFFER, B. M. The culture of organs from the embryonic chick on cellulose-acetate fabric. Exper. Cell Res. **11** (1956) 244-248.
6. TROWELL, O. A. A modified technique for organ culture *in vitro*. Exper. Cell Res. **6** (1954) 246-248.